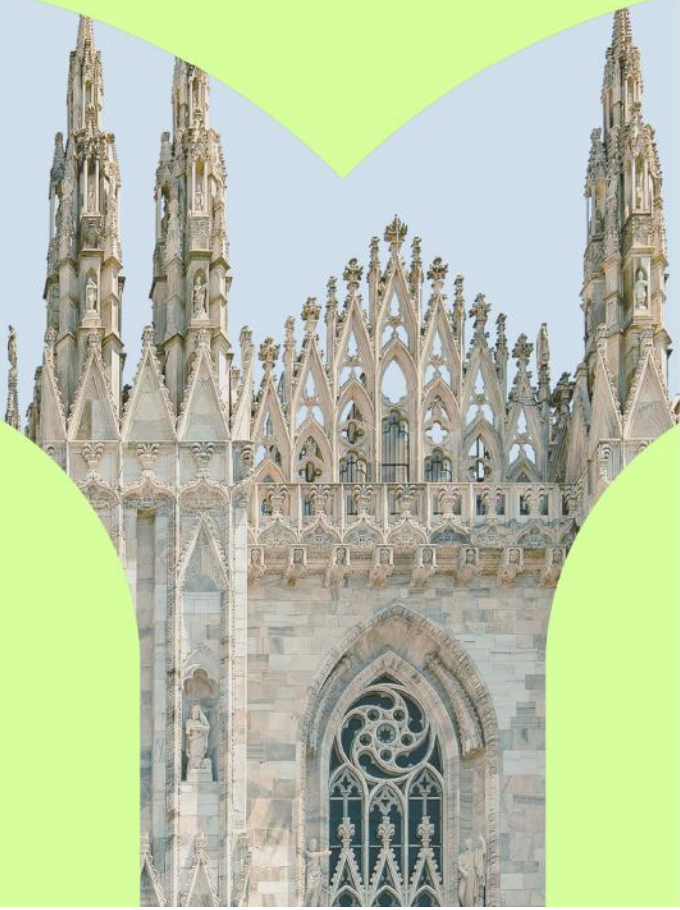
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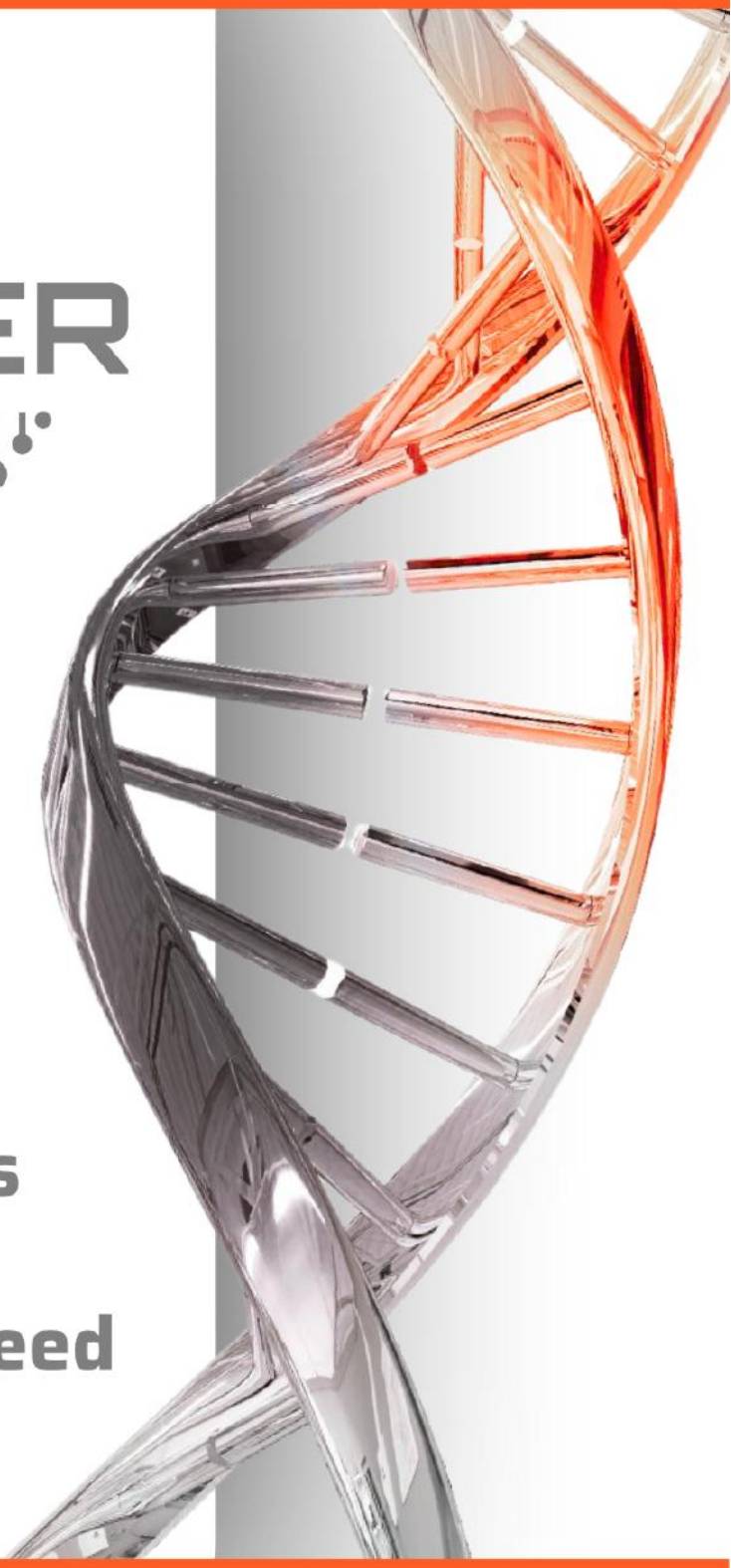
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Abstract ID: 2233 / Poster Number: 385.15P

"A novel approach for rRNA removal to enable single-cell transcriptomics in complex bacterial communities"

Presenting author: Mrs. Ines Alberola, University of Valencia, Valencia, Spain

Co-authors: *Maria Dzunkova, Rokaia Lejri*

Single-cell transcriptomics is a novel technique widely used as it enables precise analysis of cellular genetic activity. However, performing this technique in bacteria is difficult as most tools and protocols like microfluidic systems have been developed and optimized for eukaryotic cells. Besides, the unknown composition of our bacterial samples contained complicates the process. This study aimed to develop a method to remove rRNA from unknown bacteria that could be adaptable to the capsules designed for high-throughput single-cell transcriptomics, isolating mRNA effectively.

To address bacterial diversity, custom 16S and 23S rRNA probes were generated. The specific probes for this experiment were produced as follows: after performing cell lysis, 16S and 23S sections were amplified and, after purifying them, an electrophoresis and extraction of the DNA from the bands were done. A second amplification of the obtained DNA was performed, purified and digested with lambda exonuclease to obtain amplicons, that were used to isolate mRNA from bacteria before eliminate the rRNA with rNase H and purificate it with Dnase I digestion. The mRNA content and quality was analyzed with Bioanalyzer RNA chip.

We have produced our own 16S and 23S probes to eliminate the rRNA of our bacterial cells from wastewater at different stages of its treatment process from a tannery in Tunisia, which were incubated in a medium with and without Chromium (III) oxide, a common contaminant in tannery. After benchmarking with E.coli, the protocol was succesfully applied to tannery microbiome, resulting in large reduction of rRNA molecules.

Abstract ID: 3623

"Los Microbios que viven en tu escuela"/"The microbes that live in your school"- Integration of Foreign Languages and Microbiology using Outreach activities

Presenting author: Dr. Isabel Murillo, University of Bristol, Bristol, United Kingdom

Co-authors: *Carmen Meira Garcia*

This Project was created to provide an opportunity to explore the use of a foreign language, Spanish, when teaching a content-based subject, Microbiology, via a pedagogical methodology called Content Language Integrated Learning (CLIL) in two secondary schools in Bristol. Joining the effort of two academics in the discipline of foreign languages education and microbiology, we created an outreach activity for Year 9 students in secondary education. This activity gave the students the opportunity to use their knowledge in Spanish while performing novel microbiology experiments.

Method: This outreach activity is run in two one-hour lessons with over 30 students in their classroom. The first session introduced microbiology concepts and some hands-on experiments in Spanish. The second session was used to emphasise the importance of languages in the world of work and to discuss the results from experiments performed in the first session. Students assessed the importance of washing hands and swabbed their classroom to investigate what microorganisms live in their school.

Findings: We ran a two-part outreach in two schools with different levels of Spanish and Science. Based on our experience and feedback from schools, we demonstrated that students were more engaged with the hands-on experiment if they could see their own results in a second session.

We used questionnaires to assess how effective this type of approach was. We used the same questionnaire for both schools as we aimed to compare experiences from both cohorts. We also collected oral feedback from the schools' teachers. The feedback was very constructive and positive, and as a consequence, we were invited to run this activity again the following year.

The activity proved very versatile. It allowed us to adapt the level of language according to the student cohort and the school. At the same time, microbiology hands-on activities were also adjusted to the level of science knowledge. Students showed great enthusiasm when using word games and performing microbiology experiments. We found that students from both schools were equally engaged with the activities. This Project shows

that this type of outreach activity connects students with different subjects and makes science inclusive for students with varying levels of knowledge.

Conclusion: The integration of foreign languages and microbiology in outreach activities is an engaging and successful tool for schools. This kind of activity can be easily adapted



to the level of the foreign language and science of the students, creating an inclusive educational environment.

Figure 1: Outreach team on the left. Students plating out swabs from their classroom on the right.

Abstract ID: 645

"Training the Microbiome": Adaptation of a Simplified Human Intestinal Microbiome to Repeated Carbohydrate Exposure

Presenting author: Dr. Natalia Soledad Rios Colombo, APC Microbiome, University College Cork (UCC), Cork, Ireland

Co-authors: *Killian Scanlon, Neda Nezam Abadi, Paul Ross, Colin Hill*

Introduction: Organs are composed of cells that work together to fulfill specific functions. For example, the brain (a network of neurons) can learn and improve task performance through repetition. Similarly, the gut microbiome—often referred to as a "virtual organ"—is a community of microbes that collaborates to perform essential functions, such as nutrient metabolism. But can the gut microbiome also be "trained" to enhance its metabolic efficiency through repeated exposure to specific nutrients? If so, what mechanisms underlie this adaptation?

Objective: To explore these questions, we used a Simplified Human Intestinal Microbiota (SIHUMI), an in vitro consortium of diverse human gut bacteria, to assess if microbial communities exhibit improved metabolic efficiency following repeated carbohydrate exposures.

Methods: SIHUMI was exposed over three days to different doses of two carbohydrates: inulin (a complex carbohydrate) and lactose (a simple carbohydrate). During this "training" we measured pH changes, carbohydrate consumption, and microbial composition via qPCR (Figure 1). A non-conditioned community served as a control. Finally, both trained and non-trained communities were re-exposed to the same carbohydrates to evaluate whether training enhanced their metabolic responses.

Findings: Our results show that trained communities exhibit greater pH reductions and significant shifts in bacterial composition compared to non-trained controls, with these changes scaling with the training dose. This suggests that alterations in community composition may play a central role in the adaptation to repeated nutrient exposure.

Conclusion: Understanding how nutrient exposure impacts microbiome structure and function is key for developing targeted nutritional interventions aimed at optimizing the gut microbiome for specific health outcomes.

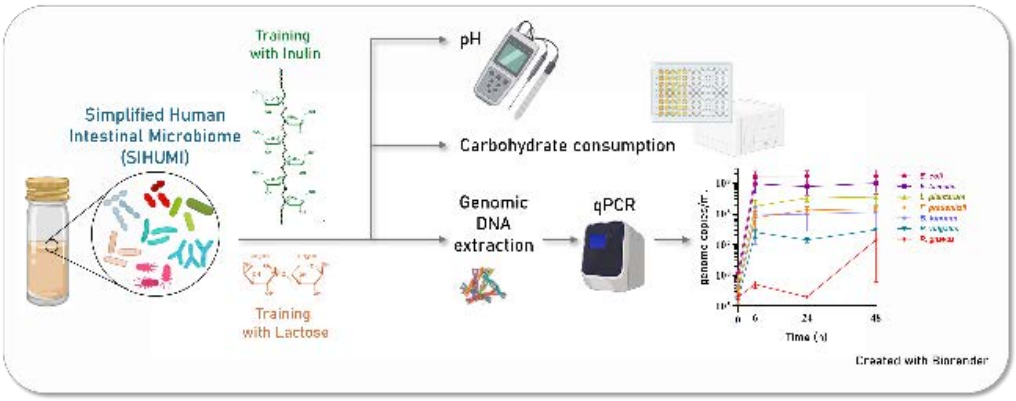


Figure 1: The Simplified Human Intestinal Consortium was exposed to both a complex (Inulin) and a simple (lactose) carbohydrate. During this "training" we measured pH changes, carbohydrate consumption, and microbial composition via qPCR.

Abstract ID: 2094 / Poster Number: 308.17P

3D Printed Yeast-Based Materials for Sustainable Architecture

Presenting author: Dr. Zeinab Hefny, Chalmers University of Technology, Göteborg, Sweden

Co-authors: *Cecilia Geijer, Yagmur Bektas, Malgorzata Zboinska*

Introduction: The integration of biological systems into architectural materials offers a promising path toward sustainable construction. Yeast are versatile microorganisms that are currently being explored in various biotechnological applications. This study investigates the development of 3D printed yeast-based materials that combine structural functionality with biological activity to address sustainability challenges in architecture.

Objective: The primary goal of this research is to create and evaluate 3D printed materials embedded with viable yeast cells for use in sustainable architectural applications. The study focuses on material compatibility, yeast viability, and the potential for long-term biological activity within the printed structures.

Methodology:

A range of materials and strains were tested to identify optimal conditions for embedding yeast cells while maintaining their viability. Experimental setups included variations in material matrix composition and yeast strains. Viability tests were conducted over a 30-day period. The ongoing study explores how different components, including cellulose, glycerol, chitin, and calcium carbonate, impact the mechanical properties and microstructural characteristics of the biomaterial and yeast viability.

Findings: The results demonstrate that yeast cells embedded in specific 3D printed matrices remain viable for at least 30 days post-fabrication. Moreover, the structural integrity of the printed materials was maintained, highlighting their potential for dual functionality as both construction material and biologically active substrate.

Conclusion: This study establishes the feasibility of incorporating living yeast into 3D printed materials for sustainable architectural applications. The findings pave the way for further research into bio-integrative construction technologies, offering innovative solutions for environmentally friendly building practices.

Abstract ID: 1922

5-Fluorocytosine inhibits biofilm formation and virulence in uropathogenic Escherichia coli and enhances β-lactam activity against pre-formed biofilms

Presenting author: Prof. Elio Rossi, University of Milan, Milan, Italy

Co-authors: *Srikanth Ravishankar, Antonietta Lucia Conte, Stacy Julisa Carrasco Aliaga, Valerio Baldelli, Karen Leth Nielsen, Maria Pia Conte, Paolo Landini*

Biofilm-associated infections pose a significant challenge in clinical settings due to the increased tolerance of bacteria to antibiotics during biofilm growth. Our study investigates the potential of the FDA-approved antimycotic 5-fluorocytosine (5-FC) as an antibiofilm agent against uropathogenic *Escherichia coli* (UPEC) strains, expanding on previous findings in the laboratory *E. coli* strain MG1655. Specifically, we assessed the efficacy of 5-FC in inhibiting biofilm formation, modulating virulence determinants, reducing bacterial adhesion, and enhancing antibiotic activity against pre-formed biofilms.

Using clinical UPEC isolates, we demonstrated that subinhibitory concentrations of 5-FC consistently inhibited biofilm formation across strains despite notable phenotypic diversity (Fig. 1A). Gene expression analyses revealed that 5-FC significantly reduced the transcription of key virulence factors, including curli fiber genes (*csgB*, *csgA*), type-1 fimbriae (*fimA*), P-fimbriae (*papC*), and α-hemolysin (*hlyA*) (Fig. 1B). This inhibition of virulence factors led to diminished UPEC adherence to bladder epithelial cells (Fig. 1C) and enhanced the survival of host cells at concentrations far below toxic levels for the host (Fig. 1D). Although 5-FC alone could not disrupt pre-formed biofilms, its combination with β-lactam antibiotics, such as ceftazidime, aztreonam, and piperacillin, markedly reduced bacterial cell viability within pre-formed biofilms. This synergistic effect was specific to β-lactams and not observed with fluoroquinolones or aminoglycosides (Fig. 1E).

In conclusion, 5-FC exhibits robust antibiofilm and antivirulence activity in UPEC strains by targeting critical biofilm and virulence determinants, reducing bacterial adherence, and protecting host cells. Importantly, its synergistic interaction with β-lactams offers a promising therapeutic strategy for eradicating bacteria within pre-formed biofilms. These findings position 5-FC as a compelling candidate for preventing and treating biofilm-associated bacterial infections, addressing a critical need in combating antibiotic resistance.

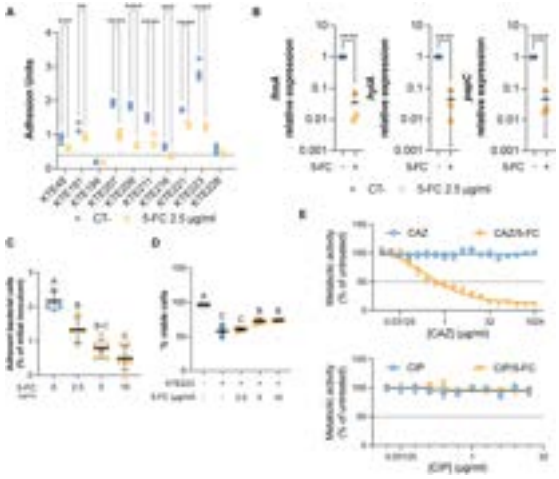


Figure 1: 5-fluorocytosine (5-FC) inhibits UPEC adhesion (**A**) and downregulates the expression of virulence genes (fimA, hlyA, papC) (**B**), reducing bacterial adhesion to bladder epithelial cells (**C**) and promoting host cell survival (**D**). 5-FC enhances the efficacy of β-lactam antibiotics (e.g., ceftazidime, CAZ) but not ciprofloxacin (CIP) against bacteria in pre-formed biofilms, as shown by reduced metabolic activity within the biofilm, measured using the fluorescein diacetate assay (**E**).

Abstract ID: 4086 / Poster Number: 330.15P

A bacterial DOT1-like methyltransferase subverts paraspeckle dynamics and host cell splicing events

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Introduction: *Legionella pneumophila* is a gram-negative bacterium ubiquitously found in freshwater environments where it parasitizes free-living amoeba, and it infects human alveolar macrophages causing a severe pneumonia called Legionnaires' disease. Uniquely, its main virulence mechanism is molecular mimicry of eukaryotic protein motifs. Secretion of these proteins allows *L. pneumophila* to subvert many host signalling pathways. Some of these effectors, named nucleomodulins, can target the host cell nucleus where they actively hijack nuclear functions.

Methods: We investigated gene conservation of DOT1 homologs across *Legionella* species and how they are distributed among organisms such as human and environmental hosts. We studied the role of *Legionella* DOT1-like protein in bacterial replication within macrophages. We determined its crystal structure via X-ray crystallography and characterized the methyltransferase activity of the *Legionella* Dot1-like protein by mass spectrometry, and different biochemical and biophysical approaches. Western blotting and high-content images screening for RNA-FISH enable the understanding of paraspeckle dynamics. RNA sequencing and flow cytometry were used to study splicing events.

Results: Here we identified and characterized a protein we named LpDot1 as it is predicted to encode a lysine methyltransferase similar to eukaryotic Dot1 that is known to catalyze the methylation of histone H3. In particular, we observed a very high aminoacidic identity with organisms sharing the same ecological niche, supporting the hypothesis that *Legionella* DOT1 protein was acquired by horizontal gene transfer. We show that LpDot1 is a virulence factor, as it promotes bacterial intracellular survival in macrophages. LpDot1's crystal structure revealed a very similar architecture to human Dot1L. However, major structural differences are noticeable at Dot1L's substrate-recognition surfaces. Indeed, we observed that LpDot1 is not a histone methyltransferase. Instead, it methylates non-histone proteins, including the splicing

factor proline-glutamine rich protein (SFPQ). *In vitro* we found that the methyltransferase activity of LpDot1 impairs SFPQ dimerization, whereas during infection it causes SFPQ degradation and consequent alteration of paraspeckles' spatial organization in the nucleus. Furthermore, such reorganization leads to a change in the alternative splicing of genes that are important for the host's immune response.

Conclusions: Altogether, our results suggest that LpDot1 methylates SFPQ and manipulates SFPQ-dependent activities to support the survival of *L. pneumophila* within infected host cells.

Abstract ID: 124 / Poster Number: 084.15P

A Combined UV-biological treatment for micropollutants degradation at very short hydraulic retention time

Presenting author: Dr. Ofir Menashe

Co-authors: *Ofir Menashe, Dror Avisar Avisar, Hadas Mamane, Eyal Kurzbaum*

Introduction: Endocrine-disrupting chemicals (EDCs) are emerging pollutants that present a significant risk to the environment, humans, and livestock. Each year, approximately 30,000 kg of natural estrogens, 700 kg of synthetic hormones from human sources, and 83,000 kg of estrogens from livestock are released into the environment. Secondary and tertiary effluents are the primary sources of these contaminants in natural water resources. A new oxidative bioreactor (OBR) system, incorporating small bioreactor platform (SBP) capsules to protect biomass and UV-LED photolysis/photocatalysis technology, has shown effectiveness in breaking down persistent EDCs in secondary effluents.

Objective: This study aimed to evaluate this innovative OBR treatment approach, developed in our laboratory, which is designed to combine biological degradation with UV irradiation (267.2, 278.8 and 285.8 nm) simultaneously to degrade EE2. **Methods:** We compared 3 different processes: (i) LEDs—physical process; (ii) SBP—biological process; (iii) OBR which combines, simultaneously, physical, and biological processes (LEDs with SBP); The effeluents were spiked by 0.5 mg/L EE2, and EE2 monitoring was made by using HPLC-UV analysis. In bioaugmentation, selected microbial flora is added to the system to accelerate the removal of organics. A volume of 1.2–1.5 mL of the culture suspension containing Rhodococcus zopfii was injected into 45 sterile SBP capsules, and the injection hole was then sealed with cellulose acetate polymer.

Findings and Conclusion: For the first 6 h, the observed EE2 degradation kinetic rates were 0.027, 0.019, and 0.019 mg/h for OBR, physical, and biological treatment, respectively. During vs (very short) HRT (up to 6 h), the combined OBR process reduced EE2 by 32%, while the physical process only reduced it by 23%, showing an advantage of the combined OBR process for short time spans. During EE2 biodegradation in vsHRT obtained the highest benefit after approximately, 2-4 hours indicating a good treatment potential by utilizing the OBR within a secondary clarifier. The advantages at vsHRT (up to 6 hours) allowing us to implement the OBR system in secondary sedimentation tanks that in most cases are having 4-6 hours of hydraulic retention time. This study is the first to examine and evaluate the effect of treatment coupling (physical and biological), using an OBR configuration, on a steroidal sex hormone (EE2), which can be modified for the

treatment of additional micropollutants. Here, the SBP-encapsulated biomass was not adversely affected by the UV light and therefore, the technology presents a significant advantage for exploring the treatment potential of UV radiation.

The OBR strucutre: 1. UV-LEDs at two wavelengths, 267.7 nm and 277.4 nm; 2. quartz beaker of maximum 3.8 L volume (diam. 135 mm ⇥ height 280 mm); 3. SBP capsules; 4. beaker-shaped grid to hold the SBP capsules; 5. cool/warm water (thermostat); 6. air diffuser; 7. and 8. inflow and outflow stream to new OBR; 9. stirrer.

Abstract ID: 2951 / Poster Number: 282.16F

A flexible and cost-effective approach for long-read amplicon sequencing with Oxford Nanopore

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Co-authors: *Bela Hausmann, Julia Ramesmayer, Petra Pjevac*

Amplicon sequencing remains the most widely used method for microbial community profiling, with various workflows developed to enhance throughput and multiplexing, thus reducing the costs per sample. With the increasing accuracy of long-read sequencing technologies, such as Oxford Nanopore Technologies (ONT), there is a growing demand to adapt short-read amplicon workflows to long-read platforms, as longer fragments (>600bp) enable higher and more accurate phylogenetic resolution. In this study, we adapt our currently published dual-barcoding two-step PCR approach designed for highly multiplexed short-read amplicon sequencing, to the ONT platform. Target gene amplification (e.g. full length 16S rRNA) is performed using headed primers, and two unique barcodes are introduced in a second PCR step. Dual-barcoded libraries are then pooled and prepared for ONT sequencing using either the standard ligation kit (SQK-LSK114) or the native barcoding kit (SQK-NBD114) and sequenced either on a MinION or a PromethION flowcell. The approach is highly flexible, allowing for the pooling of up to 548 samples of the same target gene using one single library preparation. When profiling different target genes, multiplexing capacity can be further expanded, potentially increasing the number of libraries in a run by a multifold, depending on project-specific goals such as sample type and desired sequencing depth. This flexibility makes our workflow adaptable to a wide range of microbial community profiling studies, enhancing throughput, without compromising accuracy and cost effectiveness.

Abstract ID: 3408 / Poster Number: 250.16P

A high-throughput single-cell metagenomic sequencing method to resolve microbiomes at the single-cell level

Presenting author: Dr. Jyoti Verma, Umeå University, Umeå, Sweden

Co-authors: *Hadrien Gourlé, Iryna Yakovenko, Julian Dicken, Florian Albrecht, Linas Mažutis, Johan Normark, Nicklas Strömberg, Tommy Löfstedt, Laura M. Carroll, Johan Henriksson*

Introduction: Determining the composition of a particular microbial community is a crucial question in microbiome research. Even with significant advancements in shotgun metagenomic sequencing (metaG) and bioinformatic analysis techniques, figuring out "who is there" in a microbial community is still quite difficult: when shotgun metaG sequencing reads are assembled *de novo*, the product is usually a set of contigs that cannot be categorized into strain-level genomes. However, single-cell metagenomic sequencing (scMetaG) methods have the potential to overcome the critical limitations that plague current state-of-the-art bulk metaG methods.

Objectives:

a. Develop a high-throughput scMetaG protocol to resolve microbes in complex communities.
b. Develop a bioinformatic pipeline for complete scMetaG data analysis.

Method: Our scMetaG protocol utilizes a novel microfluidics approach of the Atrandi Onyx instrument to encapsulate bacterial cells in semi-permeable capsules (SPCs), lyse, and further sequence them (Fig.1). The cell's genomic DNA stays inside the SPC, but due to the semi-permeability of the SPC, enzymes and buffers can be easily exchanged. A mock bacterial community (ATCC MSA-2003) with an even mix of 10 bacterial species was used for the optimization. Lysis being the primary source of bias, combinations of different lysis methods were tried, and species-specific primers were used to assay lysis efficiency. Furthermore, libraries were prepared following Atrandi's instructions and sequenced using Illumina Novaseq X. A complete R-based pipeline (Zorn) was designed for end-to-end analysis of scMetaG data. To manage the large amount of data, a custom file format with an integrated workflow manager (Bascet) was designed.

Findings: The mock microbial community subjected to the optimized lysis and library preparation protocol generated high-quality Illumina scMetaG data. The generated scMetaG data was analysed using the developed software. Three different modes of cell-

to-cell comparison were implemented, using existing KMER databases, *de novo*-selected KMERs, and alignment to expected reference genomes. Algorithms were implemented to detect and discard SPCs containing two or more cells, ensuring assembly of individual genomes. The pipeline was also expanded to enable tools for QC and AMR gene detection to be executed for each individual cell, with the microbiome composition visualized as an interactive UMAP.

Conclusion: The scMetaG method developed here will provide unprecedented insights into microbiomes, as it can be used to query any microbial community at single-cell-level resolution. Furthermore, the state-of-the-art single-cell technology will not only help in distinguishing the various strains of the same species but will further aid in linking extrachromosomal elements (e.g., plasmids, plasmid-mediated antimicrobial resistance genes) to their respective host.

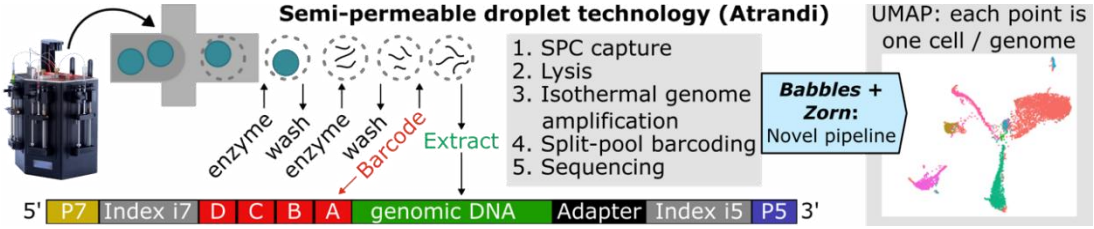


Figure 1: Workflow of our high-throughput single-cell metagenomics method. Bacterial cells enclosed inside the semi-permeable capsule were lysed and sequenced (1-5). The developed novel pipeline was used to analyse the generated Illumina scMetaG data.

Abstract ID: 1472

A Lung-on-Chip platform for delineating the interplay of infection and the epithelial innate response in non-tuberculous mycobacterial (NTM) lung disease

Presenting author: Dr. Michal Meir, The Clinical Research Institute in Rambam (CRIR), Haifa, Israel; Technion – Israel Institute of Technology, Haifa, Israel

Co-authors: *Mai Bashir, Rose Ibraheem, Adele Goldman-Pinkov, Rachel (Reli) Kakun, Arbel Artzy-Schnirman,*

Introduction: Non-tuberculous mycobacteria (NTM) cause severe chronic pulmonary infections of rising incidence in patients with chronic lung diseases such as Cystic Fibrosis (CF) or Chronic Obstructive Pulmonary Disease. NTM infections present a tremendous therapeutic challenge, with high treatment failure rates despite prolonged multi-drug therapy. Advancement in treatment and in the search for new therapies is limited by the failure of antibiotic susceptibility tests to predict clinical response, and by the lack of a reliable and accessible infection model. Organ-on-chip models aim to faithfully mimic key features of the human tissue complexity, by featuring functional cell layers in an anatomic-simulating structure, and functional tissue physiology. Specifically, lung-on-chip models enable investigation of the pathophysiology and immune responses related to pulmonary infections, and allow simulation of drug administration through the bloodstream or via inhalation while these research approaches are limited within other *in-vitro* research models such as *in-vitro* cell culture or insert.

Objectives and Methods: We aimed to establish an NTM infection model of the bronchi, based on a previously developed LoC platform, incorporating a bronchial anatomy-simulating structure, an air-Liquid Interface (ALI), mycobacterial infection and assessment of the local cytokines secretion.

Findings: We used an air-way on chip model seeded with Calu-3 bronchial cells, noted to form confluence and functional tight junctions. We infected the models with *M. abscessus* ATCC^{Mcherry} with noted proliferation within the model. However intracellular infection was limited. Accordingly, mycobacterial infection had limited effect on tissue permeability and on the cytokine secretion profile.

Conclusions: Limited mycobacterial infection of bronchial epithelium has been shown in previous immune-competent *in vivo* murine models. Our findings suggest additional processes are required to induce significant infection and cells' damage, such

inflammation induced by co-infection or recruitment of cellular immune response. Once established, the airway-on-chip model may advance understanding of the host-pathogen interplay in the pathophysiology of mycobacterial lung disease, and may serve as a tool for assessment and development of therapeutic regimens.

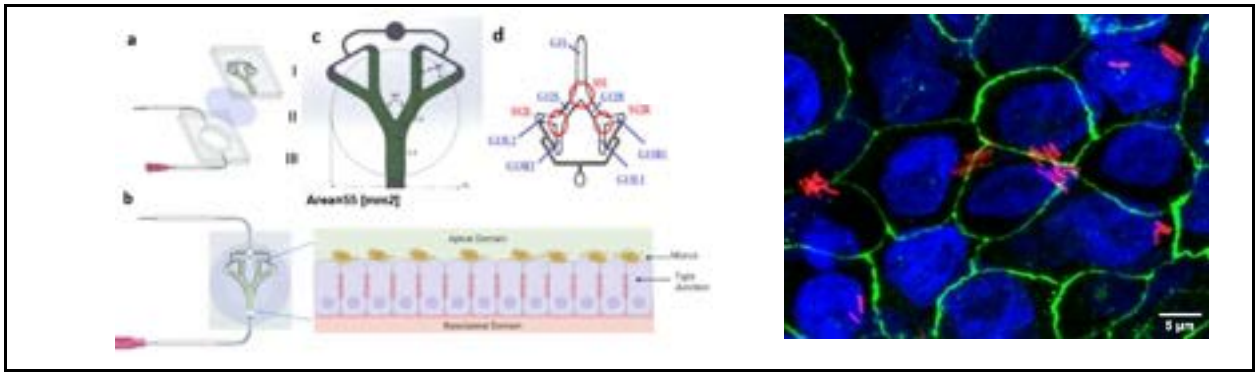


Figure 1: ***Airway-on-Chip Model represent a platform for Mycobacterium abscessus (Mab) pathogen-Host interaction: a-b*** *Bronchial Airway on Chip model platform* ***a****. Exploded view: I apical domain II basement membrane III basal domain.* ***b****. collapsed view, representative illustration of Calu-3 cells in the apical chamber of the model.* ***c.*** *Confocal microscopy y images (X60 high magnification) of Calu-3 cells within the airway-on-chip model. Cells were stained for cell nuclei (blue), ZO-1 (green), Mab (red).*

Abstract ID: 2682 / Poster Number: 423.15P

A megatransposon drives the adaptation of Thermoanaerobacter kivui to carbon monoxide

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Co-authors: *Josef Horvath, Maja Stumptner, Gerhard G. Thallinger, Stefan Pflügl*

Introduction: Acetogens are promising industrial biocatalysts for upgrading syngas — a gas mixture containing CO, H_2 and CO_2 — into fuels and chemicals. However, CO severely inhibits growth of many acetogens, often requiring extensive adaptation to enable efficient CO conversion ("carboxydotrophy"). Despite the adverse impact of CO on growth rate, acetogenic conversion of CO through the Wood-Ljungdahl pathway (WLP) provides significantly more ATP compared to H_2 and CO_2. As a result, efficient CO uptake theoretically enables the conversion of syngas into ATP-intensive fuels and chemicals (e.g., butanol), which in turn makes engineering carboxydotrophy highly desirable to develop sustainable production processes based on syngas.

The thermophilic acetogen *Thermoanaerobacter kivui* has been previously shown to be adaptable to a carboxydotrophic lifestyle, although its growth rate on CO as sole carbon and energy source remained slow (T_d ~ 40 h, versus ~ 2h for H_2/CO_2)[1]. However, the precise mechanisms underlying CO adaptation have so far remained elusive, which impedes efforts to rationally engineer carboxydotrophy.

Objective: In this work, we aimed to create a robust chassis for thermophilic bioprocessing of syngas as well as to elucidate the cognate mechanisms conferring CO tolerance and utilization.

Method: We combined adaptive laboratory evolution, bioprocess engineering and multi-omics data analysis to generate a strain rapidly growing on CO as sole carbon and energy source, as well as to quantitatively and qualitatively evaluate its physiological, genetic and metabolic features. We additionally performed genome editing to validate the function of genes putatively linked with carboxydotrophy.

Findings: We adapted the thermophilic acetogen *T. kivui* to use CO as sole carbon and energy source. Isolate CO-1 exhibited rapid growth on CO and syngas (co-utilizing CO, H_2 and CO_2) in batch and continuous cultures (T_d ~ 2.8 h). The carboxydotrophic phenotype

was attributed to the mobilization of a CO-dependent megatransposon originating from the locus responsible for autotrophy in *T. kivui*. Transcriptomics illuminated the crucial role the redox balance plays during carboxydotrophic growth. These novel insights were exploited to rationally engineer *T. kivui* to grow on CO.

Conclusion: Collectively, our work elucidates a primary mechanism responsible for the acquisition of carboxydotrophy in acetogens and additionally showcases how transposons can orchestrate evolution.

Abstract ID: 1344 / Poster Number: 261.16P

A multiclonal outbreak of carbapenem-non-susceptible Pseudomonas aeruginosa in neonatal intensive care unit

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Introduction: Pseudomonas aeruginosa (PSAE) can persist in moist environments in healthcare settings, representing a source for possible outbreaks of healthcare-associated infections.

Objective(s): Here is a multiclonal outbreak of carbapenem-non-susceptible PSAE in the neonatal-intensive-care unit (NICU) of Fondazione IRCCS Policlinico, Milan, Italy.

Method: Whole genome sequencing (WGS) was performed on PSAE strains consecutively isolated from neonates and the environment. Bacterial typing and molecular resistance mechanisms were defined by MLST-tool and AMR finder. Antimicrobial susceptibility to carbapenem was tested by broth microdilution using the Microscan-WalkAway (Beckman, Coulter). Carbapenem-non-susceptible PSAE was defined with a minimal inhibitory concentration to Meropenem (MEM) higher than 2 mg/L. Maximum Likelihood and Minimum Spanning Trees were used to determine strain correlations and identify potential transmission clusters (defined as bootstrap=100% and SNP distances <5).

Findings: Between August and September 2024, two sepsis and four colonizations by carbapenem-non-susceptible PSAE were reported in 6 neonates (including two twins) in NICU. As a result, PSAE genomic surveillance was performed in all weekly collected clinical isolates and ad-hoc collected contaminated environment samples (total isolates: 18 clinical samples from 8 neonates and 12 sink-siphons samples from nine rooms). Overall, phylogenetic analyses showed the circulation of four carbapenem-non-susceptible STs (ST446, ST395, ST253, ST179). Twelve clinical isolates accounting for 6 patients (two developing sepsis) were organized in a ST446 large transmission chain (SNP-distance:0). One isolate belonging to ST179 was genetically related to one isolate detected in moist environments carrying the blaVIM gene plus the orpD-V359L mutation, both related to carbapenem resistance mechanisms. The other three carbapenem-non-

susceptible clinical isolates were interspersed in the ST395 and ST253. The remaining 2 clinical and 11 environmental isolates were characterized by carbapenem-susceptible STs (ST155=2; ST210=1; ST244=1; ST309=3; ST235=4; ST674=2).

Conclusion: Genomic characterization of PSAE in clinical and environmental isolates allowed us to define a multiclonal diversification of the carbapenem-non-susceptible PSAE outbreak in NICU, mainly characterized by one transmission chain of probable clinical origin and one probable transmission pair involving a contaminated environment, and to implement adequate measures to limit the outbreak.

Abstract ID: 1091

A new microbial innovation for fighting cancer

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Co-authors: *Sophie Sablé, Jennifer Saliba, Béatrice Colin, Pierre-Édouard Bodet, Benjamin Musnier, Valérie Sopéna, Marina Denimal, Margot Garrabos, Isabelle Lanneluc, Ingrid Arnaudin*

Introduction: cancer is a life form characterised by competition between individual cells for access to nutrients, survival and proliferation, similar to what occurs in bacterial populations. Many characteristics and abilities acquired by cancer cells closely resemble properties observed in micro-organisms [1]. The microenvironments of cancer cells, like in biofilms, are structures with physical and chemical heterogeneities (different pH levels, different oxygen concentrations, etc), which often hinder the effectiveness of drugs in reaching and destroying cancer cells or pathogenic bacteria. In particular, bacterial biofilm formation and metastatic progression share remarkably similar biological processes. These include mechanisms such detachment, entry into the bloodstream, and colonisation of other sites, leading to the hypothesis that these mechanisms may be regulated by common chemical mediators [2]. **These striking parallels in biology between cancer and bacteria forming biofilms are inspiring and offer novel insights into the development of exciting and innovative bacteria-based anticancer treatments.** In this context, we hypothesize that bacterial strains exhibiting anti-biofilm and anti-growth activities, two critical functions for combating cancer, could serve as promising bioproducers of anticancer metabolites. **Objectives:** to test this hypothesis, we chose **to create and cultivate various consortia of several marine bacterial strains associated with these key phenotypes, and to evaluate the anti-tumor properties** of the metabolites secreted (secretomes) by these consortia on breast cancer cells. **Methods:** the growth of selected bacterial strains was first tested at two temperatures: 22°C and 30°C. Among the 34 strains, 27 strains grew better at 22°C. Strains were then assembled into several consortia and cultivated at 22°C for the bioproduction of their secretomes. Secretomes antitumor properties were evaluated *in vitro* on two types of breast cancer cell lines: MCF-7 mimicking the luminal subtype (accounts for 60% of breast cancers detected), and MDA-MB-231 mimicking the basal-like subtype, more commonly known as triple negative (associated with a high capacity for metastatic dissemination), still today in a therapeutic impasse. Each bacterial secretome was tested at four concentrations (1X, 0.5X, 0.25X or 0.1X) on the viability of these two breast cancer cell lines, and of a non-cancerous cell line for control, using the

MTT assay (figure 1). **Findings and conclusions:** most of the metabolites secreted by these consortia showed dose-dependent effects on cancer cell lines, and interestingly, some exhibited an enhanced ability to affect specifically triple negative cancer cells viability. This study provides the first evidence that assembling bacteria pre-selected for key functions into consortia could constitute new microbial innovations to fight cancer.

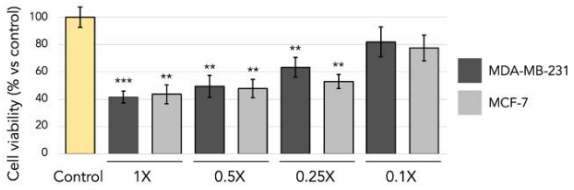


Figure 1: Cell viability of MDA-MB-231 and MCF- 7 cell lines after 24 hours of contact with bacterial secretomes from consortium 7: MTT test with different secretome concentrations (1X, 0.5X, 0.25X and 0.1X). Significant differences are indicated by * ($p<0.05$), **($p<0.01$), or *** ($p<0.001$) in comparisons between the no-treatment control (yellow) and the trials with secretome treatment.

References [1] M. Russo, A. Sogari, A. Bardelli. Adaptive Evolution: How Bacteria and Cancer Cells Survive Stressful Conditions and Drug Treatment Cancer Discov. 11, 1886-1895 (2021) [2] N. Ivanenko, Biofilm and tumor: interpretation of interaction and treatment strategies. Review. Medical Science of Ukraine (MSU), 17(1), 104-120 (2021).

Abstract ID: 1766 / Poster Number: 300.17P

A New Pan-Genome Approach for Detecting 19 Viable Probiotic Species Approved by KMFDS using Species-Specific Genes

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Co-authors: *You-Tae Kim, Jihye Yang, Li-Ha KIM, Sung-Woo Choi, Ji-Seon Hwang, Ju-Hoon Lee*

Introduction: In the case of the Ministry of Food and Drug Safety in Korea, 19 species that can be used as probiotics are designated. To measure probiotics, the same plate culture method is employed as the FDA. Accurate identification and quantification of probiotic species in commercial products remain a significant challenge due to inconsistencies between labeled and actual contents.

Objectives: Recent clinical trials have highlighted potential mismatch and contamination of probiotic strain. To tackle these concerns, we have identified species-specific genes and developed real-time PCR and NGS panel method for rapid identification and quantification.

Methods: In selecting target genes, pan-genome analysis utilized 4,345 complete genome sequences. We validated genes with BLASTN, and designed species-specific primer and probe sets in both conventional and new detection methods.

Findings: Species-specific genes were identified based on pan-genome analysis, and target genes for each species were selected and validated in silico. Primers and probes for these genes were designed and verified with conventional method of singleplex, crosscheck and multiplex PCR. Standard curves showed slopes between -3.00 and -3.50 with $R^2 \geq 0.995$. The results of PMA-qPCR and viable cell count were identical, confirming the quantification of live cells. 57 genes selected for NGS panel were also verified by using conventional PCR and Oxford nanopore sequencing.

Conclusion: According to PMA-qPCR and NGS results, the 19 probiotic species were rapidly identified and quantified even in large-scale food, suggesting that this rapid

identification method may contribute to monitoring commercial probiotics-containing products to prevent misidentification and strain contamination.

Abstract ID: 1796 / Poster Number: 220.15P

A Novel Approach to Isolate New Bacterial Strains Using Gliding Motility

Presenting author: Dr. Song-Gun Kim, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, South Korea

The bacterial group within the phylum *Bacteroidota* plays a significant role in the global carbon cycle of marine ecosystems, primarily due to its specialized ability to degrade biopolymers such as polysaccharides and proteins. While metagenomic studies utilizing high-throughput next-generation sequencing (NGS) have enriched our understanding of microbial communities, their physiology, and new metabolic pathways, isolating novel strains remains essential for testing unique physiological traits and metabolic processes, and for harnessing the microbial potential of these new resources. In this study, we employed an innovative strategy to isolate new *Bacteroidota* strains based on their gliding motility, sourcing samples from macroalgae, decaying wood, and invertebrate sediment in the waters surrounding the Korean Peninsula. Specimens were plated on low-nutrient solid media to stimulate gliding motility, allowing the bacteria to forage for survival. We utilized a stereo microscope equipped with a digital camera and monitor within a sterile aerobic and anaerobic environment to identify gliding microorganisms. Through this approach, we characterized and classified two new genera and 11 new species, demonstrating that new bacterial representatives can be isolated and cultivated by observing gliding motility, thereby enhancing our understanding of their environmental adaptation mechanisms.

Abstract ID: 1516 / Poster Number: 155.15P

A novel halophilic community from Lake Burgas in Bulgaria displayed polycaprolactone-degrading activity

Presenting author: Mr. Nikolay Krumov, Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

Co-authors: *Nikolina Atanasova, Ivanka Boyadzhieva, Tsvetelina Paunova-Krasteva, Penka Petrova*

Halophiles constitute a taxonomically diverse group of extremophilic microorganisms characterized by their ability to thrive in environments with high salt concentrations, usually ranging from 15% to 30%. They have been reported in a variety of hypersaline habitats, including seawater, lagoons, lakes, soils, and mines, and often demonstrate distinct metabolic and enzymatic properties through which they cope with extreme environmental challenges. Our research presents a novel microbial community isolated from a plastic-contaminated site in Lake Burgas, the largest body of brackish water in Bulgaria. Samples were inoculated in a nutrition medium containing 15% sodium chloride and provided with polycaprolactone, a biodegradable plastic, as the sole carbon source. The community demonstrated high esterase activity as evidenced by a spectrophotometric analysis performed over 8 weeks and biodegradation potential, determined through scanning electron microscopy (SEM), weight analysis, and cultivation on a polycaprolactone-containing agar medium. The metagenomic analysis uncovered that Hallomonas profundus prevails in the community, but the presence of other species, such as Aloalcanivorax venustensis, Chromohalobacter marismortui, and Halomonas caseinilytica, was recorded as well. Further work led to the successful isolation of a Halomonas sp. strain, demonstrating high esterase activity in polycaprolactone. Exploring the diversity of halophiles in habitats such as Lake Burgas and the biochemical properties of its constituting microorganisms can contribute substantially to our fundamental understanding of extremophiles and provide a valuable set of practical tools with potential application in conservation and environmental research.

Abstract ID: 2993 / Poster Number: 210.16P

A novel in silico system for the generation of low-toxicity antimicrobial peptides

Presenting author: Dr. Lucia Pagani, Bioaster, Lyon, France

Co-authors: *Yannick ZOETGNANDE, Derry Mercer*

Introduction: Due to the ongoing global AMR crisis (silent pandemic) one aspect of the One Health approach to combating this crisis is the development of novel antimicrobials. Antimicrobial peptides are promising candidates for combating antibiotic-resistant pathogens. However, developing antimicrobial peptides with desired characteristics, such as potent antimicrobial activity and minimal toxicity, remains a significant challenge.

Objective: To address this, we have developed a novel comprehensive computational framework for the generation of peptides and prediction of their antimicrobial and toxicity capabilities.

Methods: Our in silico architecture is composed by two main modules: a peptide generator and a predictors-modules. The first module generated peptides according to parameters set by specialists (eg length, isoelectric point, amino acid to include or limit). The generated peptides would pass through the predictors-module, for antimicrobial capabilities, Minimum inhibitory concentrations against species of interests, and cytotoxicity. Predictors leverage state-of-the-art Machine Learning techniques on 3 main blocks for: the 3D structure of the peptide (through Graph Encoder), the amino acid (via a peptide Transformer), and peptide characteristics (using a peptide Language Model). The predictors were tested against state-of-the-art models on standard datasets. Moreover, the individual contribution of the three blocks on the prediction performance was tested (ablation study).

Findings: The performance results on the predictors module demonstrate that our model surpasses the state-of-the-art models on antimicrobial and toxicity prediction across multiple experimental validated datasets by 6-12 points percentage. Our ablation study indicates that the peptide tokenizer and the peptide Language Model are the main drivers for the performance, while the Graph Encoder helps the model to reduce the variance of the performance.

Over 50k peptides were generated by the first module. Every peptide was tested by the 3 predictors module. Sequences with high antimicrobial properties and low predicted

toxicity are currently being synthetized and will be tested against pathogens of interest in in vitro assays.

Conclusion: In the current work we developed an in silico system to generate novel antimicrobial peptides. Our system is composed by a first algorithm to generate a large quantity of peptides, that are filtered for wanted characteristics (antimicrobials with low MIC for specific species, and low toxicity). Generated peptides could be scored for additional ad-hoc characteristics.

The system can play a pivotal role in the current antimicrobial resistant crisis, providing novel peptides with specific characteristics.

Results – leverages: By leveraging state-of-the-art machine learning techniques, such as graph neural networks and cross attention fusion, AIMLess integrates diverse biological and structural features, including ESM embeddings, graph-based molecular representations, and peptide sequence attention models. The framework incorporates innovative approaches, such as noise-injected embedding enhancement, to improve the generalizability of predictive models.

Abstract ID: 3093 / Poster Number: 113.15F

A novel multi omics approach for the identification of promising bacteria for plastic bioremediation

Presenting author: Dr. Silvia Petraro, Università degli studi di Parma, Parma, Italy

Plastic pollution is one of the most pressing environmental challenges, with millions of tonnes produced annually, a significant portion of which accumulates in ecosystems causing persistent damage. Current disposal methods, such as incineration and landfilling, are inadequate and often exacerbate the problem. One promising alternative is bioremediation, which employs microorganisms or plants to degrade pollutants via enzymatic activity. However, our understanding of the bacterial biodiversity involved in plastic biodegradation is limited.

This study addresses these gaps by introducing the *Plastic-Microbial BioRemediation* database (*Plastic-MBR*), a comprehensive resource integrating genetic information on bacteria capable of degrading plastic pollutants. The *Plastic-MBR* database catalogues 242 bacterial sequences associated with the degradation of 28 plastic pollutants. Using *Plastic-MBR* database, we propose and validate a multi-omics approach for the high-throughput identification of promising microbial candidates for plastic bioremediation. Through a metagenomic analysis of samples from plastic-contaminated rivers and soils, we reconstructed the distribution of microbial enzymes and associated species involved in plastic bioremediation. By integration of these findings, we identified novel candidates encoding enzymatic functions responsible for plastic degradation. Subsequently, we performed *in vitro* experiments to validate the degradation abilities of the species exploiting bacterial strains available in public repositories. These strains were genomically inspected *in silico* for the presence of the expected plastic degradation genes and subsequently were acquired and employed in *in vitro* validations. In this context, the *Plastic-MBR* database serves as a valuable resource for advancing research, offering a tool to identify and functionally characterize bacteria with potential applications in environmental plastic bioremediation.

Abstract ID: 842 / Poster Number: 208.17P

A Novel Nucleoid-associated Protein Modulates Chromosome Supercoiling in Streptococcus pneumoniae via Topoisomerase I activation

Presenting author: Dr. Monica Amblar, Centro Nacional de Microbiología. Instituto de Salud Carlos III, Majadahonda, Madrid., Spain

Co-authors: *Antonio Alexandre De Vasconcelos Junior, Diego Megias, Adela G de la Campa*

Streptococcus pneumoniae (SPN) is a major human pathogen. The World Health Organization estimates that one million children die annually from pneumococcal disease. The global spread of penicillin-non-susceptible SPN isolates is a major public health problem, and vaccines are ineffective due to the emergence of non-vaccine serotypes. The organization of the bacterial chromosome is a promising area of research, as it may provide potential new drug targets. The compaction of the bacterial chromosome to form a dynamic and functionally active nucleoid within cells, requires the concerted action of topoisomerases, which are targets of fluoroquinolones, and of nucleoid-associated proteins (NAPs). Topoisomerases play a pivotal role in shaping the chromosome, resolving topological conflicts associated with critical cellular processes such as replication and transcription (topoisomerase I and gyrase), and facilitating chromosome decatenation (topoisomerase IV). NAPs are a diverse group of proteins similar to eukaryotic histones involved in compaction that constrain supercoiling (Sc) by inducing curvature, wrapping DNA, or bridging different regions of the chromosome. To date, the only NAPs identified in SPN are HU, SMC, and StaR, a novel protein identified in our laboratory that contributes to Sc maintenance by increasing Topo I relaxation activity. However, the absence of several NAPs that are important for nucleoid organization in other bacteria suggests the potential existence of other NAPs in this pathogen.

In this study we investigated the role of the hypothetical protein Spr0488, present in pneumococcal nucleoids. This small protein, (131 amino acids) is conserved in all pneumococcal genomes and is predicted to bind nucleic acids. To determine whether Spr0488 was related to Sc, we constructed a Δspr0488 deletion strain and a Δspr0488(pR-0488) overproducer strain, and examined their growth in the presence of gyrase (novobiocina) or Topo I (seconeolitsine) inhibitors. Overproduction of Spr0488 impaired growth in the presence of novobiocina, but improved it in the presence of seconeolitsine, suggesting a role for Spr0488 in Sc. Oligomerization studies on the

purified protein using size exclusion chromatography revealed that Spr0488 is predominantly a dimer in saline solutions. We investigated its DNA binding ability using complementary fluorescent oligonucleotides, and stablished that it binds dsDNA in a non-sequence specific manner, but not ssDNA. To assess whether Spr0488 acts as a modulator of the topoisomerases, we analyzed its effect on the DNA relaxation activity of Topo I and the introduction of negative Sc activity of gyrase. Spr0488 was able to activate Topo I *in vitro*, but not gyrase. Furthermore, we employed anti-Spr0488 antibodies for immunolocalization using super-resolution confocal microscopy. The results showed that Spr0488 accumulates predominantly in one or two foci per bacterium, with either a polar localization, in the case of diplococcus or short chains, or a mid-cell localization in single cocci, suggesting a correlation with the cell cycle.

These results demonstrate the involvement of Spr0488 in Sc homeostasis through modification of Topo I activity, and highlight the existence of new factors involved in modulating chromosome compaction that remain to be discovered. Furthermore, our outcomes allow for the classification of Spr0488 as a novel NAP in SPN.

Abstract ID: 4035 / Poster Number: 334.15P

A pathway-based framework for the analysis of metagenomic data

Presenting author: Dr. Olivier Chapleur, INRAE, Antony, France; Université Paris-Saclay, Antony, France

Co-authors: *Chrystelle Bureau, Cédric Midoux*

Introduction: Metagenomic and metatranscriptomic analyses are powerful tools for deciphering the functioning of microbial communities in diverse environments. However, the complexity of the data they generate poses significant challenges, particularly when analyzing multiple samples jointly, such as in time-series or multi-condition studies. Traditional multivariate statistical approaches, while useful for summarizing such datasets, often emphasize the most divergent genes without adequately prioritizing their biological relevance. As a result, crucial biological pathways may be overlooked, outliers overemphasized, and findings misinterpreted. To address this, we present a novel framework leveraging ComDim, a multi-block chemometric method, for pathway-based analysis of metagenomic data.

Objectives: This study aims to establish ComDim as a robust analytical approach for metagenomic datasets by demonstrating its ability to identify key pathways and gene contributions that capture functional dynamics across time and conditions.

Method: Gene sequence reads are grouped by predicted function (KEGG ORTHOLOGY identifiers, KO) into blocks representing KEGG PATHWAYS, retaining only sufficiently populated blocks. ComDim computes orthogonal common dimensions (CDs) that capture the maximum shared variance across the blocks (pathways). These CDs provide global scores (ranking samples by pathway consensus), local scores (pathway-specific rankings), loadings (gene relevance within pathways), and weights (pathway importance), enabling a nuanced exploration of the data.

Findings: Time-series metagenomic data from replicated anaerobic digesters under steady-state and disturbance conditions (72 samples in total, with salt addition as the disturbance) were used as a case study. ComDim provided detailed insights into metagenomic shifts following the disturbance. In particular, it successfully identified key pathways impacted by perturbation and highlighted genes with the highest variability within each pathway. It also identified pathways that, after initial perturbation, returned to their original state, indicating functional resilience and recovery.

Conclusion: By offering an integrative, pathway-centric perspective, ComDim enhances the interpretability of complex microbiome datasets. This framework is broadly applicable to microbial community studies, facilitating improved understanding and management of microbial communities in various ecosystems.

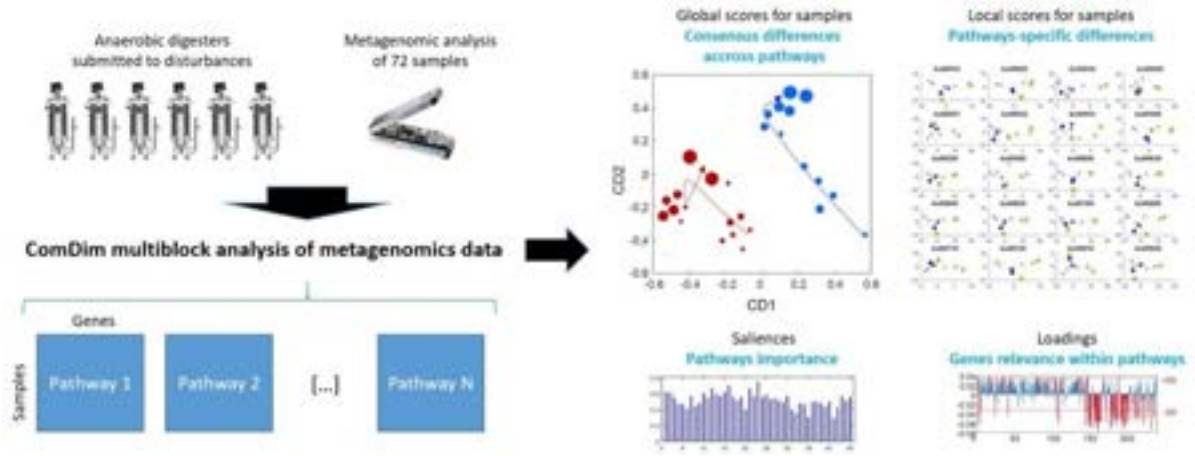


Figure 1: Schematic representation of the ComDim approach for pathway-based analysis of metagenomic data.

Abstract ID: 1534 / Poster Number: 135.15P

A plasmid harbouring a polysaccharide biosynthesis cluster partially blocks Ceduovirus infection in Lactococcus lactis

Presenting author: Ms. Claudia Rendueles, Instituto de Productos Lacteos de Asturias, CSIC, Oviedo (Asturias), Spain

Co-authors: *Javier Nicolás Garay-Novillo, Martin H. Rau, Paula Gaspar, José Ángel Ruiz-Masó, Jennifer Mahony, Ana Rodríguez, Jose Luis Barra, Gloria Del Solar, Beatriz Martínez*

Introduction: Bacterial viruses called bacteriophages (phages) remain one of the main causes of failed or low-quality dairy fermentations. Thus, phage-resistant dairy starters are essential to the continued stability of the industry. Previous adaptative evolution experiments with two *Lactococcus lactis* strains (IPLA517 and IPLA1064) resulted in the generation of derivatives resistant to the bacteriocin Lcn972 (Lcn972R), but which exhibited sensitivity to ceduoviruses.

Objective: The aim of this work was to establish the underlying phage resistance mechanism in the parental strains.

Methods: Long-read sequencing technologies and variant analysis were applied to identify mutations. A CRISPR-Cas9-based approach was used for targeted plasmid curing of the host strains. Phage infection assays in broth and solid media, as well as phage adsorption assays were conducted to analyse phage infection in detail.

Results: Genome sequencing and variant analysis revealed the loss of a 41-kb endogenous plasmid (p41), along with additional mutations, in the Lcn972R derivatives. This plasmid contains a putative exopolysaccharide (EPS) biosynthesis gene cluster with significant homology to one described in *Lactococcus garvieae*. p41 was selectively cured from *L. lactis* IPLA1064 and phage infection assays with three ceduoviruses demonstrated that curing p41 restored phage sensitivity. Phage adsorption values were consistent with the hypothesis that EPS production hinders access to the phage receptor protein Pip.

Conclusion: Our findings emphasize the role of EPSs in preventing phage infection, rarely reported for ceduoviruses in *L. lactis*, and highlight the likely occurrence of horizontal gene transfer between *L. lactis* and *L. garvieae* within the dairy environment.

Abstract ID: 1904

A prebiotic dietary pilot intervention restores faecal metabolites and may be neuroprotective in Parkinson's Disease

Presenting author: Dr. Falk Hildebrand, Quadram Institute Bioscience, Norwich, United Kingdom; Earlham Institute, Norwich, United Kingdom

Co-authors: *Janis Bedarf*

Introduction & **Objective:** Despite extensive research, current treatment of Parkinson's Disease (PD) remains symptomatic and disease modifying approaches are urgently required. A promising approach is to target the gut-brain-axis by modifying the intestinal microbiota and the herein produced metabolites.

Methods: We decided to test this approach by modifying key metabolites of bacterial intestinal fermentation: short chain fatty acids (SCFA), known to be decreased in PD patients.

A prospective, controlled pilot study was conducted in 11 couples, with one PD patient and healthy spouse as control (CO) each. Participants followed a 4-week diet rich in dietary fibre in addition to the intake of a prebiotic sirup (Lactulose). Metagenomes and metabolites of the gut microbiota, urinary metabolites and clinical characteristics were assessed.

Findings: The short-term dietary intervention significantly augmented gastrointestinal SCFA production, likely associated with increased Bifidobacteria spp. PD associated gastrointestinal symptoms improved with increasing SCFA levels. The pre-existing bacterial dysbiosis associated with PD, such as depletion of genera Blautia, Dorea, and Erysipelatoclostridium in PD, persisted within the study period. Some pathobionts, i.e. Klebsiella, were reduced after the intervention. Bacterial metabolite composition (both faecal and urine metabolomes) shifted towards the composition of the healthy control in PD after the intervention. Among these brain-relevant gut metabolic functions improved in PD patients, such as S-Adenosyl methionine (SAM), 3,4-Dihydroxyphenylacetic acid (DOPAC), Glutathione (GSH), Tryptophan and inositol related changes, involved in neuroprotective and antioxidant pathways.

Conclusions: Despite the small cohort size and short-term study period a minor dietary intervention was sufficient to improve gastrointestinal symptoms in PD and altered

metabolic parameters in a presumed neuroprotective manner, warranting further investigation in larger cohorts.

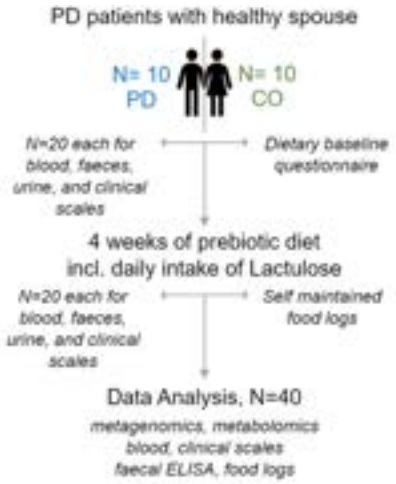


Figure 1: Study design and overview of generated data types.

Abstract ID: 3182 / Poster Number: 474.15S

A proof-of-concept drone system for efficient bioaerosol sampling and genomic analysis in intensive livestock farming

Presenting author: Dr. Marta Nerini, University of Florence, Florence, Italy

Co-authors: *Marco Merlini, Sonia Renzi, Benedetta Cerasuolo, Aldo D'Alessandro, Duccio Cavalieri, Giuseppe Rossi, Massimiliano Marvasi*

Intensive livestock farming generates bioaerosols, including potentially pathogenic bacteria, that pose significant health risks to workers and nearby residents, ranging from respiratory irritation to infections and chronic illnesses. Traditional bioaerosol analysis relies on culture-based methods, which detect only a fraction of the microbial community, leading to incomplete results. Genomic analysis offers a more comprehensive approach, but its effectiveness is limited by the low concentrations of airborne microorganisms and the lack of standardized sampling methods. To address these challenges, we introduce a drone-based system capable of collecting microorganisms within just 10 minutes of flight, enabling sufficient DNA for metataxonomic analysis. We designed an automated sampling unit for air aspiration, that directs airflow onto microbial traps coated with an adhesive layer. A proof-of-concept experiment was conducted on a cattle farm, where samples were collected from three distinct sites. Microbial DNA extraction was performed using a customized protocol combined with a viral DNA extraction kit, enabling efficient recovery of genetic material for advanced genomic analysis. The analysis of the V3-V4 region of 16S rDNA identified genera commonly associated with agricultural environments, such as *Acinetobacter* and *Bacillus*, but the system also detected potentially pathogenic genera, including *Staphylococcus*. The system demonstrated its functionality and potential for efficient bioaerosol monitoring. Further development and optimization of this system can provide insights into the dynamics of bioaerosol distribution in farm environment.

Abstract ID: 2464 / Poster Number: 438.15P

A protective protein motif shielding fragile domains from mechanical unfolding

Presenting author: Mr. Jun Sasahara, Nagoya University, Nagoya, Japan

Co-authors: *Kazushi Fujimoto, Shogo Yoshimoto, Amane Kato, Atsuo Suzuki, Katsutoshi Hori*

Introduction: Structural proteins are vital to biological resilience, serving as blueprints for biomaterial innovation. *Acinetobacter* sp. Tol 5, a nonpathogenic bacterium, adheres strongly to various surfaces via the trimeric autotransporter adhesin (TAA) AtaA. While AtaA's N-terminal domains mediate adhesion, its C-terminal domain (Chead) stabilizes the trimeric assembly under mechanical stress. Understanding how Chead withstands stress is critical for advancing protein-based material design.

Objective: To elucidate the hierarchical unfolding mechanism of Chead under mechanical stress and assess the contributions of conserved motifs to its stability.

Method: Atomic force microscopy (AFM) measured the mechanical strength of Chead, while steered molecular dynamics (SMD) simulations analyzed its structural response to force. Mutagenesis experiments identified key residues critical for stabilization.

Findings: Chead exhibited exceptional mechanical strength (~635 pN), surpassing many structural proteins. SMD simulations showed that the headCap motif acts as a protective "helmet," redistributing forces to shield the fragile Ylhead motif. Hydrogen bonds involving Q2950 were essential, as mutating Q2950 reduced mechanical strength by 33%. These results revealed a hierarchical unfolding mechanism where force redistribution delays the failure of weaker motifs.

Conclusion: Chead exemplifies an evolutionary solution to mechanical stress, integrating hierarchical unfolding, molecular clasping, and force redistribution. The headCap motif shields weaker regions like the Ylhead motif, while the Q2950 "clasp" ensures stability under extreme forces. This study advances our understanding of protein mechanics and provides a foundation for biomaterial innovation in biotechnology and materials science.

Abstract ID: 1034 / Poster Number: 080.17P

A recombinant Staphylococcus aureus transposase as a biomarker for identification of MRSA

Presenting author: Dr. Bianca Castiglioni, IBBA – National Research Council of Italy, Lodi, Italy

Co-authors: *Caterina Catalano, Pietro Roversi, Paola Cremonesi, Alessandra Mallardi*

A recombinant *Staphylococcus aureus* transposase as a biomarker for identification of MRSA

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen causing diverse infections in humans and animals. Its resistance stems from the integration of the Staphylococcal Chromosomal Cassette mecA (SCCmec) into the genome, conferring resistance to most β-lactam antibiotics. In medicine and veterinary science, rapid discrimination between MRSA and methicillin-susceptible *S. aureus* (MSSA) enables the decision on which antibiotic to use for therapy. MRSA samples are detected by MALDI-TOF mass spectrometry using a MRSA-specific peptide biomarker called PSM-mec. Unfortunately, some MRSAs have appeared for which this standard biomarker does not work (*e.g.* MRSA SCCmec type IV).

Objective: This study aims to identify novel peptide biomarkers from the Tpn IS431mec transposase, specific to MRSA SCCmec type IV, for rapid differentiation of these MRSA strains from MSSA using MALDI-TOF mass spectrometry.

Method: The Tpn IS431mec transposase gene was cloned into the pET-15b vector tagged with a C-terminal 6xHis sequence, and expressed in *E. coli* BL21 cells. The recombinant protein was purified and analysed via MALDI-TOF mass spectrometry to identify potential peptide biomarkers from MRSA SCCmec type IV.

Findings: The Tpn IS431mec transposase is a promising biomarker for MRSA type IV, which lacks the PSM-mec peptide used to identify other MRSA types. Preliminary results indicate that peptide fragments from this transposase improve the accuracy of MALDI-TOF-based detection of methicillin resistance.

Conclusion: This study highlights the potential of Tpn IS431mec transposase-derived peptides as novel biomarkers for rapid MRSA SCCmec type IV identification. These findings could enhance diagnostic precision, supporting timely interventions and improved infection control.

Abstract ID: 3319 / Poster Number: 051.17P

A regulatory small RNA modulates the non-canonical mismatch repair system in Mycobacterium tuberculosis

Presenting author: Mr. Ángel Ruiz Enamorado, Centro Nacional de Biotecnología, Madrid, Spain

Co-authors: *Renata Moreno Albiger, Jesús Blázquez Gómez*

The mismatch repair system (MMR) mainly corrects mispaired bases that arise because of replication errors. The canonical MMR system, based on MutS/MutL proteins, assures low mutation rates and its expression is tightly controlled. At a post-transcriptional level, mutS mRNA is negatively regulated by a trans-encoded small RNA (sRNA) in a RpoS dependent manner. Under stress conditions, MutS levels decrease, increasing the mutation rate to enhance the generation of adaptative mutations.

The mutS/mutL genes are highly conserved except in Actinobacteria (including Mycobacterium tuberculosis) and several groups of Archaea, which have a non-canonical MMR system with NucS as a key protein. NucS corrects mispaired bases that lead to transitions. However, little is known about how nucS expression is regulated. A recent transcriptomic study showed the possible presence of an antisense sRNA complementary to nucS mRNA, which appeared to be differentially expressed under nitrosative stress. This sRNA could be regulating nucS under stress conditions, enhancing the gain of adaptative mutations, a phenomenon of particular significance in M. tuberculosis which solely acquires antibiotic resistance through chromosomal mutations.

In this work, the mentioned sRNA was detected by retrotranscription coupled to conventional PCR, and its differential expression after nitrosative stress induction was confirmed. Transcriptional fusions to a fluorescence reporter gene allowed detecting a promoter from which the sRNA is transcribed. Our results suggest that this sRNA is a negative regulator of nucS since mutation rates increase when it is overexpressed. Additionally, expression of the sRNA is induced by isoniazide, a crucial antibiotic for tuberculosis treatment.

Abstract ID: 2918 / Poster Number: 153.17P

A small protein hidden in the SAL1 5´UTR induces the formation of protein aggregates that capture Staphylococcus aureus lipases

Presenting author: Ane Muruzabal-Galarza, PhD, Instituto de Agrobiotecnología (IdAB-CSIC), Mutilva, Spain

Co-authors: *Arancha Catalán-Moreno, Pedro Dorado-Morales, Jaione Valle, Alejandro Toledo-Arana*

Recently, the number of genomes in public databases has exponentially increased. However, automated annotation of genomes often disregards small open reading frames (smORFs) encoding less than 50 amino acids (aa). Current improvements on shotgun proteomics and ribosome profiling have unveiled hundreds of “hidden” mini-proteins in all organisms and their biological relevance is only beginning to emerge.

By combining multi-omic approaches, we have updated the small proteome of *Staphylococcus aureus*, one of the most relevant bacterial pathogens in hospitals for which new antimicrobial development is needed. Here, we present two 21-aa small proteins, LspU and LspD, amphipatic helices with similar features to phenol soluble modulins (PSMs), that were hidden in the 5’- and 3’- untranslated regions of *sal1* messenger RNA, respectively. *Sal1* encodes one of the two extracellular lipases involved in *S. aureus* pathogenesis. We demonstrated that LspU is exported through the same transporters as PSMs, suggesting they can be novel PSM-family members. Interestingly, constitutive expression of LspU produced extracellular aggregates and biofilm structures that trapped active lipases. Further composition analyses revealed that these aggregates comprised LspU, lipases and PSMs. Mutation of PSM-encoding genes avoided aggregate formation, indicating that LspU promoted the PSM-amyloidogenic state while still promoting lipase trapping to the bacterial cells.

Briefly, we propose LspU as a novel PSM that promotes the formation of extracellular structures that trap lipases closely associated to cells. This might be a useful strategy during infections, avoiding lipase dispersion to provide lipase byproducts and protection against antimicrobial fatty acids at the infection site.

Abstract ID: 3138 / Poster Number: 065.15P

A smart approach to water safety: real-time E. coli monitoring in estuarine environments

Presenting author: Ms. Lúcia Gomes, Instituto de Ciências Biomédicas Abel Salazar, University of Porto (ICBAS – UP), Porto, Portugal

Co-authors: *Ana Machado, Pedro Carvalho, Gonçalo Lima, Marta Costa, Adriano A. Bordalo*

Escherichia coli (E. coli) is a key indicator of fecal contamination in recreational waters, with major public health implications. While traditional culture-based methods to evaluate *E. coli* concentrations provide historical data and insights into contamination patterns and the influence of environmental variables, they are labor-intensive and often cause delays that hinder rapid responses to contamination events. However, these methods are labor-intensive, and often cause delays that hinder rapid responses to contamination events. Emerging technologies, such as in situ sensors based on tryptophan fluorescence, offer a challenging solution for *E. coli* detection in real-time. These sensors leverage the relationship between tryptophan and microbial activity, providing continuous data, enabling water quality forecasts. These approaches enhance temporal resolution, facilitates early detection of contamination events, and support proactive water quality management strategies. This study analyzed the water quality of an estuarine beach in the Douro River (NW Portugal) during the 2024 bathing season. Traditional culture-based methods and tryptophan fluorescence sensors at different time scales were compared. Fortnight, daily, and tidal patterns were assessed, showing water quality in compliance with EU legal standards. To ensure accuracy and reliability, tryptophan fluorescence sensors were also validated under laboratory conditions taking into account various scenarios (e.g. temperature, salinity, and level of contamination). The obtained results highlight the relevance of advanced monitoring technologies for safeguarding public health, addressing environmental, regulatory, and management demands.

Abstract ID: 1458 / Poster Number: 327.15P

A trimeric coiled-coil motif binds bacterial lipopolysaccharides with picomolar affinity – and can be used in LPS detection and removal applications

Presenting author: Prof. Dirk Linke, University of Oslo, Oslo, Norway

Co-authors: *Stefanie Barbirz, Daniel Hatlem*

Modified coiled-coils sequences can be used in biotechnology, vaccine development, and biochemical research to induce protein oligomerization. A prominent model for the versatility of coiled-coil sequences is a 30-residue peptide originally derived from a yeast transcription factor, GCN4. In our lab, we have used these peptides extensively in recombinant protein work, especially regarding bacterial virulence factors such as non-fimbrial adhesins.

Unexpectedly, the trimerizing variant of this peptide, GCN4-pII, binds bacterial lipopolysaccharides (LPS) from different bacterial species with picomolar affinity. LPS molecules are highly immunogenic, toxic glycolipids that comprise the outer leaflet of the outer membrane of Gram-negative bacteria. They are the most prominent class of bacterial endotoxins – toxic cellular components of bacteria – and the terms LPS and endotoxins are often used synonymously.

Using scattering techniques and electron microscopy, we show how GCN4-pII breaks down LPS micelles in solution. In an ELISA-like assay, we find that LPS can in principle be detected in minimal concentrations, competitive to the industry gold standard for LPS detection, the LAL assay. Our findings thus suggest that the GCN4-pII peptide and derivatives thereof could be used for novel LPS detection and removal solutions with high relevance to the production and quality control of biopharmaceuticals and other biomedical products, where even minuscule amounts of residual LPS can be lethal. I will present possible biotechnological applications of our patented peptide, including possible emergency room treatments of sepsis patients whose symptoms are based on an LPS-induced cytokine storm.

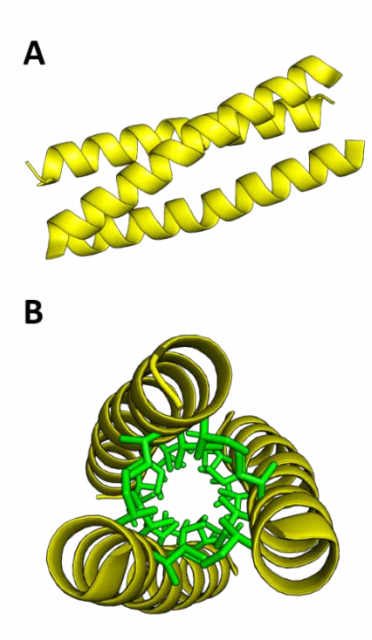


Figure 1: side view (A) and top view (B) of the GCN4-pII trimer bundle.

Abstract ID: 3352 / Poster Number: 179.17P

A tyrosine residue regulates the deacylation efficiency in the putative beta-lactamase BlaE (MSMEG_3978) of Mycobacteria smegmatis

Presenting author: Mr. Aditya Prasad Panda, Indian Institute Of Technology Kharagpur, Kharagpur, India

Co-authors: *Anindya Sundar Ghosh*

Introduction: Our understanding of beta-lactamases is limited to a few clinically isolated enzymes, leaving much of their environmental diversity unexplored. Studying beta-lactamases from environmental bacteria can enhance our understanding of their evolutionary relationships.

Objective: Identifying the molecular basis for the activity of a putative beta-lactamase BlaE (MSMEG_3978) of *Mycobacterium smegmatis*.

Methods: The membrane-bound and truncated *blaE* (*msmeg_3978*) forms were cloned into pBAD18cm and pET28a. In-silico analysis identified a critical tyrosine residue (Y170), which was substituted with alanine via site-directed mutagenesis. The antibiotic susceptibilities of the *E. coli* Δ*ampC* mutant (AM1OC) expressing mutated and native *blaE* were assessed. Purified proteins were evaluated spectroscopically, with kinetic behaviours determined using fluorescent penicillin, Bocillin-FL, and thermal stability assessed through T_{50} values.

Findings: Overexpression of the blaE gene in *E. coli* AM1OC increases the minimum inhibitory concentration (MIC) of various beta-lactam antibiotics: 2- to 4-fold for penicillin, 4-fold for third-generation cephalosporins, 8-fold for first- and second-generation cephalosporins, and 2-fold for carbapenems. No significant MIC changes were noted for $blaE_{Y170A}$ in AM10C. Both native and mutated BlaE proteins bind to Bocillin FL, indicating penicillin-interacting enzymes. The native protein can hydrolyse Nitrocefin, while the Y170A mutation impairs this ability. Kinetic assessments show higher efficiency for cephalosporins over penicillins for BlaE. The deacylation efficiency, a marker of beta-lactamase activity, is impaired in the mutated protein, and no compensatory gain in DD-Cpase activity was observed in vitro.

Conclusion: BlaE appears to be a beta-lactamase in the Bush-Jacoby group 1 cephalosporinases, potentially regulated by residue Y170.

Abstract ID: 2929 / Poster Number: 155.17P

A whole-genome sequencing approach for the molecular characterization of multiple carbapenemase-encoding Klebsiella pneumoniae isolates

Presenting author: Mr. Riccardo Bollini, University of Pavia, Pavia, Italy; Humanitas University, Milan, Italy

Co-authors: *Laura Peiretti, Ilaria Rossomanno, Davide Bavaro, Zian Asif, Maddalena Casana, Linda Bussini, Cristina Scuderi, Raffaella Renzulli, Giorgio Da Rin, Sergio Marchini, Erminia Casari, Michele Bartoletti, Sara Caloni, Valeria Cento*

Genomic surveillance of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is essential in hospitals to track the spread of highly resistant strains and inform infection prevention and control (IPC) strategies. Whole-genome sequencing (WGS) provides insights into the distribution of high-risk clones and resistance mechanisms, aiding local risk mapping and antimicrobial stewardship. This study characterized CRKP isolates with multiple resistance markers in a hospital setting using WGS.

Between January and August 2024, nine CRKP isolates harboring multiple resistance markers were collected from the microbiology biobank of our hospital. WGS was performed using Illumina NextSeq-2000, with bioinformatic analyses identifying resistance genes (Kleborate), sequence types (MLST), and phylogenetic relationships (Snippy, IQ-tree, and ChewBBACA).

All isolates were resistant to carbapenems, 88.9% to meropenem/vaborbactam and ceftazidime/avibactam, and 22% to cefiderocol. WGS revealed bla_{NDM-1} in all isolates, combined with bla_{KPC-3}, bla_{KPC-2}, bla_{OXA-48}, bla_{KPC-97}, or $bla_{OXA-232}$, carried by 2 plasmids/isolate, alongside genomic *ompK35* and *ompK36* mutations (7/9). Cefiderocol-resistant isolates were characterized by 2 novel *cirA* frameshift mutations. Six isolates belonged to ST6668, two to ST147, and one to ST14, with four ST6668 forming a tight cluster (median allelic distance: 5), potentially suggesting a common origin. All isolates were classified as low-virulent.

The study reveals the spread of *Klebsiella pneumoniae* co-producing different carbapenemases, characterized by high clonality, and an extensive resistance. Two putative novel cirA frameshift mutations conferring cefiderocol-resistance in CRKP were highlighted, further emphasizing the importance of an integrated genotypic and phenotypic analyses for AMR surveillance to guide IPC practices.

Abstract ID: 3976

A workflow for ONT long-reads analysis reveals genome-wide methylation motifs and functional patterns

Presenting author: Mrs. Alessia Marotta, Università di Genova, Genova, Italy

Co-authors: *Emanuele Bosi*

DNA methylation is one of the many mechanisms of epigenetic regulation in both prokaryotes and eukaryotes. It has been long recognized that DNA methylation in prokaryotes plays numerous crucial roles ranging from well-known restriction-modification systems defending against exogenous DNA to cell cycle regulation, DNA repair, phase variation, and gene expression regulation, including virulence and stress response. However, the literature on this topic remains fragmented and disjointed and research in this field is still far from reaching its full potential. There are currently few tools available for processing methylation data generated by Nanopore sequencing. Moreover, many existing tools do not integrate methylation data with genomic features such as coding regions, promoters, or other functional elements. Sequencing data do not inherently store information about the methylome. The occurrence and abundance of different motifs must be determined by mapping reads to an annotated genome. Finally, file formats are not directly compatible across different tools. The primary objective of this work was to deliver a pipeline for exploring and analyzing bacterial methylation in Nanopore sequencing and combine genome-wide methylation profiles with genomic features. As a case study, *Vibrio aestuarianus* was used, and to the best of our knowledge, this work presents the first genome-wide methylation patterns analysis of Vibrio utilizing Nanopore sequencing. Methylated bases distribution was analyzed in relation to genomic features such as genes, promoters, and regulatory regions. A Gene Set Enrichment Analysis was conducted to assess the functional implication of DNA methylation on gene regulation. The pipeline we present automatically performs data processing, annotation, and visualization, including circular plots (Figure 1) to represent the spatial distribution of methylation across the genome. By achieving these objectives, our work provides a standardized workflow for the genome-wide analysis of bacterial methylation patterns, to accelerate our understanding of the epigenomic mechanisms underlying gene expression and microbial adaptation.

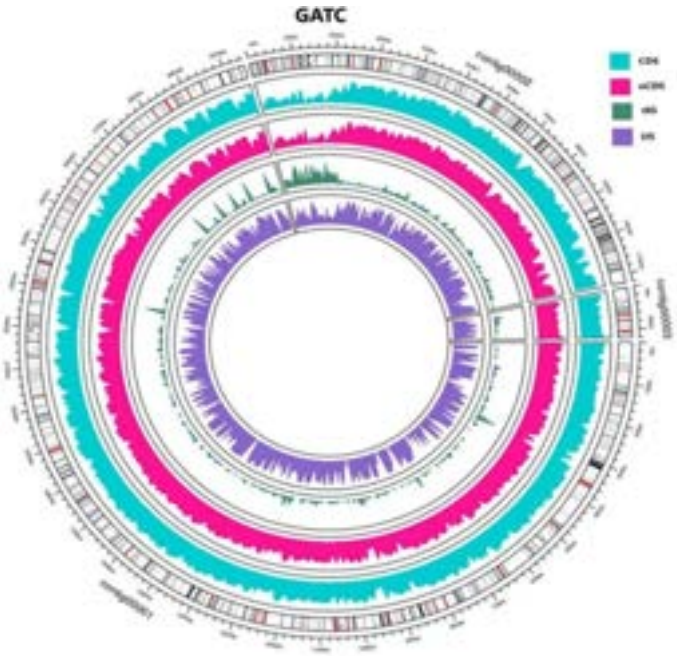


Figure 1: Example of circular density plot of GATC motif in *V. aestuarianus*. The outer circle represents the genome annotation of the contigs of the strain (black lines indicate the position of CDS). Histograms represent, respectively, CDS, nCDS, tIG and US

Abstract ID: 2669 / Poster Number: 058.15P

Abundance and diversity of airborne antibiotic resistome and mobilome in Belgrade metropolitan area during summer season: a metagenomic approach

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Co-authors: *Danka Matijasevic, Milka Malesevic, Lazar Gardijan, Stefan Stanovcic, Nemanja Kljajevic*

Introduction: The importance of the environment for the transmission of antimicrobial resistance is increasingly acknowledged. The airborne transmission route of antibiotic resistance genes (ARGs) is particularly significant since ARGs-carrying bioaerosols could travel long distances and remain in the atmosphere for a long period of time. In addition, mobile genetic elements (MGEs) can promote the spread of ARGs in the environment.

Objective: To reveal the potential of the atmosphere in the dissemination of ARGs and MGEs, the antibiotic resistome and mobilome of airborne metagenomes collected during summer season 2024 at seven locations in the Belgrade metropolitan area were analysed.

Method: Outdoor air was sampled at seven locations using hydrophobic polypropylene membrane filters (air volume of 30 m^3). Airborne DNA samples were subjected to shotgun metagenome sequencing (Illumina Novaseq X plus) and bioinformatic analysis (Novogene, UK). ARGs and MGEs were annotated using CARD, Integrall, Isfinder and Plasmid databases, respectively.

Findings: The tested locations were selected according to the air pollution during heating season (highly polluted: Borca, Despota Stefana, Lestane, Zemun Ugrinovacka, Veliki Crljeni; low polluted: Barajevo, Zeleno brdo). Metagenomic analysis revealed that the air sampled at Zemun Ugrinovacka showed the highest abundance of ARGs, while the lowest level was observed in sample Barajevo. The most abundant ARG was bla_{TEM}, while transposon Tn*Swi1* was dominant in four samples. MGEs content profiles varied among the samples, although Veliki Crljeni and Lestane showed similar patterns of MGEs relative abundance.

Conclusion: This study revealed that Belgrade air could be a potential dissemination route for antimicrobial resistance.

Abstract ID: 1753 / Poster Number: 245.17P

Abundance and diversity of comammox bacteria during biological removal of nitrogen from municipal organic waste leachates

Presenting author: Dr. Sergio Martínez Hernández, Universidad Veracruzana, Xalapa, Mexico

Co-authors: *Roberto Carlos Moreno Quirós*

Introduction: Leachate from municipal solid waste contains high-risk contaminants for humans and the environment, such as ammonium, so its treatment is a priority. Ammonium can be removed by various microbial groups, including the recently discovered comammox microorganisms.

Objective: This work analyzed the diversity and abundance of comammox microorganisms in a bioreactor treating leachates from municipal organic waste. Studies on this subject are still very scarce.

Method: Assays were carried out in batch reactors (1L) inoculated with sludge (2 g of volatile suspended solids (VSS)/L) from sediments of a leachate storage tank, dissolved oxygen concentrations between 0.5-4 mg DO/L, temperature of 30 °C, pH of 7, and 100 rpm. Reactors were fed first with 100 mg of synthetic ammonium/L and then with diluted leachates. Ammonium, nitrite, nitrate, DO, and chemical oxygen demand (COD) were measured periodically. The microbial community was analyzed by high-throughput sequencing.

Findings: Results showed ammonium consumption efficiencies of 98% and nitrate yields of 0.84. COD consumption efficiencies were of 78% (initial concentration around 6840 mg/L). The microbial community showed the coexistence of several genera of bacteria and archaea related to the nitrogen cycle showing the following predominance: *Nitrospira*, *Nitrosomonas*, *Nitrosopumilus*, *Nitrososphaera* and *Candidatus kuenenia*. Microorganisms of the genus *Nitrospira*, where comammox bacteria are found, represented about 1% of the total community. The predominant comammox species were *Candidatus Nitrospira nitrificans*, *Candidatus Nitrospira inopinata* and *Candidatus Nitrospira nitrosa*.

Conclusion: These findings contribute to the knowledge of the diversity and abundance of comammox microorganisms in wastewater treatment systems.

Abstract ID: 4140

Abundant non-canonical DNA viruses in seawater revealed by high-throughput single particle sequencing

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Viruses and other extracellular genetic elements play essential roles in marine communities. However, methods to capture their full diversity remain limited by the constraints of bulk sequencing assemblers or pre-sorting throughput. Here, we introduce environmental micro-compartment genomics (EMCG), which vastly improves the throughput and efficiency of single-particle genomic sequencing obtained from small volumes by compartmentalizing particles of a sample into picoliter-sized, semipermeable capsules for in-capsule DNA amplification and barcoding. From 300 nanoliters of seawater, EMCG obtained genomic sequences of 2,037 particles. The microbiome composition agreed with other methods, suggesting quantitative recovery. The virus-like assembly lengths indicated most were near-complete. Many belonged to the *Naomiviridae*, lacked metagenomic representation, and aligned to outlier contigs of abundant, putative host lineages, suggesting their use of noncanonical DNA and overlooked ecological significance. The reported EMCG offers new opportunities for high-throughput, quantitative, and cost-effective genome analyses of individual cells and extracellular particles in complex microbiomes.

Abstract ID: 2170 / Poster Number: 371.17P

Accelerating sewage sludge composting: A fungal consortium targeting bulking agent decomposition

Presenting author: Dr. Namrata Joshi, University of Warsaw, Warsaw, Poland

Co-authors: *Lukasz Drewniak*

Wastewater treatment generates sewage sludge that poses environmental challenges due to its high organic content, pathogens, and toxic pollutants. However, its direct composting is impractical due to excessive moisture, requiring the addition of lignocellulosic bulking agents to enhance aeration, moisture absorption, and compost quality. This study investigated the impact of a fungal biopreparation on the composting process of sewage sludge with wheat straw as a bulking agent. The biopreparation utilized in this study consists of a fungal consortium, including *Aspergillus fumigatus* ZS_AF, *Penicillium fuscoglaucum* JAM-1, and *Trichoderma deliquescens* TE, specifically designed to accelerate lignocellulosic biomass degradation. The primary objective was to evaluate its effectiveness in enhancing wheat straw decomposition during sludge composting.

Field trials were conducted under real environmental conditions to assess the biopreparation's performance, considering microbial interactions and enzymatic activities. Key physicochemical parameters, such as dry mass, organic matter content, and cation exchange capacity, were analyzed. Microbial indicators, including dehydrogenase activity and the abundance of *Actinomycetes*, were also monitored to assess microbial response and compost quality.

The results demonstrated that biopreparation application significantly enhanced organic matter mineralization, leading to improved compost maturity. A notable increase in dehydrogenase activity and *Actinomycetes* proliferation indicated enhanced microbial functionality and stability within the composting system.

In conclusion, integrating fungal biopreparation into sewage sludge composting with wheat straw effectively accelerates decomposition, promotes organic matter stabilization, and enhances microbial activity. These findings underscore the potential of biopreparations as an effective approach for optimizing sludge composting, contributing to sustainable waste management practices.

Abstract ID: 2075 / Poster Number: 137.15P

Acetic acid-induced alterations in H+ and K+ fluxes in Saccharomyces cerevisiae ATCC 9804: Implications for stress adaptation and industrial fermentation

Presenting author: Gohar Azbekyan, Scientific-Research institute of Biology, YSU, 0025 Yerevan, Armenia; Department of Biochemistry, Microbiology, and Biotechnology, 0025 Yerevan, Armenia

Co-authors: *Anahit Shirvanyan, Karen Trchounian*

Ion homeostasis is crucial for proper cellular functioning and adaptation to stress conditions. Acetic acid (AA), commonly found during industrial processes, serves as a fermentation inhibitor, however, its action mechanisms on yeast are not well understood. This study aims to investigate the influence of AA stress on H^+ and K^+ fluxes of *S. cerevisiae* ATCC 9804 industrial wine strain under aerobic conditions at pH 3.0. Specific growth rate (SGR) was determined by monitoring yeast culture growth, and ion fluxes were analyzed using the potentiometric method. The results show that 10 mM AA had no significant effect on yeast SGR (0.4 h^{-1}), while 40- and 50 mM AA reduced the SGR of ATCC 9804 by 3-fold and ~5-fold, respectively. The *N, N'*-dicyclohexylcarbodiimide (DCCD)-sensitive proton flux increased ~3-fold under 10-50 mM AA stress (0.2 mmole H^+ min^{-1} CFU^{-1}), highlighting the role of proton pumps, particularly yeast plasma membrane ATPases (Pma), in adaptation to AA stress. Interestingly, DCCD completely impairs K^+ flux across the plasma membrane, resulting in K^+ efflux. DCCD-sensitive K^+ flux (8.0 mmole K^+ min^{-1} CFU^{-1}) was inhibited by 50% and 70% under 10- and 20 mM AA stress, which can be due to AA-induced proton influx, which activates the Pma to pump out the excess protons and maintain intracellular pH, resulting the depolarization of the membrane potential and a reduction of the potassium transport to balance the electrochemical gradient. The results can enhance the robustness of yeast and improve industrial production efficiency.

Abstract ID: 668 / Poster Number: 017.17F

Acetyl Modifications Alter the Colonic Fermentation of Plant Xylan

Presenting author: Ms. Su-Mei Lu, National Taiwan University, Taipei, Taiwan

Co-authors: *Yu-Wen Chiao, Ming-Hsu Chen*

O-acetyl groups commonly modify plant xylan, altering its hydrophobicity and physiological roles. Few reports have explored the connection between the degree of acetylation (DA) of xylan and its colonic fermentability, alongside related changes in human gut microbial community. Therefore, in this study, we aimed to investigate how varying DAs influence xylan degradation. We chemically modified beechwood xylan (BWX) to achieve DAs of 0.0, 0.5, 1.1, 1.6, and 1.9, designated as $BWX_{DA0.0}$, $BWX_{DA0.5}$, $BWX_{DA1.1}$, $BWX_{DA1.6}$, and $BWX_{DA1.9}$, respectively. Structural analysis included glycosylic composition and functional group profiling, followed by fermentation trials inoculated with human fecal microbiota. Results demonstrated that acetylation significantly influenced xylan degradation in the human colon. Higher acetylation decreased gas production and medium acidity, while lower acetylation reduced microbial diversity. Specific microbial genera, including *Bacteroides*, *Bifidobacterium*, *Blautia*, *Faecalibacterium*, *Escherichia-Shigella*, *Parabacteroides*, *Fusicatenibacter*, *Agathobacter*, and *Acidaminococcus*, showed shifts in abundance. Treatments of $BWX_{DA0.0}$ enriched *Bacteroides*, *Blautia*, and *Fusicatenibacter,* whereas *Bacteroides xylanisolvens* thrived with $BWX_{DA0.5}$ and $BWX_{DA1.1}$ treatments. Acetylation also reduced short-chain fatty acid production during fermentation. These findings highlight the role of DA in modulating microbial accessibility to xylan, with implications for dietary interventions targeting gut health.

Abstract ID: 2253 / Poster Number: 252.17P

Acid tolerance response in foodborne pathogen Yersinia enterocolitica 4/O:3 strains originating from pigs

Presenting author: Prof. Nevijo Zdolec, Faculty of veterinary medicine University of Zagreb, Zagreb, Croatia

Co-authors: *Marta Kiš, Snježana Kazazić*

Yersinia enterocolitica is one of the priority biological hazards in pork safety that can be controlled primarily at the slaughterhouse level. However, its acid resistance must be evaluated in the context of meat safety and the effectiveness of chemical decontamination as a possible intervention measure to reduce risk.

In this study, Yersinia enterocolitica 4/O:3 strains (n=10) isolated from pig's tonsils at slaughter were adapted to mild pH conditions by propagating them in Tryptic Soya Broth containing 1 and 2% glucose. The adapted strains were exposed to 2% lactic acid and acetic acid solutions (final pH=3.5-4.0) and the survival rate was calculated. In addition, MALDI-TOF MS was used to detect the differences between strains in proteins for acid stress response.

When strains were cultured with 2% glucose, the population reduction by lactic acid was on average 0.7 log higher than with TSB and 1% glucose TSB. Individually, acid adaptation resulted in better survival of three and five strains after treatment with 2% lactic acid and acetic acid solution, respectively. The results obtained indicate that the adaptability of Y. enterocolitica 4/O:3 to a slightly acidic environment is strain-dependent. The intensity of the MALDI-TOF MS peaks was also associated with the different acid tolerance of the strains. Acid resistance had no effect on the identification accuracy of Y. enterocolitica.

In the practise of meat decontamination, prior exposure of Y. enterocolitica 4/O:3 strains to an acidic environment may result in a better decontamination effect of lactic acid and a weaker effect of acetic acid.

Abstract ID: 3161

Acinetobacter ecology and antibiotic resistance along a patient-river continuum

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Co-authors: *Marie-Sarah CAYETTE, Thibault STALDER, Sandra DA RE, Elodie COUVE-DEACON*

Acinetobacter baumannii, a major nosocomial pathogen, represents a significant threat to public health due to its antibiotic multi-resistance and its ability to persist on surfaces (1). While *A. baumannii* is extensively studied, *non-baumannii Acinetobacter* species are increasingly recognized in human infections (2). *Acinetobacter spp.* can acquire antibiotic resistance genes through horizontal gene transfer and establish in hospital sewer system raising concerns about environmental dissemination. This study aims to assess the presence of *Acinetobacter* along a patient to environment continuum.

Acinetobacter strains were isolated on selective media from samples taken from hospitalized infected patients, their clinical environment (sink/shower drains during patient's stay and at least two weeks after discharge), hospital effluents, the wastewater treatment plant and the river. Isolates were identified by mass-spectroscopy, characterized for clonal profile, presence of integrons and antibiotic susceptibility profile.

A total of 397 isolates (244 clones) were isolated from all compartments (Figure 1), with three main species in patients (*A. baumannii*, *A. pittii* and *A. ursingii*) and two in the clinical environment (*A. ursingii* and *A. johnsonii*). For three patients, we identified clones shared between patients and their environment. Among the 397 isolates, four carried a class 1 integron: three were isolated from the same drain, and one, a multi-drug resistant strain, from the hospital effluent. Moreover, our study showed significant colonization of sink-drains by *Acinetobacter* genus, with both clinical and environmental species. Our results suggest that sinks may act as hotspots for genetic exchange between *Acinetobacter spp.*, and for transmission of strains to both effluents and patients (via aerosolization). To better characterize these mechanisms, whole genome sequencing of the strains of interest is currently underway.

This study provides valuable insights into the distribution and characteristics of *Acinetobacter* species along a patient-to-river continuum, with a focus on their persistence in clinical environments and potential for antibiotic resistance gene transfer.

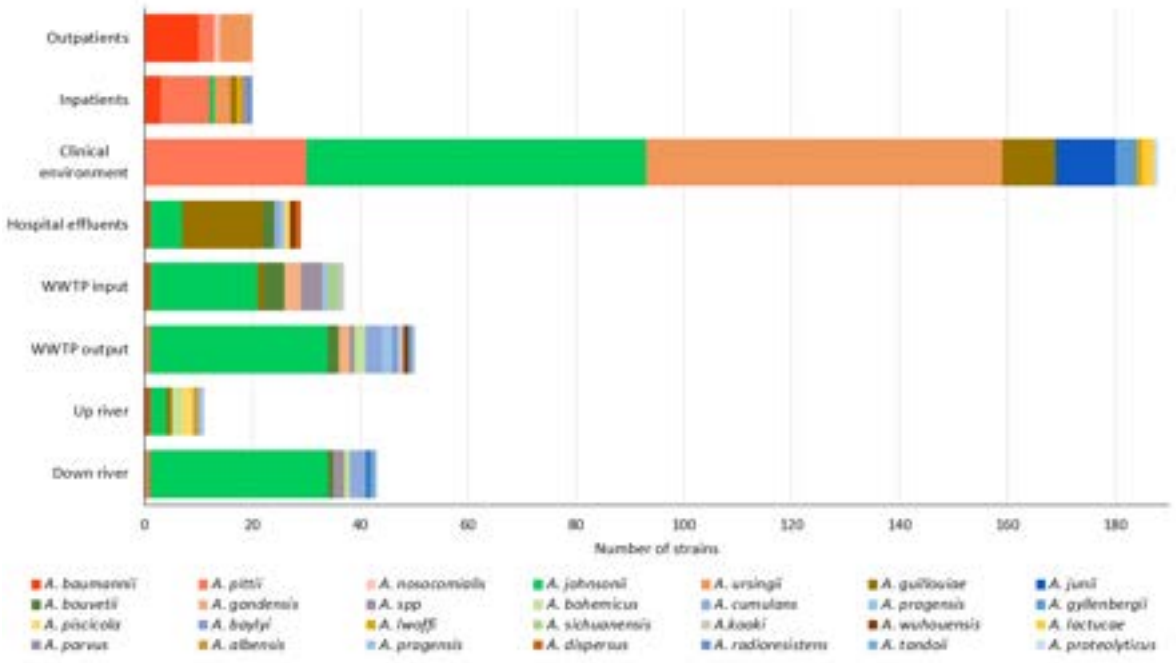


Figure 1: Distribution of the different Acinetobacter species isolated in the various compartments of the patient-river continuum.

Abstract ID: 2593 / Poster Number: 314.16F

Actinobacterial strains modulate growth and metabolome of mediterranean crops

Presenting author: Mrs. Silvia La Scala, University of Palermo, Palermo, Italy

Co-authors: *Sara Amata, Teresa Faddetta, Loredana Abbate, Carla Rizzo, Antonio Palumbo Piccionello, Alessandra Carrubba, Rumyana Karlova, Giuseppe Gallo, Paola Quatrini*

Microbial-based biostimulants (MBBs) represent promising solutions to improve crop quality adopting sustainable practices. Actinobacteria acting as plant-growth promoting bacteria (PGPB) are good candidates for MBB formulations. The two actinobacteria *Streptomyces violaceoruber* and *Kocuria rhizophila* were assayed as PGPB in laboratory and in open field experiments on the Mediterranean plant oregano to investigate their effects on plant growth and plant metabolite production. Inoculated and uninoculated plants were grown from seed to plantlets in controlled conditions and PGPB root colonization was assessed by SEM. On the other hand, plant explants from a Sicilian oregano field were inoculated in pots, transplanted in open field, and monitored until flowering by Soil Plant Analysis Development (SPAD) and growth parameter measurements. Flowers were analysed by GC-MS to characterize the volatilome and the essential oil profiles. *S. violaceoruber* inoculated seeds showed a higher germination rate and SEM confirmed the colonization on leaf and root surface. In field, *S. violaceoruber* inoculated plants had highest SPAD, while *K. rhizophila* positively influenced plant heights. Bioactive metabolites modulation was assessed by GC-MS volatilome analyses on flowers, revealing that the actinobacterial consortium increased 10-fold the amount of p-cymene, a monoterpenoid with high therapeutic potential, if related to the control. Oregano essential oils have different terpenoids content based on the PGPB inoculum. Actinobacteria can exert, hence, a positive effect on oregano plant-growth parameters and modulates plant metabolome. Based on these findings, a study on tomato plants was implemented to assess general effects of actinobacterial strain inoculation on other plants of agronomic interest.

Abstract ID: 2453 / Poster Number: 393.16P

Actinomycetes from the Rice Microbiome as Potential Biocontrol Agents for Sustainable Agriculture

Presenting author: Mrs. Melissa Gonzalez, CENIBiot, CeNAT, CONARE, San José, Costa Rica

Co-authors: *Jonathan Parra*

Rice is one of the most consumed crops worldwide. However, its cultivation faces significant challenges, from low nutrient availability to fungal diseases, which often means elevated use of fertilizers and pesticides. This work aims to study bacterial communities associated with rice plants that can contribute to plant health and suppress diseases, which could, in turn, reduce reliance on agrochemicals. The bacterial microbiome of two rice varieties was characterized across three growth stages (vegetative, reproductive, and ripening) in the endosphere, rhizosphere, and surrounding soils, revealing the presence of actinomycetes in the endosphere. Since actinomycetes are a well-known source of potentially beneficial compounds, a collection of actinomycete strains associated with rice plants was tested for antifungal activity against key rice pathogens. Some isolated strains displayed antifungal activity against *Curvularia* sp., the causative agent of leaf spot, and *Gaeumannomyces* sp., responsible for take-all disease. Whole-genome sequencing was conducted on the strains with the highest antifungal activity to explore their biosynthetic potential. Moreover, the most promising strain was used in an *in planta* assay to evaluate its potential as a biocontrol agent. Overall, the results of this project show that actinomycetes from the rice microbiome may be a promising alternative to conventional agrochemicals. Future work will include field trails to assess the strains' ability to promote plant health, enhance disease resistance, and support sustainable agricultural practices.

Abstract ID: 4106 / Poster Number: 393.15P

Activation with Er,Cr:YSGG Laser Improves the Antimicrobial and Antibiofilm Effects of Low-Concentration Sodium Hypochlorite in Root Canals

Presenting author: Dr. Pablo Betancourt, UNIVERSIDAD DE LA FRONTERA, TEMUCO, Chile

Co-authors: *JOSEP ARNABAT-DOMINGUEZ*

Introduction The initiation and persistence of endodontic infections caused by residual biofilm after chemical disinfection contribute to secondary bacterial infections. Alternative methods for disinfecting treated root canals are of great interest. **Objective** The objective was to assess the antibacterial effectiveness of low-concentration sodium hypochlorite (NaOCl) activated by Er,Cr:YSGG laser-activated irrigation (LAI) against a 10-day-old intracanal *Enterococcus faecalis* biofilm. **Method** Biofilms were formed inside the root canals and divided into 7 groups (n13): 0.5% NaOCl + Er,Cr:YSGG; Saline + Er,Cr:YSGG; 0.5% NaOCl + syringe irrigation(SI); 2.5% NaOCl + SI; 5% NaOCl + SI; positive and negative controls. **Findings** Bacterial survivors were counted and specimens visualized under scanning electron and confocal laser scanning microscopy. Treatments with 0.5% NaOCl + Er,Cr:YSGG and 2.5% NaOCl + SI gave a significant reduction in the number of CFU/mm2. Moreover, scanning electron microscopy and confocal laser scanning microscopy imaging confirmed and reinforced bacteriological data. **Conclusion** Thus, Er,Cr:YSGG LAI demonstrated its ability to enhance the intracanal distribution of 0.5% NaOCl after 60 seconds of activation, achieving the same level of effectiveness as 2.5% NaOCl. This finding is clinically relevant, as using lower concentrations may help minimize undesirable effects.

Abstract ID: 3726

Active viruses shape microbial host community dynamics after a moisture and nutrient perturbations in grassland soils

Presenting author: Mr. Grant Gogul, University of Califronia, Davis, Davis, United States

Co-authors: *G.Michael Allen, Ikaia Leleiwi, Steven J. Blazewicz, Jennifer Pett-Ridge, Joanne B. Emerson, Gareth Trubl*

Viruses are thought to play a key role in soil ecosystems by shaping their environment through the viral shunt—the turnover of nutrients via microbial lysis. In dry soils experiencing a wet-up that triggers viral infection, virus-host dynamics are hypothesized to follow 'cull-the-winner' theory where viruses reduce, but do not eliminate their host populations. However, there is still a disconnect between which virions of the viral community in dry soils respond to a wet-up and which one become an active viral infection. While phosphorus is known to affect viral infections in other systems, how it impacts soil viral communities is still unknow. To further explore how phosphorus and wet-up impacts virus-host dynamics, we established a soil microcosm experiment where water with and without phosphorus was added to dry grassland soils and sampled weekly at 4 time points (dry soils, 7, 14, and 21 days) for 16S rRNA gene, viromic (size selected fraction), and metatranscriptomic analyses. In the virome, we identified 13,840 vOTUs, approximately 31% of which were actively infecting a microbial host (active viruses), although each had a distinct viral community composition. Viral communities displayed lineage-specific viral response to the addition of water and phosphorus. Notably, predicted virions decreased after the initial wet-up while predicted virocells (cells that are infected by a virus) increased, suggesting that wet-up induced viral infections. Phosphorus amended soils additionally had a significant increase in virocells and changed the viral community composition of active viruses, highlighting that phosphorus plays an important role in viral infection. To explore the role of phosphorus in virus-host dynamics, bacterial hosts were predicted for 37% of active viruses and 5,331 ASVs where identified. At each time point the most abundant phyla, *Actinomycetota* and *Pseudomonadota*, remained stable accounting for over 70% relative abundance of viruses with a host and over 50% of relative abundance of bacteria communities. The stability of host abundance coinciding with an increase in virocells, supports "cull-the-winner" virus-host dynamics. These dynamics may be driven by the emergence of new

viruses that prevent dramatic increases in host abundance, with phosphorus likely promoting infection, but not virion production.

Abstract ID: 3246 / Poster Number: 289.15P

Adaptable Ochrobactrum sp. GPr1 13 converts pyruvate to lactate under oxic conditions

Presenting author: Dr. Katharine J. Thompson, University of Stuttgart, Stuttgart, Germany

Co-authors: *Fady Isaac, Esteban Góngora, Sara Kleindienst*

The soil microbiome is a complex environment comprised of microhabitats where microorganisms adapt to rely on specific substrates to survive. The genus *Ochrobactrum* is ubiquitous across many environments, including those contaminated with pollutants like glyphosate, and is considered to be highly adaptable. Despite their ubiquity, which has earned them the nickname anthropogenic genus, little is known about how these microorganisms adapt, especially in the soil environment. Here we demonstrate the adaptability of one strain of *Ochrobactrum* (strain GPr1 13) via two separate mechanisms. First, although *Ochrobactrum* strain GPr1 13 is dependent on glutamate as a carbon (C) source for optimal growth, we found that few population members were able to use other carbon sources (e.g., sarcosine or proline), albeit through linear rather than exponential growth. Slower and linear growth could ensure the survival of the *Ochrobactrum* population until the preferred C-source is available. Furthermore, the strain converted approximately 25 % of a non-preferred C-source, pyruvate, to lactate over 15 days under oxic conditions. This is considered to be a fermentative reaction, yet, GPr1 13 could utilize this in the presence of oxygen as a maintenance strategy to survive until a more preferred C-source is available. To assess whether these findings are specific to GPr1 13 or more broadly applicable to the genus *Ochrobactrum*, a pangenome analysis of verified *Ochrobactrum* genomes is ongoing. Ultimately these results demonstrate a set of response systems for an adaptable species to survive within the soil environment.

Abstract ID: 2236 / Poster Number: 251.17P

Adaptation of Thermus thermophilus as a new tool for the degradation of plastics

Presenting author: Dr. Bruna Fernanda Silva De Sousa, UAM/CSIC, Madrid, Spain

Co-authors: *Álvaro Villamayor, Marta Failde, María Josefa Hernaiz, José Berenguer, Mario Mencía*

Background: The accumulation of plastic waste in ecosystems is a first magnitude world problem. The key to the problem is that the rate of production of plastics of mankind far exceeds the rate at which natural processes mineralize or convert to biomass the plastic residues. To put forward a possible alleviation strategy, degradation of plastic by microorganisms has been tested on polyethylene terephthalate (PET) and Polystyren (PS). *Thermus thermophilus* (Tth) is a bacteria that grows between 50 and 80ºC, has a natural ability to capture extracellular DNA very efficiently, have a mostly aerobic respiratory metabolism and are easily manipulated in the laboratory.

Objectives: Use all the above-mentioned qualities of Tth to adapt it and convert in a new tool for the degradation of plastics at high temperatures, to obtain biomass and monomeric organic molecules that can be valorized by coupling them to the synthesis of useful chemical products.

Methods: We are expressing a plastic-degrading enzymes in Tth while selecting by evolution variants capable of biofilm formation on plastics and growth in carbon-limited media and tolerance to several green solvents that could separate the polymers from plastic.

Results: We already have biofilm-forming Tth strains that grow in the presence of PET and PS films, or grow on PET and PS monomers as a carbon source and on the othe hand we have tested the tolerance of the bacteria to green solvents, like 2-methyltetrahydrofuran (2MetHF), which can deform PS. These strains are currently being characterized and further optimised for plastic degradation.

Abstract ID: 2764

Adaptation to salt in Archaea vs. Bacteria: a new "osmoregulatory" point of view

Presenting author: Alicia García-Roldán, PhD, University of Sevilla, Sevilla, Spain

Co-authors: *Taylor Priest, Fernando Puente-Sánchez, Rafael R. de la Haba, Cristina Sánchez-Porro, Shinichi Sunagawa, Antonio Ventosa*

Hypersaline environments are extreme habitats, where life manages to persist despite significant challenges. These environments, characterized by high salt levels and nutrient scarcity, have been previously considered to harbour a low diversity of *Archaea* and *Bacteria*. Rising salinity levels not only decrease diversity but reshape the community, tipping the scales in favor of the *Archaea*. Microbial capacity to survive in such extreme conditions is driven by different strategies, including specialized osmoregulation processes and dedicated metabolic pathways. However, our knowledge on these adaptation strategies is derived largely from studies on isolated microbes or on select hypersaline environments, with a focus on extreme salt concentrations. As such, we know little about salt adaptation strategies employed in environmental microbial populations and how they shape the dynamics of populations across a range of salt concentrations.

Marine salterns are a unique form of hypersaline environment that can serve as a natural laboratory to study such questions, as they comprise a series of ponds that range in salinity from seawater to salt saturation. In this study, we sampled across a salinity gradient in two marine salterns in order to i) explore microbial taxonomic and functional diversity across a salinity gradient in marine salterns; ii) determine whether microbial communities exhibit a tipping point along the salinity gradient, at which diversity is significantly reduced and *Archaea* become dominant; iii) elucidate the mechanisms of osmoregulation and metabolic adaptations of microbial populations that shape the community at different salinities.

34 metagenomes from the Isla Cristina and Isla Bacuta salterns (Huelva, southwester Spain), with salinities between 13% and 44% (w/v), were sequenced by Illumina NovaSeq. We taxonomically profiled the metagenomes into species-level units using the mOTUs database and assessed how diversity and community turnover shifted across the salinity gradient. MAGs from these samples were recovered and their metabolic capabilities were compared with MAGs from nine seawater metagenomes in the surrounding area of the studied salterns, together with over 800 reference genomes of

cultured halophilic taxa. This analysis enabled an exploration of the osmoregulatory mechanisms and their distribution across phyla, as well as the metabolic interactions within the community.

Other studies have hypothesized that diversity significantly decreases at salt concentrations between ~20% to ~30% (w/v), a range regarded as a critical point in of hypersaline communities. Contrary to our expectations, diversity did not drastically decrease, although it underwent relevant compositional variations. Moreover, *Archaea* dominance increased with salinity as suspected, but this was unexpectedly linked to an increase in *de novo* synthesis genes involved in the “salt-out” osmoregulatory strategy, which have so far been related to the domain *Bacteria*. A detailed analysis of more than 1500 genomes confirmed that the distribution of osmoregulatory mechanisms does not always follow the classical domain-based paradigm where *Archaea* are strictly associated with the "salt-in" strategy.

Our results highlight the unexpected role of *Archaea* as the main contributors to the osmolyte pool in hypersaline environments, blurring the phylogenetic boundaries in salt adaptation strategies and suggesting that they could be facilitating stress tolerance at the community level.

Abstract ID: 3172 / Poster Number: 333.15P

Adaptative strategies of Pseudomonas in oxygen-limited environments

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Co-authors: *M. Salomé Duarte, Sónia Barbosa, João Sequeira, Michael Ho, Christian Vicera, Alcina Pereira, Giin-Yu Amy TAN, Po Heng Henry LEE, Andreia Salvador, Ana J. Cavaleiro*

Pseudomonas species are found in wastewater treatment systems operating under anaerobic and micro-aerobic conditions. Although their role is not fully understood, they appear to be key players in the biodegradation of organic matter, particularly recalcitrant compounds such as lipids or hydrocarbons. They thrive under limited oxygen conditions, which is a competitive advantage over other microorganisms. Gaining a deeper understanding on the role and behaviour of *Pseudomonas* spp. is essential for optimizing wastewater treatment processes such as anaerobic digestion (since at real-scale strictly anaerobic conditions are not consistently maintained) or micro-aerated systems.

This study aims to detect changes in gene expression by *Pseudomonas* species under micro-aerobic conditions. *Pseudomonas* sp. strain I1, isolated from micro-aerated bioreactors treating wastewater, were cultivated in sealed batch bottles containing acetate (10 mM) as the sole carbon source. Oxygen concentration in the headspace was adjusted to 0% (anaerobic control), 0.1%, 0.2%, 0.5%, 1%, 2% and 21%. The experiment was conducted for approximately six hours, during which oxygen and acetate concentrations were monitored at 0h, 3h and 6h by GC and HPLC, respectively. RNA was isolated and sequenced. Bioinformatics analysis was performed with MOSCA pipeline. Upregulation and downregulation of genes were assessed relatively to the 21% oxygen condition.

Significant differences were observed in genes related with the electron transport chain, TCA and quorum sensing pathway. In particular, the membrane-bound electron transport chain in *Pseudomonas* spp. can employ five oxygen reductases, two of which (cbb3-1 and cbb3-2) exhibit high affinity for oxygen (~0.25 µM O_2). Our results demonstrated a significant upregulation of these enzymes under all conditions, relatively to 21% O_2. A similar upregulation trend was observed in the bc1 complex, which reduces a cytochrome c-type protein that subsequently serves as electron donor to the cbb3 reductases. In contrast, terminal oxygen reductases with low oxygen affinity (~4 µM O_2),

showed low expression. These results highlight the importance of *cbb3*-type oxygen reductases in the response of *Pseudomonas* to low O_2 concentrations.

Our findings also reveal that succinyl-CoA synthetase is strongly repressed under limited O_2 conditions, while the genes encoding succinate dehydrogenase (sdhD) and fumarate reductase (frdD) are highly expressed. This pattern suggests a potential re-routing of metabolic flux from the TCA cycle towards the Glyoxylate Cycle, as supported by the overexpression of isocitrate lyase under 0.5%, 1% and 2% O_2. The activation of the Glyoxylate Cycle may confer an adaptive advantage by enabling the bypass of CO_2-generating steps in the TCA cycle, thereby preserving carbon skeletons for biosynthetic processes critical under O_2-limiting conditions.

The quorum sensing pathway has been shown to play a crucial role in oxidative stress survival by regulating rhamnolipid production, which forms a protective foam on the medium´s surface, limiting oxygen entry. However, genes for rhamnolipid biosynthesis and key detoxifying enzymes, such as catalase and alkyl hydroperoxide reductase (ahp), were expressed at very low levels under anaerobic and micro-aerobic conditions. The expression of enzymes responsible for flagellar assembly decreased under lower oxygen conditions, probably linked to a decrease in cell mobility, as a consequence to the increased energy demand for survival under stress, prioritizing other metabolic processes.

This study highlights some adaptative mechanisms of *Pseudomonas* under oxygen-limited conditions, which are responsible for the endurance of these microorganisms in diverse anaerobic and micro-aerobic environments.

Abstract ID: 3510 / Poster Number: 428.17S

Adapted Microbial Communities in Lab-Scale Membrane Bioreactors: Exploring Community Evolution and Stress Responses for Effective Pharmaceutical Bioremediation

Presenting author: Ms. Francesca Demaria, FHNW, Muttenz, Switzerland

Co-authors: *Marcel Suleiman, Philippe Corvini, Pilar Junier*

Pharmaceuticals are emerging contaminants (ECs) that given their biological activity and, in some cases, persistence and recalcitrance to biodegradation, cause adverse effects on ecosystems and living organisms. Moreover, the pharmaceutical market is expected to grow, indicating increasing pollution scenarios. Microbial communities have been identified as being able to degrade these persistent compounds and represent a promising approach for a more sustainable removal of these compounds in wastewater (**Figure 1**). However, little is known regarding the adaptation of such microbial consortia for a bio-based solution to be used *in situ* in wastewater treatment plants. One of the challenges in bioremediation is the fluctuation of wastewater composition and low concentration of pharmaceuticals in sewage inflow. Therefore, this study assesses how a lab-adapted and efficient micropollutant-degrading microbial consortium can remove micro-pollutants under fluctuating concentrations, which mimics the variability in micropollutant concentration occurring in a wastewater treatment plant. The study was conducted on six lab-scale membrane bioreactors (MBRs), continuously aerated and fed with different compounds (Atenolol, Caffeine, Diclofenac, Enalapril, Ibuprofen, Paracetamol). After an adaptation of two months in which the communities were exposed at higher concentration (100 mg/L pharmaceutical mixture), microbial communities within MBRs increased the efficiency of pollutant removal and reached 100%. When the mixture of pharmaceuticals concentration was decreased to 1 mg/L, the adapted microbial communities were still able to degrade 100%. This level of performance remained in five out of six MBRs when the concentration of pharmaceuticals was set again to 100 mg/L. Therefore, the experiments conducted demonstrate that MBRs are a good evolutionary tool for bioremediation purposes resulting in the enrichment of degrading populations due to the infinite sludge retention time. Moreover, the changes in the composition of the communities allows identifying those populations. Finally, the adapted microbial community was exposed at lower concentration of pharmaceuticals (µg/L), showing positive results and demonstrating its potential for use in environmental bioremediation strategies *in situ*.

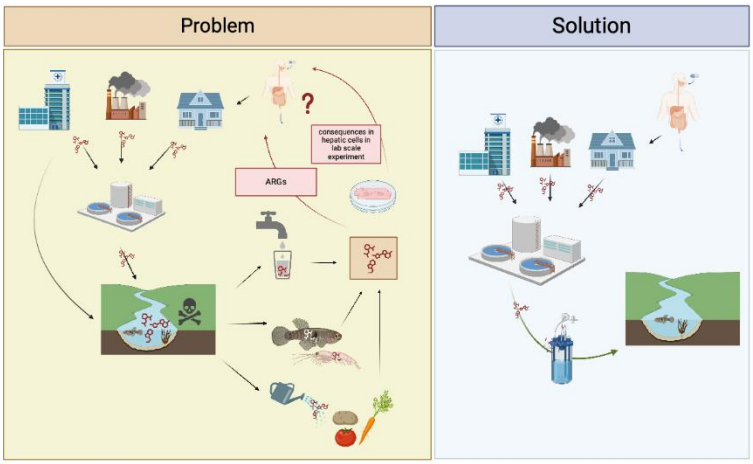


Figure 1: *Representation of the persistent environmental issue of pharmaceutical pollution: pharmaceuticals released into water sources bioaccumulate in fish, contaminate vegetable peels, and are detected in drinking water, contributing to the proliferation of antimicrobial resistance genes (ARGs) with unknown impacts on human health. On the right, a proposed bio-based solution using membrane bioreactors (MBRs) demonstrates potential for mitigating these pollutants.*

Abstract ID: 3058 / Poster Number: 165.17P

Adaptive mechanisms driving blaNDM-1 amplification and resistance evolution

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The *bla*$_{NDM-1}$ carbapenemase gene poses a global health challenge due to its antimicrobial resistance phenotype, widespread distribution, and ability to transfer genetically across hosts. This study investigates the molecular mechanisms and consequences underlying *bla*$_{NDM-1}$ gene dynamics within a prevalent genetic context. Specifically, we examined multiple 9 kb tandem repeat units on the pCW-NDM-1 plasmid, which contain the *bla*$_{NDM-1}$ gene flanked by homologous regions with IS*CR1* elements—an uncharacterized insertion sequence with a putative rolling-circle transposition mechanism.

Evolution experiments under varying antibiotic concentrations were conducted to assess the adaptability and reversibility of copy number amplifications in this genetic region. Additionally, knockout mutants targeting bacterial regulatory pathways and structural components of the amplified region were generated. Gene copy number changes in evolved populations were analyzed using quantitative PCR, while their effects on meropenem resistance were evaluated via broth microdilution. The genetic location of *bla*$_{NDM-1}$ copies was determined through Southern blotting and Nanopore sequencing.

Our results demonstrate that stable adaptive amplification of *bla*$_{NDM-1}$, leads to increased carbapenem resistance phenotype, and is independent of IS*CR1* activity but strongly dependent on bacterial homologous recombination mechanisms. Notably, this amplification produced independent circular 9 kb genetic structures. These findings highlight the critical role of genetic amplification dynamics in enhancing bacterial survival under selective pressure. Moreover, the accumulation of small *bla*$_{NDM-1}$-carrying genetic structures may significantly impact the horizontal transfer and dissemination of the carbapenemase gene, a hypothesis that requires further investigation. This study provides valuable insights into adaptive resistance mechanisms that threaten the efficacy of last-resort antibiotics.

Abstract ID: 1532 / Poster Number: 001.16S

Addition of spores or vegetative cells of Neobacillus vireti to soil microcosms promotes reduction of nitrous oxide emissions

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Introduction and Objectives: Fertilised agricultural soils are the source of more than 50 % of anthropogenic emissions of the potent greenhouse gas nitrous oxide (N_2O), contributing significantly to global warming. The only known microbial N_2O sinks are bacteria and archaea that contain the enzyme nitrous oxide reductase (NosZ), which reduces N_2O to N_2, and bioaugmentation with NosZ-carrying bacteria has been shown to have the potential to reduce soil N_2O emissions. However, this approach is challenging due to the variability of different soils and the need for added microorganisms to persist and efficiently reduce N_2O in the soil environment. In this study, we present a novel approach using spores of the N_2O-reducing soil bacterium *Neobacillus vireti* to augment soil slurry microcosms.

Method and Findings

In pure culture experiments using a tryptic soy broth medium supplemented with nitrate, both vegetative cells and spores (after germination) were able to reduce N_2O to N_2 (Figure 1A). Specific turnover rates for N_2O and O_2 and apparent K_M values for N_2O were calculated. The biokinetics of soil slurry microcosms, which were inoculated with 10^8 *N. vireti* spores per ml and incubated at 20°C, showed an enhanced turnover of N_2O to N_2 compared to a control experiment without spore addition, reflecting the N_2O consumption of the native soil microbiota (Figure 1B). It was also shown by microrespirometry that *N. vireti* cells are capable of reducing N_2O over a wide temperature range from 5 to 30°C.

Conclusion: The results indicate the potential to use spores to introduce metabolically active *N. vireti* cells into soils, which could enable their future use as a persistent and low-cost fertiliser additive to mitigate N_2O emissions at ambient temperatures. It is also shown that *N. vireti* spores can germinate under hypoxic conditions.

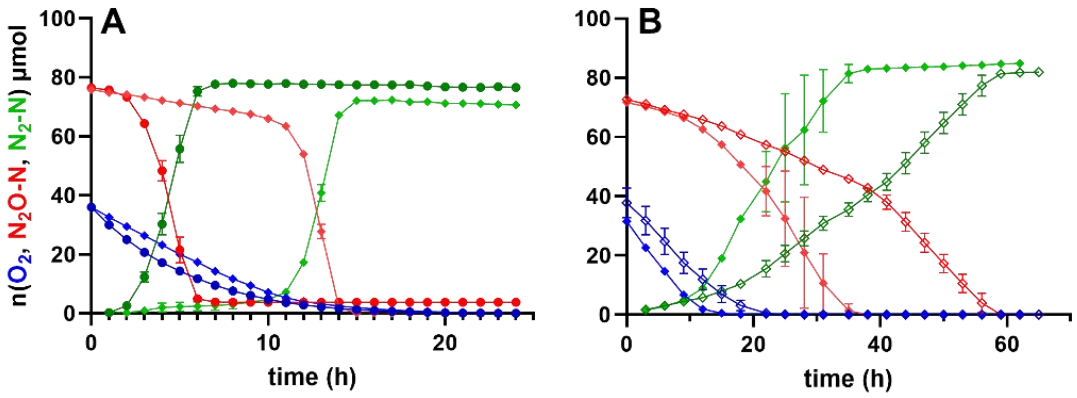


Figure 1: Consumption of added N_2O and O_2 and production of N_2 by vegetative or germinated *Neobacillus vireti* cells in pure culture (A) or soil slurry microcosm (B) experiments at 20°C. A, the cultures were inoculated with $5*10^7$ cells (circles) or spores (diamonds), the latter resulting in delayed N_2O consumption, due to germination, with a time lag of about 7 to 8 hours. B, soil microcosm experiments with (closed diamonds) or without (open diamonds) addition of *N. vireti* spores. The addition of spores resulted in an improved N_2O consumption rate. All values refer to µmol per vial (50 ml medium or slurry in 120 ml flasks) and were determined by gas chromatography using headspace samples.

Abstract ID: 2631 / Poster Number: 163.15P

Addressing Microbiome Modernization in Urbanized Children Through Targeted Biodiversity Interventions Leveraging Natural Ecosystems

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Co-authors: *Lucia Foresto, Elena Radaelli, Giorgia Palladino, Simone Rampelli, Marco Candela*

Introduction: In modern urban populations, the decline in gut microbiome (GM) diversity has been associated with the rising prevalence of autoimmune diseases in children, due to a failure in the GM-dependent immune education processes. In this context, innovative strategies to restore GM diversity in urban children are urgently needed. A significant amount of research highlights the critical role of interactions with natural ecosystems in supplying the human GM with the diversity needed to promote health.

Objective: Within this framework, the possibility to restore (“rewild”) the loss of GM diversity in modern urban populations through the recovery of beneficial interactions with natural ecosystems has been proposed. Despite their potential, experimental studies in this direction remain limited.

Method: To provide some glimpses in this direction, here we conducted a nature-based biodiversity intervention demonstrating that the GM of urban children was effectively subject to rewilded after a 15-days camping in the woods in the Dolomites Natural Park, Italy.

Findings/Conclusion: As an outcome of the successful rewilding, we observed the increased GM diversity, the higher abundance of fibrinolytic butyrate producers, and the increased production of anti-inflammatory metabolites, such as short chain fatty acids (SCFAs). Also, the relationship between nature exposure and GM response was carefully assessed, uncovering the mechanisms linking nature interaction, GM responses, and health. Our findings may pave the way to sustainable and equitable approaches of "nature prescribing" for the prevention of autoimmune diseases in the urban western populations.

Abstract ID: 3256 / Poster Number: 028.17F

Administration of dietary lactate leads to changes in fecal short-chain fatty acids in pre-diabetic subjects

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Co-authors: *Clarissa Schwab, Jiri Hosek, Jin Xie, Esben Søndergard, Natasa Zubanovic*

The short chain carboxylic acid (SCCA) lactate can be taken up through food, for e.g. fermented products or is a product of intestinal bacterial fermentation. Recent research suggests that oral administration of lactate may influence appetite regulation, but the impact on the gut microbiome remains poorly understood.

In this study, we investigated the effect of orally administrated lactate on fecal microbiota composition and fermentation metabolites of twelve pre-diabetic volunteers consuming either a lactate solution or a placebo. Fecal samples were collected at two time points post-intervention to assess changes in fecal SCFA profiles with HPLC-RI, and microbiota composition with long read 16S rRNA gene nanopore sequencing.

Our results show that dietary lactate can cause a short-term shift in fecal SCCA profiles. The proportion of propionate increased in 75% of fecal samples ('propionate responders') after lactate intervention, while the proportion of butyrate was lower.

Abundance of members of the *Romboutsia* genus, usually associated with the small intestine microbiome, and members of the *Veillonellaceae* family, which are linked to utilization of lactate in the small intestine, was indicative of community profiles of propionate responders, while abundance of members of the *Lachnospiraceae* family was indicative of non-responders' profiles. Taken together, our data suggest that orally consumed lactate is metabolized by propionate producers mainly found in the small intestine.

Lachnospiraceae were previously correlated with insulin resistance, while high abundance of propionate in the stool has been previously correlated with satiety, suggesting a microbiota-dependent response to oral administration of lactate.

Abstract ID: 3464

Advances in biological poly(lactic acid) (PLA) degradation and upcycling

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Co-authors: *Benedikt Wynands, Nick Wierckx*

Bio-based plastics, like poly(lactic acid) (PLA), pose challenges in traditional recycling. To address this, we propose a consolidated bioprocess (CBP) integrating PLA degradation and upcycling into a one-pot reaction by combining insights from enzymology, microbiology, and bioprocess technology. We focus on intersections of these fields to reveal CBP strategies with increasing efficiencies as shown in the Venn-diagram in Figure 1.

Initially, commercially available PLAses were characterized *in vitro* to identify optimal conditions and limitations, including phosphate absence and lactic acid product inhibition. Results highlighted the need to continuously withdraw lactic acid for sustained PLA depolymerization. CBPs achieve this by deploying microbes that metabolize lactic acid as a carbon source. Enzymes remained active across 30 – 60 °C, with peak PLA-depolymerization rates at 60 °C, necessitating thermophilic organisms for efficient degradation.

To find suitable microbes for CBPs, thermophiles obtained either commercially from DSMZ or isolated from industrial composting facilities were screened for their PLA-degrading ability and lactic acid growth. Promising wild-type strains were cultivated in bioreactors, identifying critical physiological parameters for shorter lag phases and faster growth, which in combination shortened the overall cultivation time.

Additionally, *in sillico* analysis of putative PLAses in the genomes of these thermophiles revealed several PLAse candidate enzymes. The most promising enzyme containing a predicted secretion signal peptide was overexpressed and purified, showing *in vitro* PLAse activity, which was however outperformed by commercial PLAses. Consequently, the best CBP with emphasis towards fastest PLA-degradation combines the fastest thermophilic lactic acid metabolizer with external addition of the most efficient PLAse.

This concept was adapted to mesophilic organisms engineered to produce 4-coumaric acid, a precursor for aromatic compounds. These strains achieved only approx. 15% lower yields on lactic acid compared to glucose, operating efficiently at 37 °C instead of the usual 30 °C, ensuring the best trade-off between cell viability and enzyme activity.

Finally, the study compares strategies for PLA degradation and valorization, including pure enzymatic depolymerization, thermophile deployment for rapid degradation, and engineered mesophiles to emphasize carbon source valorization.

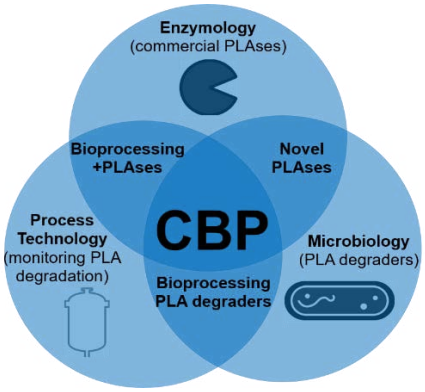


Figure 1: *Venn-diagram depicting the intersections of scientific disciplines necessary for realizing a consolidated bioprocess with PLA as carbon source.*

Abstract ID: 3654 / Poster Number: 291.16F

Advancing Bioremediation Strategies by Spatially Organizing Hard-to-Culture Microbes Using a Novel Isolation Technique

Presenting author: Mr. Dmitrii Deev, Jožef Stefan Institute, Ljubljana, Slovenia

Co-authors: *José Carlos Castilla Alcántara, Alberto Soto Cañas, Maja Zugan, Tomaž Rijavec, Rocio Barros, Aleš Lapanje*

Polycyclic aromatic hydrocarbons (PAHs) pose a serious threat to global environmental health. While significant progress has been made in developing bioremediation strategies for low-molecular-weight hydrocarbons, degradation of high-molecular-weight hydrocarbons (HMWHs) remains more challenging. Most conventional approaches rely on standard microbiological practices, utilizing either bottom-up or top-down strategies to assemble or isolate HMWH-degrading bacterial consortia, respectively. However, these approaches often result in the complete omission of: (i) viable but non-culturable (VBNC) species, (ii) the preservation of natural cell-to-cell distances, and(iii) the consideration of the low accessibility of HMWH to cells due to its hydrophobic nature. To overcome these drawbacks, we have developed a novel top-down method, uLUME, for isolating native HMWH-degrading consortia that we further evaluate its performance with conventional enrichment methods. Our approach is based on (i) using porous vaterite particles loaded with pyrene and benzo[a]pyrene, simulating natural PAH-polluted soil particles, (ii) adhering native bacterial consortia to these particles, (iii) stabilisation and compartmentalisation in alginate beads via a microfluidic approach. Growth on the vaterite surfaces was monitored through optical density (OD_{600}) and reduction of resazurin. The most efficiently growing consortia were selected and subjected to further characterization. Microbial community composition and metagenomic properties were characterized by DNA sequencing. PAH utilization was quantified using radiorespirometry with ^{14}C-labeled silicone O-rings. Results show that the top-down consortia achieved markedly higher degradation rates, utilizing up to 30 ng/mL of pyrene and 60 ng/mL of benzo[a]pyrene after 14 days—29.03% and 50.76% more than consortia isolated via classical enrichment. Accordingly, this approach showed promising results not only for the isolation of strains but also for studying cross-metabolic processes and spatial distribution.

Abstract ID: 3418 / Poster Number: 336.17P

Advancing dairy quality control: exploring flow cytometry for natural whey starters

Presenting author: Paolo Bellassi, PhD, Università Cattolica del Sacro Cuore, Cremona, Italy

Co-authors: *Alesandra Fontana, Lorenzo Morelli*

Introduction: The flow cytometer is an advanced tool for the assessment and enumeration of bacterial viability. The publication of ISO 19344 in 2015 standardized its application in probiotics and fermented milks, paving the way for its use in food fermentations, including natural whey as a lactic starter for Italian hard cheeses.

Objective: The purpose of this study was to evaluate the application of flow cytometry as a rapid and efficient method of enumerating LAB in natural whey starters with a comparison to standard microbiological methods and acidifying capacity.

Method: Natural whey starter samples were analysed using a BD Accuri™ C6 Plus flow cytometer with protocols A and B (ISO 19344:2015) to identify live, dead and damaged cells. Parallel culture analysis was performed on Agar whey, MRS and M17 culture media. Acidifying capacity was evaluated by monitoring milk acidity with 3% whey starter, initially and after 4 hours at 52 °C.

Results: Results indicated a promising linearity between flow cytometry and classical culture techniques (R^2>0.6), although flow cytometry appears to overestimate culture data. The ability to differentiate viable from non-viable cells could provide valuable insights into the physiological status of LAB and help predict their acidifying capacity under Italian hard cheese production conditions.

Conclusions: In conclusion, flow cytometry is a valuable tool for counting LAB in natural whey starter. The rapidity and high degree of accuracy of this method open doors for quality monitoring in dairy production processes.

Abstract ID: 2790 / Poster Number: 390.15P

Advancing prokaryotic genus demarcation within the families Halomonadaceae and Oceanospirillaceae assisted by network analysis

Presenting author: Dr. Rafael R. de la Haba, University of Sevilla, Sevilla, Spain

Co-authors: *Jorge Val-Calvo, David R. Arahal, Cristina Sánchez-Porro, Maria Chuvochina, Philip Hugenholtz, José A. Vázquez-Boland, Antonio Ventosa*

Prokaryotes taxonomy has benefited from the advent of high-throughput microbial genome sequencing, enabling genome-based phylogenetic reconstructions and the estimation of robust metrics and criteria for species circumscription. Despite these advances, the establishment of genus boundaries based on phylogenomic data and overall genome relatedness indexes remains inconsistent, often relying on group-specific thresholds leading to some degree of subjectivity.

Previous research conducted within the family *Halomonadaceae* using core and pangenome analyses, along with the identification of clade-specific signature genes, led to the proposal of six new genera and the reclassification of several species-level taxa. The present study aims to reevaluate the convenience and robustness of this reorganization by incorporating species previously excluded due to unavailable genomic data. Additionally, this work extends the analysis to include genera of the sister family *Oceanospirillaceae*, some of which have often been regarded as members of the *Halomonadaceae*.

To achieve these objectives, we employed a novel genus demarcation framework that combines normalized phylogenomic tree clustering with validation of relationships between taxa by network analysis of maximum-likelihood distances and genome relatedness indexes (ANI, AAI, and alignment fraction).

Our findings demonstrate that the integration of this innovative method with clade-specific signature genes provides a robust basis for establishing a non-subjective delimitation of genera and families within the order *Oceanospirillales*. Furthermore, this methodology may hold promise for application to other taxonomic groups, fostering greater consistency in prokaryotic taxonomy.

Abstract ID: 1689 / Poster Number: 097.17P

Advancing rapid diagnostics: Detection of antibiotic resistance using MALDI-TOF MS

Presenting author: Mrs. Daria Janiszewska, Nicolaus Copernicus University in Torun, Toruń, Poland

Co-authors: *Michał Złoch, Paweł Pomastowski, Marzena Kociołek, Małgorzata Szultka-Młyńska*

Introduction: The increasing prevalence of antibiotic-resistant bacteria poses a significant threat to effective treatment of severe infections, including respiratory diseases and sepsis. Rapid and accurate detection of resistance is crucial to initiate targeted therapies and improve patient outcomes. Traditional diagnostic methods are often time-consuming, delaying critical interventions.

Objective: This study aimed to evaluate the utility of MALDI-TOF MS techniques, specifically MBT-STAR and MBT-ASTRA, for rapid detection of antibiotic resistance in bacterial isolates from respiratory and bloodstream infections.

Methods: Clinical isolates from respiratory and bloodstream infections were analyzed using MALDI-TOF MS systems, including the MALDI Biotyper and EXS2600. Resistance profiles were assessed by detecting beta-lactamase activity and metabolic changes under antibiotic exposure. Optimized protocols were benchmarked against conventional resistance profiling methods for validation of sensitivity and specificity.

Findings: MALDI-TOF MS demonstrated the ability to identify bacterial species and detect antibiotic resistance within hours, significantly reducing diagnostic time compared to traditional methods. Both MBT-STAR and MBT-ASTRA showed high efficiency in detecting resistance in pathogens isolated from respiratory and systemic infections, such as sepsis. This highlights the critical role of rapid diagnostics in managing multidrug-resistant infections. Additionally, the study evaluated resistance to beta-lactam antibiotics, macrolides, and fluoroquinolone chemotherapy agents. For the first time, MBT-ASTRA was used to analyze resistance to levofloxacin and azithromycin in *Staphylococcus aureus*, advancing the precision of resistance monitoring in these pathogens.

Conclusion: MALDI-TOF MS-based diagnostics represent a transformative approach for the rapid detection of antibiotic resistance in severe infections, enabling timely therapeutic decisions and improving patient outcomes.

Abstract ID: 4036 / Poster Number: 306.15P

Aerobic anoxygenic phototrophic bacteria are part of core microbiomes of arctic and polar plants

Presenting author: Riitta Nissinen, PhD, University of Turku, Turku, Finland

Aerobic anoxygenic phototrophic bacteria (AAPB) are photoheterotropic bacteria that rely on organic carbon but utilize solar radiation for energy via anoxygenic photosynthesis. They are well known from aquatic ecosystems, but have been detected also in soil ecosystems and plant phyllospheres. We combined culturomics with bacteriochlorophyll-based near-infrared fluorescence imaging to screen culturable bacterial communities of over 100 plant species from Greenland, Svalbard, Finland, Patagonia and maritime Antarctica. AAPB were present in the phyllospheres of all plant species sampled, and they were highly abundant in many key plant species in boreal climate. Further, we consistently detected these bacteria in the leaf and stem endospheres of perennial species. This suggests the potential ecological significance of AAPBs to their plant hosts in cold, seasonal climates. We isolated, verified and identified >1500 AAPB strains. Majority of these plant associated AAPB represented alphaproteobacteria, with genera Sphingomonas and Methylobacterium dominating. Additionally, several novel bacterial genera and family were detected. Taxonomic distribution of AAPB was impacted by plant species, season and location. AAPB were highly abundant in the late fall and early winter in Finland. Methylobacterium species were most common in boreal plants and in the summer, but were virtually absent from high Arctic and maritime Antarctic plants. Sphingomonas species dominated Arctic and Antarctic samples, with climate and host specificity at species level. Genome analysis of 100 of these strains revealed a diverse set of photosensors in addition to photosynthesis gene cluster.

Abstract ID: 2781 / Poster Number: 071.16P

Age-related dynamics of predominant methanogenic Archaea in the human gut microbiome

Presenting author: Ms. Rokhsareh Mohammadzadeh, Medical University of Graz, Graz, Austria

Co-authors: *Alexander Mahnert, Tejus Shinde, Christina Kumpitsch, Viktoria Weinberger, Helena Schmidt, Christine Moissl-Eichinger*

Background

While the role of bacteria in the gut microbiome is well-documented, specific changes in the composition of methanogens during extreme aging remain unclear. This study was designed to explore the association of predominant methanogenic archaea within the human gut and aging.

Methods: Shotgun metagenomic data from the stool samples of young adults (n=127, Age: 19-59 y), older adults (n=86, Age: 60-99 y), and centenarians (n=34, age: 100-109 years) were analyzed.

Results: Our findings reveal a compelling link between age and the prevalence of high methanogen phenotype, while overall archaeal diversity diminishes. Surprisingly, the archaeal composition of methanogens in the microbiome of centenarians appears more akin to that of younger adults, showing an increase in *Methanobrevibacter smithii*, rather than *Candidatus* Methanobrevibacter intestini. Remarkably, *Ca*. M. intestini emerged as a central player in the stability of the archaea-bacteria network in adults, paving the way for *M. smithii* in older adults and centenarians. Notably, centenarians exhibit a highly complex and stable network of these two methanogens with other bacteria. The mutual exclusion between *Lachnospiraceae* and these methanogens throughout all age groups suggests that these archaeal communities may compensate for the age-related drop in *Lachnospiraceae* by co-occurring with *Oscillospiraceae*.

Conclusions: This study underscores the dynamics of archaeal microbiome in human physiology and aging. It highlights age-related shifts in methanogen composition, emphasizing the significance of both *M. smithii* and *Ca*. M. intestini and their partnership with butyrate-producing bacteria for potential enhanced health.

Abstract ID: 662 / Poster Number: 104.15F

Agricultural ecosystem restoration increases soil microbial diversity and reduces nitrogen losses

Presenting author: Ms. Sweta Kumari, Ben Gurion University of the Negev, Sde Boker, Israel

Co-authors: *Max Kolton, Orah Felicia Rein*

Microbial communities regulate ecosystem services and sustain ecological balances. However, human population growth, higher food demands, and environmental changes impair these balances. Intensive agricultural production has degraded soil health. Therefore, soil restoration is necessary to enhance ecosystem services and recover biodiversity. Despite the central role of microbes in ecosystem functioning, assessments of their contributions to restoration are limited. Thus, to quantify microbial contributions to soil restoration and ecosystem services, we compared the effects of soil management practices on soil properties, microbial activity and biodiversity, and ecosystem functions. A 1 km stretch of the Nahalal river bank, Israel, was divided into ten plots, and the effect of a Restoration Treatment (mix of 8 seeded native plants) on soil microbial diversity, activity, and ecosystem functionality was compared with those of a conventional Agriculture Treatment (wheat crop). After 3 years of restoration efforts, a significant increase in microbial abundance, diversity, and activities was observed in a Restoration Treatment compared to a conventional Agriculture Treatment. Restored soils hosted a greater number and diversity of culturable bacteria and higher levels of available soil phosphorous, while agricultural soils exhibited higher potential nitrification rates and greater nitrogen losses. Furthermore, restored soils showed potential to utilize and store the complex carbon efficiently, suggesting long-term carbon sequestration. Restored plots were also characterized by significantly higher water content and lower soil surface temperatures during the dry summer. These findings enable policymakers to develop and incorporate new guidelines into future soil ecological restoration programs.

Abstract ID: 2029

Agricultural intensification reduces selection of putative plant growth-promoting rhizobacteria in wheat

Presenting author: Dr. Tessa E. Reid, Rothamsted Research, Harpenden, United Kingdom

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Introduction: The complex evolutionary history of wheat has shaped its associated root microbial community. However, consideration of impacts from agricultural intensification has been limited.

Objective: This study investigated how endogenous (genome polyploidization) and exogenous (introduction of chemical fertilizers) factors have shaped beneficial rhizobacterial selection.

Method: We combined culture-independent and -dependent methods to analyze rhizobacterial community composition and its associated functions at the root–soil interface from a range of ancestral and modern wheat genotypes, grown with and without the addition of chemical fertilizer.

Findings: In controlled pot experiments, fertilization and soil compartment (rhizosphere, rhizoplane) were the dominant factors shaping rhizobacterial community composition, whereas the expansion of the wheat genome from diploid to allopolyploid caused the next greatest variation. Rhizoplane-derived culturable bacterial collections tested for plant growth-promoting (PGP) traits revealed that fertilization reduced the abundance of putative plant growth-promoting rhizobacteria in allopolyploid wheats but not in wild wheat progenitors. Taxonomic classification of these isolates showed that these differences were largely driven by reduced selection of beneficial root bacteria representative of the Bacteroidota phylum in allopolyploid wheats. Furthermore, the complexity of supported beneficial bacterial populations in hexaploid wheats was greatly reduced in comparison to diploid wild wheats (Figure 1).

Conclusion: We therefore propose that the selection of root-associated bacterial genera with PGP functions may be impaired by crop domestication in a fertilizer-dependent manner, a potentially crucial finding to direct future plant breeding programs to improve crop production systems in a changing environment.

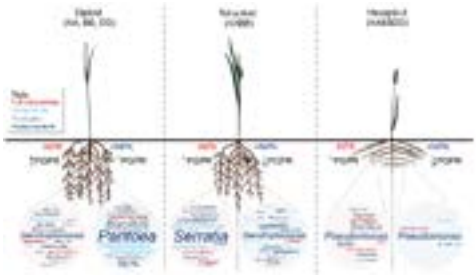


Figure 1: PGPR selection has reduced with agricultural intensification; in this schematic representation, the number of enriched genera with PGP traits (word clouds) associated with wheats dramatically reduced with genome polyploidization and fertilization (+NPK), and word clouds were made using wordart.com from differentially abundant ASVs classified to genus level associated with each ploidy level and fertilization type; genera are color coded by phyla.

Abstract ID: 4003 / Poster Number: 456.15P

Airborne microbiome – characterization of microorganisms inhabiting particulate matter of urban areas and their impact on human health

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Co-authors: *Arkadiusz Kajdasz, Anna Philips*

The airborne microbiome, composed of microorganisms inhabiting particulate matter (PM), remains underexplored despite its potential impact on human health. High concentrations of urban PM (PM2.5, PM1) pose significant respiratory health risks and act as carriers for bacteria, including pathogens and antibiotic resistance genes (ARGs), exacerbating public health concerns.

This study investigates airborne microbial communities in two urban areas with distinct emission characteristics: Zabrze, an industrial city with high coal combustion emissions, and an infectious disease hospital in the less industrialized Racibórz. A total of 300 PM samples, collected across different seasons, were analyzed using whole-metagenome high-throughput sequencing to characterize microbial taxonomic profiles. Moreover, we detect ARGs and assess the influence of PM's physio-chemical properties and meteorological factors on community structure.

Preliminary methodological research was conducted mainly to optimize the DNA extraction protocol from PM filters. However, it yielded promising results revealing diverse microbial communities, including common environmental bacteria, plant-associated microbes, and human-related species. Pathogenic bacteria, such as Acinetobacter spp. and Escherichia coli were detected, along with ARGs linked to tetracycline resistance, suggesting that PM contributes to the spread of antibiotic resistance in the environment.

Understanding the complexity of airborne microbial communities with ARGs and their relationship with PM composition and meteorological conditions is crucial for assessing air quality and public health risks such as disease transmission, and antibiotic resistance spread. Our findings confirm these relationships and highlight the need for further research, which could aid in redefining air quality standards and public health policies.

Abstract ID: 2321 / Poster Number: 055.15P

Airborne Pathogens and Virulence Factors in Belgrade Metropolitan Area Metagenomic Insight

Presenting author: Dr. Milka Malesevic, Institute of Molecular Genetics and Genetic Engine, Belgrade, Serbia

Co-authors: *Danka Matijasevic, Lazar Gardijan, Stefan Stanovcic, Nemanja Kljajevic, Dusan Radojevic, Katarina Novovic*

Introduction: Air pollution has been recognized as one of the greatest threats to human health. The particulate matter-$PM_{2.5}$, main air pollution indicator, is found to contain numerous bacterial pathogens. Considering that Serbia is struggling with excessive antibiotics use and misuse, a high percentage of multidrug-resistant (MDR) bacterial isolates, and poor air quality, made Serbian capital Belgrade interesting research model for the effects of air pollution on the abundance and diversity of airborne pathogens.

Objective: The presence of MDR pathogens, virulence factors as well as pathogen-host interactions are studied in the airborne metagenome samples from seven locations of Belgrade metropolitan area collected during the summer season of 2024.

Method: Air samples were collected from seven Belgrade locations using hydrophobic polypropylene membrane filters (air volume of 30 m^3). Total DNA was analysed by shotgun metagenomic sequencing, followed by bioinformatic processing (Novogene, UK). Taxonomic diversity/abundance, virulence factors and pathogen-host interactions were analysed using MEGAN, VFDB, and PHI databases, respectively.

Findings: Important human and animal pathogens were found in analysed metagenomes: Acinetobacter schindleri, Streptococcus pneumonie, Streptococcus oralis, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Ewingella americana, Burkholderia multivorans, Legionella pneumophila, Salmonella enterica and Brachybacterium muris. Genes encoding for motility, adherence, immune modulation, exotoxins and biofilm formation were also found. Additionally, PHI analysis revealed the presence of different type of infections caused by pathogenic bacteria – nosocomial and urinary tract infections, pneumonia, salmonellosis, soft rot.

Conclusion: This study indicated that Belgrade air could serve as a reservoir for pathogenic bacteria, virulome and numerous types of infections.

Abstract ID: 3191 / Poster Number: 081.16P

Akkermansia species dynamics in treatment of inflammatory bowel diseases by fecal microbiota transplantation

Presenting author: Klara Filek, PhD, Medical University of Graz, Graz, Austria

Co-authors: *Marija Durdevic, Patrick Schimmel, Christoph Högenauer, Christine Moissl-Eichinger, Gregor Gorkiewicz, Christian Diener*

Introduction: Fecal microbiota transplantation (FMT) is a promising therapy for inflammatory bowel diseases (IBD), but its mechanism of action remains unclear. While *Akkermansia muciniphila* is recognized as a biomarker of FMT success, recent research identified several novel *Akkermansia* species with unknown roles in IBD.

Objectives: The objective was to determine the prevalence and potential associations of novel *Akkermansia* species with FMT response in IBD patients.

Methods: Longitudinal fecal samples of IBD patients (P) that received FMT from donors (D) were analysed using 16S rRNA gene amplicon (P:44, D:24) and metagenomic shotgun sequencing (P:24, D:19).

Results: In amplicon data, *A. muciniphila* was detected in 45% of donors and 38% of patients. Novel species, *A. biwaensis* and *A. massiliensis* were detected at low prevalence; in 13% of donors and 16% of patients. Metagenomic analysis recovered more *Akkermansia* species, *A. muciniphila* subgroups, and uncultivated *Akkermansia* species (54-91% across donors and patients). Significant differences in the relative abundance of *A. massiliensis* and uncultivated *Akkermansia* were observed between donors and non-responders post-FMT (adjusted $p < 0.05$). Post-FMT remission was associated with lower accumulation of other Verrucomicrobiota, suggesting a displacement by various Akkermansia species.

Conclusion: This study emphasizes the complex dynamics of *Akkermansia* in IBD post-FMT and suggests that species other than *A.muciniphila* may be critical for treatment response. We show that 16S sequencing severely underestimates the prevalence of *Akkermansia* species compared to metagenomic sequencing. Further work will focus on clarifying their functional roles, and to assess their potential as biomarkers and therapeutics.

Abstract ID: 2282

Alginate-εPLL core-shell hydrogel beads as a tool for microbial encapsulation and co-culture

Presenting author: Ms. Alba Amaro da Cruz, University of Granada, Granada, Spain

Co-authors: *Miguel García Román, Ignacio Moya Ramírez*

Microbial communities are ubiquitous in nature and are notable for their resilience, robustness against environmental perturbations, and capability to perform complex functions that single-strain systems cannot achieve. These traits make them specially promising for biotechnological applications. However, engineering stable and reproducible synthetic microbial consortia remains challenging due to competitive dynamics and disparities in species growth rates.

In this study, we aim to develop a microbial encapsulation method that ensures the complete entrapment and growth of several species, therefore making possible the co-culture of species with differing growth rates. To achieve this, we encapsulated four microbial strains in alginate hydrogel beads (HB) coated with polymers using a layer-by-layer methodology. We optimized the coating agent and number of layers to effectively prevent microbial escape. Additionally, we characterized the HB, monitored microbial growth inside them, constructed spatially organized co-cultures, and evaluated the long-term storage of microorganism-loaded HB.

Our results show that hydrogel beads coated with εPLL (εPLL-HB) successfully ensure microbial containment and support growth of both bacterial and yeast species. Additionally, εPLL-HB enabled us to assemble spatially organized co-cultures and maintain a balance between the populations of microorganisms with significantly different growth rates. Furthermore, they provided protection against toxic compounds in lignocellulosic-derived media and maintained their encapsulation efficacy and microbial viability after long-term storage.

The superior microbial containment, structural stability, and chemical resistance of εPLL-HB, combined with their low cost and ease of preparation, make them a versatile and valuable tool for engineering synthetic microbial consortia. These features highlight their broad potential for application in diverse biotechnological processes.

Abstract ID: 2879 / Poster Number: 150.17P

Alleviating the Burden of Biofilms: Design and Synthesis of Dual-acting Antimicrobials

Presenting author: Rhiannon Baxter, Institute for Molecular Bioscience, Brisbane, Australia

Co-authors: *Lily Kenchington-Evans, Anthony Verderosa, Mark Blaskovich*

Antimicrobial resistance poses a significant threat to global health and is exacerbated by the propensity for pathogenic species to form biofilms. Biofilms are a community of homo- or hetero-generous microorganisms encased in an extracellular polymeric matrix that further impedes antibiotic diffusion to susceptible bacterial cells. Additionally, there is a dearth of antibiotics in the clinical pipeline, resulting in the urgent need for novel antibacterial agents, including ones to target and eradicate biofilms. This project aims to address the above by the design and synthesis of dual-acting antimicrobials known as 'hybrids', which combine separate moieties that provide antibacterial and antibiofilm activity. Our approach to hybrid development involves installation of readily derivatisable chemical handles to existing antibiotics, which are then linked to the antibiofilm component using 'click' chemistry. This 'click' approach allows for the rapid synthesis of hybrids and investigation of the most efficacious combinations. Here, we have synthesized more than 20 hybrids from six antibiotic classes with ammonium or phosphonium moieties acting as the antibiofilm agent of interest. Hybrids are evaluated against Gram-negative and -positive populations to determine minimum inhibitory concentrations (MIC) and minimum biofilm eradication concentration (MBEC). Several hybrids across four antibiotic classes have retained activity against planktonic populations and testing for antibiofilm activity is ongoing with these lead candidates. If successful, these dual-acting hybrids have the potential to advance the management of chronic infections caused by bacterial biofilm communities and restock the antibiotic pipeline.

Abstract ID: 612 / Poster Number: 036.15P

Alleviation of Dysmenorrhea Using Bifidobacterium animalis subsp. lactis SBla010 Isolated from Adult Feces

Presenting author: Ms. Jinok Kwak, Dankook university, Dongnam-gu, Cheonan-si, South Korea

Co-authors: *Hyunok Doo, Yejin Choi, Juyoun Kang, Haram Kim, Yeongjae Chae, Suyoung Lee, Hyunjin Yang, Sheena Kim, Hyen Bum Kim*

Introduction: Dysmenorrhea, characterized by painful cramps, impairs women's quality of life. While pharmacological treatments are available, they often come with side effects and limited long-term efficacy. Probiotics, beneficial microorganisms that confer health advantages to the host, have gained attention for their potential to enhance the health of the female reproductive system.

Objective: To investigate the effect of oral administration of Bifidobacterium animalis subsp. lactis SBla010 (B.animalis SBla010) on pain frequency, hormonal changes, and the vaginal microbiome in a mouse model of dysmenorrhea.

Method: ICR mice were orally administered B.animalis SBla010 at 1x10^9 CFU/ml three times weekly for four weeks. After probiotic treatment, dysmenorrhea was induced. Analyses were then conducted to assess the pain frequency, hormonal fluctuations, and vaginal microbiome of the ICR mice.

Findings: Mice receiving B.animalis SBla010 exhibited a significant reduction in writhing counts, indicating decreased pain frequency. The uterine endometrial thickness of the probiotic-treated mice was significantly reduced. Additionally, serum and uterine tissue $PGF_2\alpha$ levels decreased, while PTGER2 gene expression in uterine tissue was upregulated. Moreover, the abundance of Proteus bacteria in vagina, which can cause endometriosis, was reduced in the treated group. These physiological changes correlated with enhanced uterine health.

Conclusion: Oral administration of B.animalis SBla010, isolated from adult feces, effectively alleviated dysmenorrehea symptoms in a mouse model. This probiotic strain enhanced vaginal health and modulated prostaglandin hormonal levels, highlighting its potential as an alternative treatment for dysmenorrhea. Further clinical studies are warranted to validate its efficacy and explore therapeutic applications in humans.

Abstract ID: 991 / Poster Number: 195.16P

Alleviative effects of the oral administration of a bacterium that produces conjugated linoleic acid on allergic dermatitis in mice

Presenting author: Mr. Hiroki Kaneko, Meiji University, Kawasaki, Japan

Co-authors: *Narito Asanuma, Yusei Umezawa*

Introduction: Conjugated linoleic acid (CLA), which is the isomers of linoleic acid (LA), has been reported to exhibit anti-inflammatory properties. Atopic dermatitis is one of the most common chronic relapsing inflammatory skin disorders. CLA may have a beneficial effect for amelioration of allergic dermatitis. It might be more beneficial to absorb CLA produced from dietary LA by bacteria in the host intestinal tract.

Objective: We investigated the effect of oral administration of the CLA-producing bacterium on a murine model of the allergic dermatitis.

Method: To compare the ability to produce CLA, some bacterial isolates were incubated in the presence of LA, and the conversion of LA to CLA was assayed. The high-CLA producer was identified and administrated to mice as the probiotic. Allergic dermatitis was induced to mice with oxazolone at the ears by repeated application.

Findings: The isolate with the highest ability to produce CLA belongs to Clostridium cluster XIVa and the closest species was Pseudobutyrivibrio ruminis. Administration of this bacterium alleviated the symptoms of allergic dermatitis in mice fed a diet rich in LA. The development of skin roughness or exfoliation was suppressed, and the raised ear thicknesses was decreased. These effects were associated with the decrease in the expression of pro-inflammatory cytokines in ears.

Conclusion: Administration of the high-CLA producing bacterium may alleviate symptoms of allergic dermatitis in mice fed a high-fat diet rich in LA. This result might be caused by the intestinal production of CLA by this bacterium.

Abstract ID: 833 / Poster Number: 273.17P

Alteration of metabolic functions associated with biohydrogen production in food wastewater treatment plants

Presenting author: Dr. Sang-Hoon Lee, Korea University, Seoul, South Korea

Co-authors: *Na-Kyung Kim, Do-Hyung Kim, Parthiban Anburajan, Hee-Deung Park, Jeong-Hoon Park*

The production of biohydrogen from food wastewater offers advantages: it generates green hydrogen while treating wastewater with high organic content. However, the unique characteristics of food wastewater such as elevated levels of organic matter, low pH, and high salt concentrations pose challenges for optimizing biohydrogen production. Unlike in the context of municipal wastewater treatment plants (WWTP), these conditions also lead to specialized microbial communities capable of surviving in harsh bioreactor environments. To investigate disparities in biohydrogen production performance, batch-type experiments were conducted using anaerobic sludge samples obtained from food WWTPs. These tests revealed a comparatively lower biohydrogen yield when anaerobic sludges from food WWTPs were used as inoculants in bioreactors, in contrast of those collected from municipal WWTPs. To gain insights into the microbial communities primarily responsible for biohydrogen production in food WWTPs, 16S rRNA gene-based sequencing analyses were carried out. The key microbial communities implicated in biohydrogen production were found to differ significantly, particularly at the species level within the genus Clostridium, regardless of the source of anaerobic sludge. Further, to explore the distribution patterns of functional genes related to biohydrogen production, metagenomic analyses were performed. The abundance of the genes encoding ferredoxin (Fd) was one of the highest among the entire annotated genes together with the [FeFe]-hydrogenase, indicating that the oxidation of Fdred by [FeFe]-hydrogenase was the main pathway for Clostridium to use hydrogen production while Pyruvate is oxidized to acetyl-CoA. These results suggested that different metabolic functions might contribute in biohydrogen production in food WWTPs.

Abstract ID: 1337 / Poster Number: 286.17P

Altering the glucose uptake system of Serratia marcescens enables 2-butanol production in a co-culture

Presenting author: Dr. Anna Maria Erian, BOKU University, Vienna, Austria

Co-authors: *Hannes Rußmayer, Michael Sauer, Hans Marx*

With its high energy content, 2-butanol holds significant potential as a biofuel. One of the few microorganisms naturally capable of producing it is *Lentilactobacillus diolivorans*, which converts meso-2,3-butanediol into 2-butanol under oxygen-limited conditions. However, *L. diolivorans* does not produce 2,3-butanediol itself but relies on an external supply. Conversely, other bacteria, such as *Serratia marcescens*, produce meso-2,3-butanediol in substantial quantities but do not produce 2-butanol. Therefore, taking advantage of the complementary metabolisms of these bacteria and co-cultivating them appears promising for microbial 2-butanol production.

To effectively control growth and production of different microorganisms within a co-culture, utilizing different carbon sources is advantageous. Consequently, we targeted the glucose uptake system of *S. marcescens* to reduce its affinity for glucose and prioritize the uptake of an alternative substrate while saving glucose for *L. diolivorans* as a growth substrate and electron donor. We knocked out genes associated with glucose uptake in *S. marcescens*, assessed the impact on substrate utilization, and examined the co-cultivation with *L. diolivorans*. Additionally, we developed a cultivation and feeding strategy in bioreactors, achieving titers of more than 14 g/L 2-butanol. Our findings establish a foundation for further advancements in co-cultivations involving *S. marcescens* and glucose-consuming microorganisms.

Abstract ID: 3227 / Poster Number: 397.16P

Alternative media as a cost-effective solution for bioconcrete production

Presenting author: Ms. Henrietta Ottová, University of Chemistry and Technology, Prague, Prague, Czech Republic

Co-authors: *Petr Holeček, Václav Nežerka, Barbora Malíková, Hana Stiborová*

The recycling of waste concrete fines through microbially induced calcite precipitation (MICP) presents a promising solution to reduce the carbon footprint of the construction industry, which is responsible for up to 10% of global CO_2 emissions. However, scaling up this technology requires addressing its high cost, with up to 60% arise from the use of commercial cultivation media for MICP-capable bacteria. To overcome this challenge, we explored feather hydrolysate as a cost-effective alternative medium. Approximately 5 million tons of feathers are produced as waste by the poultry industry each year. Using a machine learning model, we optimized the feather hydrolysis process, focusing on parameters such as hydrolysis duration, temperature, and hydroxide or feather concentration. The optimization aimed at enhancing the growth of bacteria *Sporosarcina pasteurii* DSM 33 and *Bacillus cohnii* DSM 6307. With the optimized feather hydrolysate, we successfully produced bioconcrete samples and analysed their mechanical and chemical properties, including crystal types and thermogravimetric analysis. The results revealed that bioconcrete samples prepared with feather hydrolysate were comparable in quality to those produced using commercial media. Furthermore, building on this success, we are now exploring other industrial by-products, such as brewery yeast and spent grain, as alternative media. These results demonstrate the potential of this approach to lower costs and advance the sustainable application of MICP in the construction industry.

Abstract ID: 2959 / Poster Number: 111.15F

Amoebae and bacteria quantification on a French Atlantic estuary and the role of environmental factors

Presenting author: Gaëlle Bednarek, PhD, Université de Poitiers, Poitiers, France ; La Rochelle Université, La Rochelle, France

Co-authors: *Yann Héchard, Hélène Agogué, Vincent Delafont*

Amoebae are phagotrophic protists that primarily feed on bacteria and are found in various environments. Some bacteria can resist digestion by amoebae, and several studies have demonstrated that this can enhance bacterial pathogenicity.

Our study aims to investigate whether marine amoebae, which are currently poorly characterized, could serve as vectors for pathogenic bacteria belonging to the genera *Vibrio*, *Mycobacterium*, *Legionella* and *Pseudomonas* in coastal waters. Also, we aim to evaluate the influence of environmental parameters on amoebae and bacteria presence.

Sixty samples were collected, monthly over a year, along a salinity gradient, at five different stations, from the Charente River to the Pertuis Charentais (Atlantic estuary). Various environmental variables were measured, including water parameters and meteorological data.

Microbial genera dynamics monitoring (qPCR) revealed variability in population composition and abundance, depending on the sampling stations and the season. The genera *Naegleria* and *Acanthamoeba* were the most prevalent amoebae. Regarding bacteria, the genera *Vibrio* and *Pseudomonas* were the most frequently detected. Besides, amoebal cultures led to the isolation of a dozen amoebae belonging to different genera such as *Acanthamoeba* and *Vannella*.

Salinity and oxygen parameters strongly influence amoebae and bacterial communities. Significant negative correlation between salinity vs. oxygen and *Legionella spp*.; and significant positive ones between *Acanthamoeba*, *Naegleria vs. Vibri*o, *Pseudomonas and Legionella* were identified through statistical analysis.

In conclusion, amoebae were found in each station throughout the year. Salinity and oxygen appear to strongly influence microbial communities, and some amoebae were correlated to bacteria presence.

Abstract ID: 3573 / Poster Number: 263.16S

An active wound dressing based on bacterial cellulose containing immobilized bacteriophages

Presenting author: Anna Zywicka, PhD, West Pomeranian University of Technology in Szczecin, Szczecin, Poland

Introduction: Antimicrobial resistance is one of the most important global threats to public health. Therefore, for years there has been an intensive search for treatment methods that could be an alternative to traditional antibiotic therapy. Phage therapy is a leading candidate among the proposed solutions. Phage therapy is a form of antibacterial therapy carried out using specific viruses bacteriophages) that lead to lysis bacterial cells (O'Connell et al. 2021). There is also great interest in combining phages with various types of carriers in order to improve and expand their potential applications, e.g. as dressings for difficult-to-heal wounds.

An example of a biomaterial that meets all the requirements of an ideal dressing is bacterial cellulose (BC). t is a natural polymer synthesized, among others, by non-pathogenic bacteria of the *Komagataeibacter genus*. BC is characterized by high purity, crystallinity, mechanical strength and water retention capacity. Moreover, it is a biodegradable and, above all, biocompatible material (Choi et al. 2022). Due to its three-dimensional structure, BC can be an ideal carrier for the immobilization of bioactive substances and various types of human cells, bacteria and viruses (Żywicka et al. 2019).

Objective: The aim of the study was to immobilize phages on BC and then analysed the effectiveness of the BC-based active dressing against bacteria forming biofilm.

Methods: In this study two phages were used against strains capable of forming biofilm: methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In order to immobilize phages on BC-based carrier adsorption method was be used. For this purpose, a purified and sterile BC pellicle was incubated with phages in SM buffer. After incubation, the effectiveness of immobilization was analysed. To obtain the appropriate level of phages per unit surface of the carrier, for each phage all parameters of the immobilization procedure was optimized.

In order to analysed the effectiveness of BC-based dressing conitaining immobilized phages Antibiofilm Dressing's Activity Measurement (A.D.A.M. test) (Junka et al., 2021) and artificial wound bed model (Ciecholewska-Juśko et al., 2022) were used. Those tests allows to check in vitro a dressing's suitability against biofilm-related wound infections. The effectiveness of biofilm eradication (the number of live bacteria after incubation with

the dressing) was assessed using the MTT test, which is a colorimetric assay for assessing cell metabolic activity.

Results: The results obtained in this study showed that BC is an ideal carrier for phage immobilization. The effectiveness of immobilization depended primarily on the properties of BC such as crystallinity and porosity. The effectiveness of the BC-based active dressing against bacteria forming biofilm was also confirmed. The analysis showed that the degree of biofilm eradication depended on the type of phage and the number of particles immobilized on the carrier. In the case of *S. aureus*, a biofilm reduction by 35% was observed compare to control (BC-based dressing without phage), and 42% of in the case *of P. aureuginos*.

Conclusion: Application of BC-based dressing containing immobilized phages could accelerate the treatment process of difficult-to-heal wounds in the future, especially in cases where the etiological factor of the infection is antibiotic-resistant strains or those capable of forming biofilm.

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Abstract ID: 1863 / Poster Number: 192.16P

An antisense Peptide-conjugated Peptide Nucleic Acid (PPNA) for peptidoglycan recycling inhibition reduces AmpC hyperproduction and β–lactam resistance in Pseudomonas aeruginosa

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Co-authors: *Isabel M. Barceló, Jordi Sansó Sastre, Elena Jordana Lluch, Antonio Oliver, Carlos Juan*

Introduction: Given the high antimicrobial resistance levels in *Pseudomonas aeruginosa* (PA), new therapeutic strategies are urgently needed. Antisense Peptide-conjugated Peptide Nucleic Acids (PPNAs) are emerging alternatives in this regard, and consist of synthetic N-(2-aminoethyl)-Glycine-based oligomers linked to cell-penetrating peptides, that block target proteins translation by hybridizing with their mRNAs leading to their degradation. Our designed PPNA targets NagZ, a key factor in the interplay between peptidoglycan recycling, AmpC β-lactamase production and virulence.

Objective: To assess the potential of a NagZ-targeting PPNA to reduce AmpC hyperproduction and β-lactam resistance in PA, and thus rehabilitate β-lactams' power.

Method: NagZ-PPNA [(RXR)4XB-O-CATGAAAAGTCC, PNA-Bio, USA] concentrations from 16 to 0.125μM were tested regarding their capacity to reduce *nagZ* and *ampC* mRNAs (real-time RT-PCR) and ceftazidime resistance (microdilution) in the well-known high-level AmpC hyperproducer mutant PAdacBΔD. An unspecific PPNA and PAO1 and PAdacBΔDnZ strains were used as controls. PPNAs Minimum Inhibitory Concentrations (MICs) were determined by microdilution. Experiments were performed in 96-well non-binding plates sealed with gas-permeable foils (37ºC-180rpm-14h, Müller-Hinton broth). PPNAs cytotoxicity was tested on A549 cells using commercial Lactate-Dehydrogenase quantification kits.

Findings: NagZ-PPNA (MIC=16μM) at 2μM reduced *nagZ* and *ampC* mRNA levels in PAdacBΔD up to ≈25% of control values, causing low cytotoxicity (≈10% of A549 cells

death). PAdacBΔD ceftazidime MIC decreased from 64 to 8 mg/l after combination with NagZ-PPNA 2µM.

Conclusion: This proof-of-concept study shows that NagZ-PPNA at low concentration significantly decreased AmpC production and ceftazidime resistance in a clinically relevant PA mutant with low eukaryotic toxicity, suggesting interesting therapeutic potentials.

Abstract ID: 942

An archaeal CBASS system clears viral infection without killing the host

Presenting author: Prof. Uri Gophna, Tel Aviv University, Tel Aviv, Israel

Introduction: Cyclic-oligonucleotide-based anti-phage signalling systems (CBASS) protect bacterial populations through host cell death before phage maturation. This defense strategy is reasonable in bacteria, as cells in late stages of lytic infection face inevitable death when the phage completes its lifecycle. However, archaea in their natural habitats frequently encounter non-lethal viruses that establish chronic, long-term coexistence with their hosts. In such scenarios, abortive infection should be disadvantageous, as the cost of immunity may exceed that of infection. Yet paradoxically, CBASS are similarly prevalent in archaea and bacteria.

Objective: We tested whether CBASS is beneficial or deleterious to the archaeal host during chronic productive viral infection.

Methods: We cloned the type II-C CBASS from Haloferax strain Atlit 48N and expressed it heterologously in the laboratory strain Haloferax volcanii DS2. We then challenged *H. volcanii* DS2 with HFPV-1, a model virus known to establish chronic productive infection in this host.

Findings: Expression of type II-C CBASS in *H. volcanii* DS2 did not affect HFPV-1 plating efficiency or cause immediate cell death, as measured by live-dead assay, but did reduce growth rate. This growth effect was specific, and could be abolished through mutation of the system's cyclase active site, required for production of the signal molecule. Unexpectedly, most CBASS-expressing cells eliminated the chronic virus infection within 100 generations in liquid culture, while control cells maintained the infection. NAD^+-depletion, the proposed mechanism for this system's function, occurred only in stationary phase cells, and caused mortality only after weeks of incubation on solid medium.

Conclusion: Our findings demonstrate that CBASS can benefit archaea predominantly exposed to chronic viral infections, potentially explaining the widespread presence of these systems in archaeal populations.

Abstract ID: 3953 / Poster Number: 220.17P

An Australian perspective on methods to quantify the in vitro antimicrobial activity of honey

Presenting author: Assoc. Prof. Katherine Hammer, The University of Western Australia, Perth, Australia

Co-authors: *Kathryn Green, Md Lokman Hossain*

Honey is well-known for demonstrating antimicrobial activity in vitro. It is also well-known that the level of antimicrobial activity varies according to the floral source of the nectar from which the honey is made. These antimicrobial properties and differences have been shown in vitro using a range of methods, including both broth (liquid medium) and agar (solid medium) assays. In some instances, these activity measurements are used in the marketing and promotion of honeys.

Our research aims to develop robust in vitro methods for investigating the antibacterial activity of honey and honey products (Fig 1). Further, we aimed to use these methods to evaluate the antimicrobial activity of many different varietal honeys, and honey products.

A broth (liquid) assay was developed for quantifying the antibacterial activity of honey, and an agar overlay assay was developed for evaluating the activity of honey products. Results showed that the antimicrobial activity of honey is a function of the honey composition, the microbial species being tested, and concentration of honey applied. For example, Gram negative bacteria are more susceptible to the osmotic effects of the sugar component of honey compared to Gram positive bacteria, whereas conversely, Gram positive bacteria are more susceptible to phenolic or antimicrobial peptides than are the Gram negative bacteria.

In conclusion, differences in antibacterial activity between different types of honey, and activity of honey formulations, can clearly be demonstrated in vitro. However, the relevance such differences to clinical outcomes, such as wound healing time, has not been determined. Whilst animal models may be useful for evaluating clinical efficacy (Fig 1), it is preferable to utilise and/or develop in vitro wound-healing models to generate further proof-of-concept data before progressing to animal or human clinical trials. This would build on the methodologies already developed in our laboratory and would provide further evidence for the clinical use of honey.



Figure 1: Suggested steps in evaluating the antimicrobial activity and efficacy of honey

Abstract ID: 2980

An ecological and stochastic perspective on persisters resuscitation

Presenting author: Mrs. Tania Alonso-Vásquez, University of Florence, Florence, Italy

Co-authors: *Michele Giovannini, Gian Luigi Garbini, Mikolaj Dziurzynski, Giovanni Bacci, Ester Coppini, Donatella Fibbi, Marco Fondi*

Resistance, tolerance, and persistence to antibiotics have mainly been studied at the level of a single microbial isolate. However, in recent years it has become evident that microbial interactions play a role in determining the success of antibiotic treatments, in particular by influencing the occurrence of persistence and tolerance within a population. Additionally, the challenge of resuscitation (the capability of a population to revive after antibiotic exposure) and pathogen clearance are strongly linked to the small size of the surviving population and to the presence of fluctuations in cell counts. Indeed, while large population dynamics can be considered deterministic, small populations are influenced by stochastic processes, making their behaviour less predictable.

Our study argues that microbe-microbe interactions within a community affect the mode, tempo, and success of persister resuscitation and that these are further influenced by noise. To this aim, we developed a theoretical model of a three-member microbial community, including one hypothetical pathogen, sensitive to a given antibiotic, and able to form persister cells. We analysed the role of cell-to-cell interactions on pathogen clearance, using both deterministic and stochastic simulations. In addition, we used these results to model real data of a microbial community coming from a wastewater depuration plant in Prato, Italy (Gestione Impianti Depurazione Acque S.p.A).

Our findings highlight i) the importance of ecological interactions and population size fluctuations (and hence the underlying cellular mechanisms) in determining the resilience of microbial populations following antibiotic treatment; and ii) the possible relevance of these models in the study and improvement of water depuration systems.

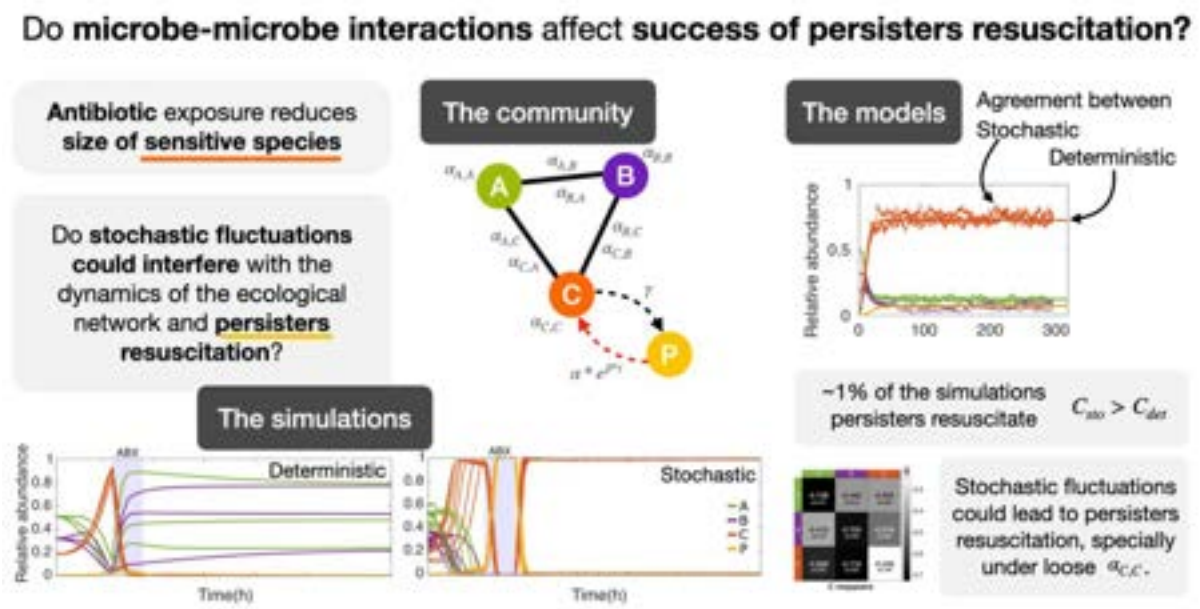


Figure 1: Description of the workflow followed and the main results. Stochastic fluctuations in microbe-microbe interactions can influence the resuscitation of persisters following antibiotic exposure. The deterministic and stochastic models show an agreement in community dynamics, but stochastic simulations suggest rare events (~1%) where persisters successfully resuscitate.

Abstract ID: 1571 / Poster Number: 376.15P

An integrated and coordinated set of morpho-ultrastructural, physiological, and proteomic changes facilitated Anabaena sp. PCC 7120 respond to and recover from acute cadmium toxicity

Presenting author: Dr. Yogesh Mishra, Banaras Hindu University, Varanasi, India

Co-authors: *Akanksha Srivastava, Subhankar Biswas, Sandhya Yadav, Vaibhav Srivastava*

The constant increase of cadmium (Cd) in the environment has made it necessary to shift research from low to high Cd concentrations since it poses a serious risk to all organisms. Cyanobacteria, the earliest photoautotrophs, exhibit excellent adaptation capacities towards most abiotic stresses, including Cd. A model filamentous cyanobacterium, *Anabaena* sp. PCC 7120, has been widely used to study cyanobacterial adaptability to various abiotic stresses. However, how it adjusts to high Cd toxicity is not well understood. Thus, we exposed the *Anabaena* 7120 to acute Cd stress (100 µM Cd for 48 hrs) and then transferred into the fresh medium for post-stress recovery. We then assessed the dynamics of morpho-ultrastructure, physiology, cytosolic proteome, thylakoid membrane proteome, and some Cd-detoxifying genes expression after acute Cd stress, as well as during early, mid, and late phases of post-stress recovery. Results showed that acute Cd stress enhanced the ROS generation, altered the morphology and ultrastructure, and reduced the photosynthetic efficiency. *Anabaena* 7120 respond to acute Cd stress and recover during post-stress recovery by (i) increasing CO_2 access, Calvin-Benson cycle, glycolysis and pentose phosphate pathway (PPP), (ii) inducing cellular defense and repair system, (iii) enhancing biosynthesis of specific amino acids, (iv) regulating protein quality control machinery, (v) increasing NDH-1L dependent cyclic electron flow (CEF), (vi) increasing Cd-chelating ability and (vii) activating Cd-efflux transporters. These modulations occurred in a highly integrated and coordinated manner that allowed *Anabaena* 7120 to export Cd out of the cell and repair acute Cd-induced cellular damage.

Abstract ID: 2921

An underground microbial hotspot for carbon cycling at the interface of Earth's atmosphere and terrestrial subsurface

Presenting author: Dr. André Soares, University of Duisburg-Essen, Essen, Germany

Co-authors: *Sarah Esser, Noel Kleber, Indra Banas, Komal Kaur, Joern Starke, Julia Banholzer, Rainer Straub, Herbert Jantschke, Louisa Rothe, Alexander Probst*

Session: Microbial Roles in Aquatic, Soil, Geological, and Extreme Environments

Even in modern times, karstic caves remain mysterious systems whose microbial ecology is still understudied mostly given the physical and logistic constraints that underlie its access. Yet, they represent unique underground environments under constant stygian darkness and devoid of nutrients, while in constant interaction with Earth's atmosphere. Prokaryotic presence in karstic caves however, is globally prevalent and highly diverse, impacting key elemental cycles namely via active carbon dioxide fixation, methane oxidation and nitrogen fixation. Moonmilk is a secondary speleothem (cave formation) made up of a tight mesh of calcium carbonate spicules in an aqueous medium, giving it a soft, 'cream-cheese'-like texture. In an otherwise barren underground environment, moonmilk provides an oasis for microbial life to thrive in. Despite this, the microbial ecology of moonmilk is as-yet virtually unexplored, as are the microbial metabolisms underlying its biogeochemical potential, namely carbon dioxide and methane cycling. Here, we leverage short-read metagenomics and correlative light and electron microscopy (CLEM) data generated from three caves in the German Swabian Alps to uncover the diversity, structure and encoded function of moonmilk microbial communities. Manual curation of hundreds of metagenome-assembled genomes (MAGs) resulted in the first direct functional survey of this underexplored underground environment. Carbon fixation, sulfur and nitrogen cycling pathways spanned multiple bacterial and archaeal phyla, illuminating the key biogeochemical roles of moonmilk in cave systems. We were further able to infer on lifestyles and phenotypes of prokaryotes inhabiting this environment via CLEM, paving the way to high-resolution, taxonomy-aware microscopic detection of bacterial and archaeal symbioses and syntrophies relevant to the cycling of CO_2. A wide diversity of carbonate anhydrases (CA) was found across bacterial and archaeal MAGs, reflective of the importance of moonmilk and cave microbiomes in carbon cycling in Earth's karstic caves. Despite their remoteness and challenging conditions presented, microbial life in karstic caves plays meaningful roles in global biogeochemical cycling.

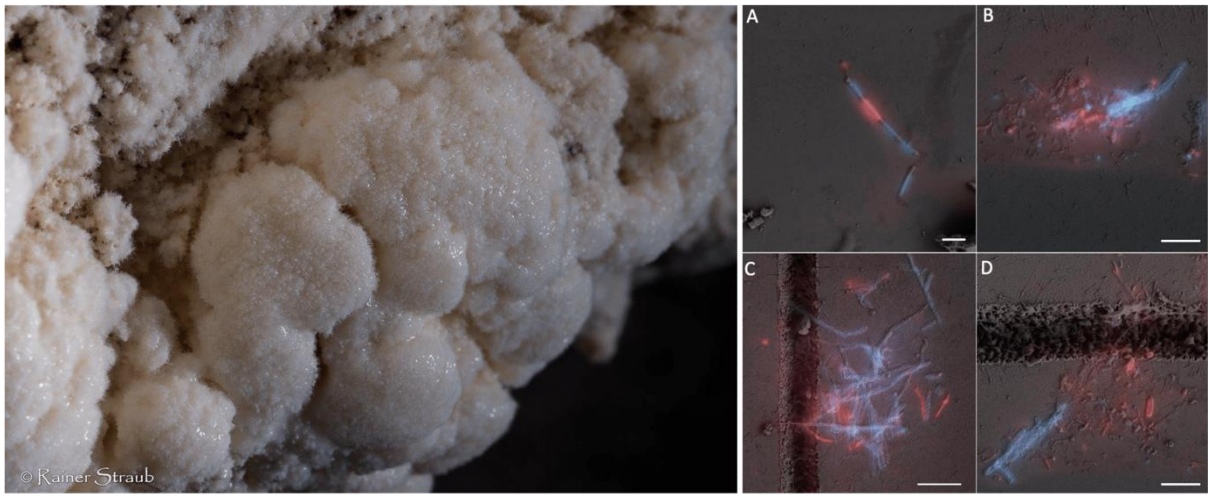


Figure 1: A macroscopic view of moonmilk (left-hand side) in a cave (Credit: Rainer Straub), and CLEM visualization of filamentous bacteria and clusters of bacterial cells inhabiting moonmilk (right-hand side). Blue fluorescence signal corresponds to DAPI, while red signal corresponds to the labelling of bacterial 16S rRNA. Scale bars A = 3 µm, B-D = 10 µm (Credit: Noel Kleber).

Abstract ID: 3010 / Poster Number: 375.15F

Analysis of a tellurite-resistant mutant of Rhodococcus to investigate mechanisms of stress response and resistance to toxic metal(loid)s

Presenting author: Prof. Martina Cappelletti, University of Bologna, Bologna, Italy

Co-authors: *Stefania Di Silvestro, Andrea Firrincieli, Alessandro Presentato, Raymond J. Turner*

Introduction: Bacteria of the *Rhodococcus* genus can withstand high level of toxic metal(loid)s, such as tellurite (TeO_3^{2-}). Their interaction with metal(loid)s has applications in environmental biotechnology, including bioremediation, nanostructure production for biomedical sensors, industrial uses, and rare metal recovery. Research has shown that *Rhodococcus aetherivorans* BCP1 can transform the oxyanion tellurite into its elemental form (Te^0), producing nanostructures that are less toxic and recoverable from the environment. However, the molecular basis for *Rhodococcus* resistance and tolerance to tellurite remains unclear, as does the role of carotenoids in non-photosynthetic bacteria.

Objective: This study investigates the interaction between *Rhodococcus* cells and tellurite to explore the stress response and transformation mechanisms contributing to metal resistance.

Method: A comparative analysis between BCP1 and its tellurite hyper-resistant mutant (BCP1-Wh) was conducted by examining planktonic/biofilm growth, tellurite uptake, and oxidative stress response. Whole-genome sequencing and RNA-seq are also under investigation for full description of the mutant response.

Findings: BCP1-Wh showed high resistance to tellurite under both planktonic and biofilm growth conditions. No significant differences were observed between BCP1 WT and the mutant in terms of tellurite uptake and ROS production. However, the mutant showed increased resistance when exposed to oxidative stressors like hydrogen peroxide. Whole-genome sequencing indicated that the mutant BCP1-Wh carries a mutation at the level of a gene involved in carotenoid biosynthesis.

Conclusion: This work provided new understanding on the association between carotenoid biosynthesis and metal stress response and resistance in bacteria. In addition to unveil novel mechanisms of tellurite resistance, these findings have possible applications in bioremediation and metal recovery.

Abstract ID: 3620 / Poster Number: 413.15P

Analysis of Microbial Communities in the Koněprusy Limestone Caves

Presenting author: Mrs. Kristýna Kliková, University of Chemistry and Technology, Prague, Czech Republic

Co-authors: *Henrietta Ottová, Tereza Branyšová, Petr Holeček, Dana Koňáková, Václav Nežerka, Kateřina Demnerová, Hana Stiborová*

Microbially Induced Calcite Precipitation (MICP) is a biomineralization process in which microorganisms produce calcium carbonate $CaCO_3$ crystals through their enzymatic activity. These crystals are used to recycle waste concrete fines (WCF) that results in reduction CO_2 emissions in the construction industry. While bacterial strains such as *Sporosarcina pasteurii* and *Bacillus cohnii* are commonly used for MICP, their cultivation is costly and requires specific conditions. Oligotrophic microorganisms from limestone caves, adapted to nutrient-poor environments, offer a sustainable alternative.

To explore this potential, microbial communities in the Koněprusy Limestone Caves (Czech Republic) were analysed. Samples included karst formations, karst-pool water and karst fragment samples. DNA (all microorganisms) and RNA (metabolically active microorganisms) were isolated from karst formations and karst-pools using polyurethane sponges. The amplicons for Illumina MiSeq sequencing were prepared using two-step PCR with specific primers for 16S rRNA gene. The obtained sequences were assigned to microbial species and data were statistically evaluated. RNA and DNA results were compared to assess microbial diversity.

Karst fragment samples were analyzed using XRD and EDS. For a comprehensive analysis of microbial diversity in caves, microbial influence on karst structure ($CaCO_3$ crystal types and shapes) and the elemental composition of karst formations was evaluated as a function of cave location and sample type (swabs, water). These findings will be used for the targeted isolation of cave that could replace the collection strains and improve the mechanical properties of WCF composites.

Abstract ID: 4084 / Poster Number: 034.15P

Analysis of the biodiversity of the microbial variability in gleba of Tuber aestivum Vittad

Presenting author: Dr. Niccolo' Militello, Università Cattolica del Sacro Cuore, Piacenza, Italy

Co-authors: *Maria Elena Antinori, Filippo Vaccari, Giovanna Soffritti, Matteo Busconi, Edoardo Puglisi*

Truffles (*Tuber* spp.) are the fruiting body of hypogeous fungi belonging to the ascomycete division that grows by establishing symbiotic associations with the roots of several plants. Among the different truffle species, some are prized for their unique aroma and flavour, however over the last few decades, there has been a continuous trend of decreasing productivity of natural truffle grounds. Several reasons underlie this reduction, often the consequence of anthropic activities, including climate change.

The role of bacteria within truffles is a complex area of study that has received an increased level of interest. Truffles form symbiotic relationships with plant roots, facilitating nutrient exchange and enhancing plant health. Moreover, research studies support an important role of the microbial community in the production of some of the aromatic compounds of truffles facilitating the identification of the fruiting body by mycophagous animals and thus favouring the spread of the species.

One hundred DNA samples were extracted from the gleba of summer truffles (*Tuber aestivum* Vittad.) coming from ten Italian regions (Apulia, Basilicata, Calabria, Campania, Emilia Romagna, Lazio, Marche, Piedmont, Tuscany, and Umbria). Shotgun sequencing is being employed in parallel with 16S rRNA gene amplicon sequencing to analyse the biodiversity of bacterial communities colonizing the inner tissue of the samples. We expect to analyse and compare a heterogeneous population of bacteria with the aim to highlight the implications of our findings for truffle spreading and organoleptic quality.

Data collection is ongoing, comprehensive data and detailed results will be presented in the poster.

Abstract ID: 812 / Poster Number: 021.17F

Analysis of the Growth, Biofilm Formation and Enterotoxin Production of Staphylococcus aureus Under Different Conditions

Presenting author: Prof. Dong-Liang Hu, Kitasato University School of Veterinary Medicine, Towada, Japan

Co-authors: *Zuo Hu, Hisaya Ono*

Staphylococcal food poisoning (SFP) is a worldwide issue globally and ranks as one of the top bacterial foodborne illnesses in developing countries. In Japan, while its occurrence has decreased, cases are still reported year-round, particularly from May to October. Notable incidents, such as the 14,000-case outbreak in Osaka caused by contaminated skim milk and the September 2023 outbreak involving boxed meals affecting 554 individuals across 29 prefectures, underscore its significant societal impact.

This study aimed to analyze the growth, biofilm formation, and enterotoxin (SE) production of *Staphylococcus aureus* under varying environmental conditions. *S. aureus* FRI-S6 was cultured in BHI-YE medium supplemented with NaCl (2.5%, 5%, 10%) or glucose (1%, 4%, 16%) at 37℃ or 25℃. Bacterial growth (OD550), biofilm formation, and SEA production were assessed.

At 37℃, 5% and 10% NaCl significantly inhibited bacterial growth, while 4% and 16% glucose suppressed growth after 48–72 hours. NaCl inhibited biofilm formation dose-dependently, but glucose had no effect. SEA production was significantly suppressed by 2.5% NaCl, whereas glucose showed no impact. At 25℃, similar trends were observed; however, 1% glucose increased SEA production.

These findings indicate that while bacterial counts may remain similar under different conditions, SE production can vary significantly depending on the environment. Understanding these variations is crucial for improving food safety strategies. Further research is ongoing to elucidate the regulatory mechanisms of SE production.

Abstract ID: 3541

Analysis of the Intestinal Resistome in Amerindian and European Populations: A Comparative Study Using Targeted Metagenomics

Presenting author: Dr. Miguel D. Fernandez-de-Bobadilla, Institute of Biology and Functional Genomics, Salamanca, Spain; Ramon y cajal health research institute, Madrid, Spain

Introduction: The resistome encompasses the entire pool of antimicrobial resistance (AMR) genes, including those for antibiotics, metals, and biocides, offering insights into microbial resilience and the transmission dynamics of resistance. While most studies focus on hospital populations in developed nations, they often overlook minor bacterial populations, limiting the detection of rare resistance genes. This study compares the intestinal resistome of Amerindian individuals from French Guiana (minimal antibiotic exposure and high mercury exposure) with European individuals, using the high-sensitivity, targeted metagenomic platform ResCap (Lanza et al., 2018).

Materials and Methods: We analyzed the intestinal resistomes of 96 Amerindian (Group A) and 15 European (Group B) individuals using ResCap, a technology that targets 8667 canonical resistance genes, including those for antibiotics, metals, and biocides, as well as plasmid markers (relaxases). Data analysis was performed with a custom pipeline utilizing Bowtie2 and KMA, and statistical validation was carried out in R.

Results: The resistome of the Amerindian group (A) exhibited greater diversity and abundance of resistance genes across all categories compared to the European group (B). Notably, the Amerindian resistome overrepresented relaxases from families MOBQ, MOB, and MOBV, while the European resistome showed a dominance of MOBP1 plasmids. Resistance to beta-lactams, macrolides, chloramphenicol, tetracycline, and trimethoprim was more prominent in Group A, whereas glycopeptide resistance (vanBXYZ) was mainly found in Group B. The Amerindian group also displayed a significant presence of metal resistance genes, particularly those conferring resistance to mercury and copper.

Conclusions: This study represents the first comprehensive application of ResCap to investigate the resistome of human populations with varying exposure to antimicrobials, highlighting ecological and geographical differences in resistance gene distribution. Our results underscore the role of environmental and anthropogenic pressures, such as mercury contamination in French Guiana, in shaping microbial resistomes. This work

establishes a baseline for understanding resistome dynamics and provides a novel approach to exploring AMR in non-industrialized communities. Furthermore, the data offers valuable insights into the genetic redundancy and adaptability of gut microbiomes, which may influence the emergence and spread of resistance upon exposure to medical interventions.

Keywords: Resistome, metagenomics, antimicrobial resistance, Amerindian, European, targeted sequencing, ecological pressures, mercury, genetics.

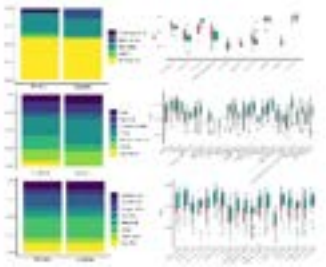


Figure 1: **Gene diversity and gene depth**. Panels A), B), C), and D) display the percentage of ARGs, BRGs, MRGs, and REL genes in the population, shown on a logarithmic scale. Panels F), G), H), and I) show the sequencing depth (or read depth) for each gene category, also represented on a logarithmic scale.

Abstract ID: 2784 / Poster Number: 254.15P

Analysis of the role of two orphan methyltransferase genes in Rhodococcus opacus PD630

Presenting author: Mr. Umberto Genchi, University of Bologna, Bologna, Italy

Co-authors: *Andrea Firrinceli, Alduina Rosa, Davide Roncarati, Martina Cappelletti*

DNA methylation in bacteria is a process carried out by DNA methyltransferases (MTases) that catalyze the transfer of methyl groups to adenine or cytosine bases in DNA. These enzymes are divided into two categories: DNA methyltransferases associated with restriction enzymes, known as “Restriction-Modification systems,” and those not associated with restriction enzymes, referred to as “orphan” MTases.
In this work we describe two orphan methyltransferases found in *Rhodococcus opacus* PD630 and analyze their role in the growth of this bacterium that is known to accumulate lipids in the form of triacylglycerols (TAGs) using different organic compounds.

Two orthologous genes of orphan methyltransferases were identified in the PD630 genome. These are the orthologs of *dcm* gene in *E. coli* and *SCO1731* gene in *Streptomyces coelicolor*. The deletion mutants of these MTases were constructed using a two-step recombination method. Phenotypic and methylomic profiles of the WT strain and mutants were analysed by using phenotypic microarray assays and Oxford Nanopore sequencing, respectively.

Methylomic analyses allowed the identification of the target consensus sequences of the two MTases. In some cases, the methylation sites were located in promoters of genes involved in biodegradation processes and stress response. First results from phenotypic tests indicated some difference in the growth profiles of the mutants in the presence of oxidative stressors and in late stationary phase of growth.

This work provides first insights into the role of two orphan methyltransferases in DNA methylation and growth in *Rhodococcus opacus* PD630.

Abstract ID: 11 / Poster Number: 052.17P

Analyzing the effects of benzodiazepines exposure on Pseudomonas aeruginosa infection-related parameters: biofilm formation on endotracheal tubes and rat model of ventilator-associated pneumonia (VAP)

Presenting author: Ms. Maria Escobar Salom, Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain; Microbiology Department, University Hospital Son Espases (HUSE), Palma, Spain; Centro de Investigación Biomédica en Red, Área Enfermedades Infecciosas, Madrid, Spain

Co-authors: *Carlos Juan Nicolau, Isabel M Barceló, Elena Jordana-Lluch, Anna Tomàs, María Tortosa, Antonio Oliver*

Introduction: Antibiotic-resistant Pseudomonas aeruginosa (PA) is among the top VAP-causing species, entailing high morbidity-mortality and economic costs. Some publications suggest that patient sedation with benzodiazepines is a risk factor for VAP development, although the underlying mechanisms are unclear.

Objective: To study the impact of benzodiazepines exposure on PA as potential enhancer for its VAP-associated success through in vitro and in vivo models.

Methods: Formation of biofilm on endotracheal tubes after 5 days of incubation comparing medium with or w/o benzodiazepines (midazolam or diazepam, 10 mg/l) was quantified by crystal-violet straining and confocal microscopy (Syto_9 dye). A VAP model was developed with ≈10–12 weeks Wistar rats (4 males +4 females/group), treated with ketamine/dexmedetomidine 75/0.25 mg/kg or ketamine/dexmedetomidine/midazolam 75/0.125/5 mg/kg, and ventilated for 1h using a SAR-1000 device. Besides controls, infection groups were intra-tracheally inoculated with ≈5E7 PAO1 strain cells before ventilation. 24h later, animals were sacrificed and their lungs homogenized for bacterial counts and IL-8 ELISA as inflammatory marker.

Findings: Biofilm-related parameters values after benzodiazepine exposure were incremented ≈50% compared to controls (T tests P values<0.05). Mean PA counts in midazolam- vs non-midazolam-treated rats were 1.5E9 and 6.5E8 bacteria/lung and IL-8 values were 466.3 vs 412.2pg/mL (differences not statistically significant).

Conclusion: Benzodiazepines boosted PA capacity to form biofilms on endotracheal tubes, which could act as reservoir to promote VAP. Current animal models use short

intubation-ventilation periods, which explains why our infection-related parameters were similar whichever was the treatment. Extended periods of ventilation/benzodiazepines exposure are needed to confirm our biofilm-related results.

Abstract ID: 922

Anoxic Energetic Convergence Nodes in High-Altitude Hydrothermal Systems

Presenting author: Mr. Pablo Paquis, Universidad Católica del Norte, Antofagasta, Chile; Università degli Studi di Palermo, Palermo, Italy

Co-authors: *Coral Pardo-Esté, Joseline Tapia, July Z. Flores, Vilma Pérez, Verónica Molina, Marcela Cornejo-D'Ottone, Pablo A. Pérez, Wade Jeffrey, Sergio Calabrese, Paola Quatrini, Franco Tassi, Martha B. Hengst*

The hydrothermal systems of Chile's Altiplano plateau, situated at 4,000 metres elevation, operate under reduced atmospheric pressure (~0.6 atm), providing exceptional natural laboratories for examining extreme biogeochemical processes. These conditions mirror early Earth settings, yet the mechanisms governing microbial metabolic adaptations in these systems remain poorly understood, particularly regarding their responses to combined thermal and pressure constraints.

Our research characterised precise thermal zones where multiple metabolic pathways achieve equivalent thermodynamic yields, termed Energetic Convergence Nodes (ECN, Figure 1). We examined six hydrothermal pools (53-75°C) through integrated analytical approaches (Figure 1a), combining water and sediment analyses with thermodynamic modelling of anoxic carbon and sulfur reaction pathways (Figure 1b). Organic matter underwent isotopic analysis through continuous-flow mass spectrometry, with findings validated through bootstrap analysis (Figure 1c). The methodology incorporated intensive field monitoring of physicochemical parameters alongside laboratory analyses of major ions, trace elements, and dissolved gases.

Distinct primary convergence nodes emerged at 63-64°C, where carbon reduction and sulphate reduction pathways achieved equivalent thermodynamic yields (uncertainty <0.2%). These nodes (Figure 1d) correspond with systematic transitions in organic matter composition, marked by carbon isotope depletion (-17.2‰ to -27.7‰) and sulfur isotope enrichment (-19.4‰ to positive values). Carbon-to-sulfur ratios decreased markedly (10.0 to <1.0), whilst $\delta^{13}C$ and $\delta^{34}S$ values showed significant negative correlation ($\tau = -0.52$, $p < 0.01$). Our data revealed that dissolved CO_2 concentrations exceeded CH_4 by two orders of magnitude, indicating complex metabolic processes particularly in hydrogenotrophic methanogenesis and anoxic methanotrophy.

Our findings establish ECN as quantitative markers for metabolic transitions in extreme environments, demonstrating microbial communities' preference for physiological stability over energy yield maximisation. These insights advance our understanding of early Earth conditions whilst providing frameworks for identifying potential biosignatures in extraterrestrial hydrothermal systems. The relationship between metabolic flexibility and environmental constraints offers crucial perspectives for examining biological adaptation in extreme settings, with implications extending to the search for life beyond Earth.

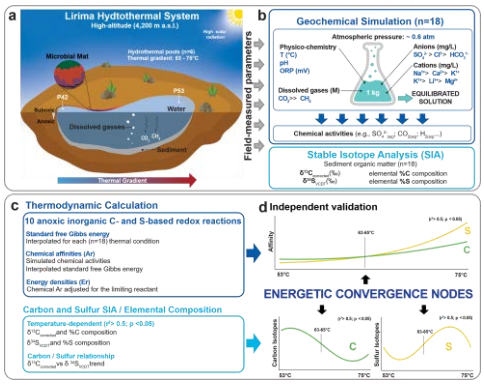


Figure 1: Hydrothermal system characterisation and energetic convergence analysis. a, High-altitude thermal pool structure showing redox zonation and geochemical gradients. b, Field parameters used in equilibrium modelling under reduced atmospheric pressure. c, Integration approach for thermodynamic and isotopic data analysis. d, Temperature-dependent convergence nodes (63-64°C) validated through carbon and sulphur isotopic transitions.

Abstract ID: 1149 / Poster Number: 337.15S

Antagonism as a foraging strategy drives nutrient transfer in microbial communities

Presenting author: Dr. Astrid Stubbusch, Monash University, Clayton, Australia

Co-authors: *François Peaudecerf, Kang Soo Lee, Lucas Paoli, Julia Schwartzman, Roman Stocker, Marek Basler, Olga Schubert, Martin Ackermann, Cara Magnabosco, Glen D'Souza*

Introduction: Microbial activity is a key driver of biogeochemical cycles. In natural ecosystems, the growth of microorganisms is often limited by nutrient availability.

Objective: We discovered that bacteria can overcome this limitation by acquiring nutrients through lysing neighboring cells via contact-dependent antagonism (Fig. 1A), and magnified this evolved micro-scale strategy to assess its global impact on nutrient cycling.

Method and findings

Using single-cell live imaging (Fig. 1B) and isotopic markers (Fig. 1C), we found that during starvation the type VI secretion system, which transfers toxins into neighboring cells, increases growth by enabling the uptake of nutrients from lysing cells. In dense cell groups, the gradual lysis of just a few target cells allows for the growth of a new antagonist cell. We then investigated the role of this antagonistic foraging in the environment by assessing genomic adaptations in antagonists and their prevalence in diverse ecosystems. We found that antagonists encode a reduced repertoire of metabolic genes and that they are prevalent in natural environments like global oceans and soils, in built environments like waste water treatment plants, and in host-associated habitats like the rhizosphere.

Conclusion: Our findings suggest that bacterial antagonism has the potential to substantially contribute to nutrient transfer within microbial communities across various ecosystems, impacting the cycling of nutrients and carbon globally.

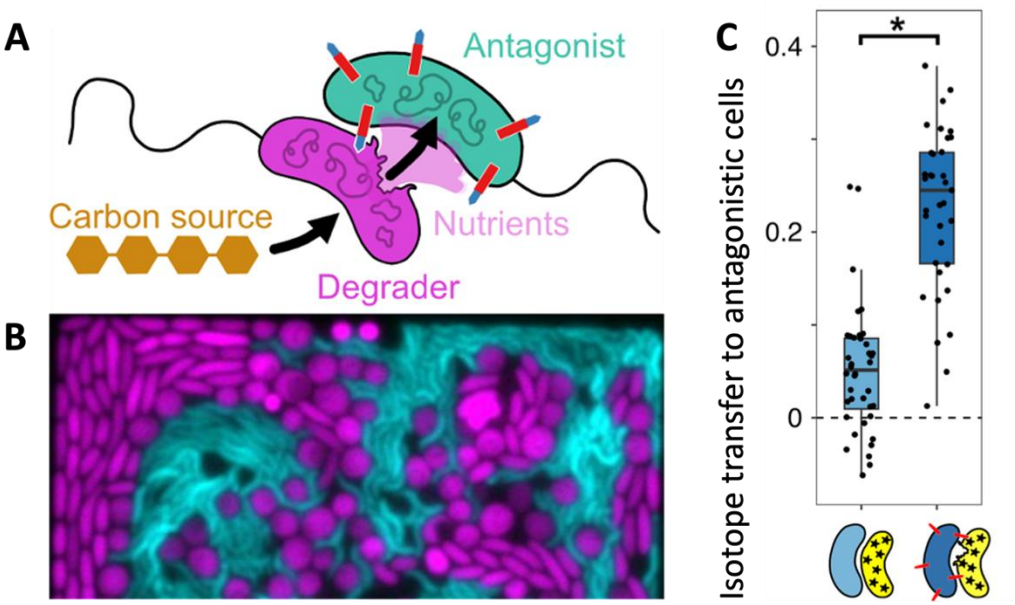


Figure 1: Schematic (A), fluorescent microscopy image (B), and stable isotopic tracing (C) of antagonistic foraging. (A and B) Antagonistic cells (cyan) lysed target cells (magenta), enabling them to access nutrients for their growth. (C) The transfer of isotopically labelled molecules from target cells (yellow) to antagonistic cells (dark blue) was significantly larger than to mutants without functional antagonistic system (light blue).

Abstract ID: 267 / Poster Number: 180.15P

Antagonism or Synergy: Unraveling the intertwined relationship between Bacillus and Trichoderma against plant pathogens

Presenting author: Jiyu Xie, PhD, Nanjing Agricultural University, Nanjing, China; Leiden University, Leiden, Netherlands

Co-authors: *Xinli Sun, Qirong Shen, Ákos T. Kovács, Zhihui Xu*

Agricultural practices using the combination of Bacillus and Trichoderma demonstrate their growth promotion and biocontrol effects. We investigated the interactions among Bacillus velezensis SQR9, Trichoderma guizhouense NJAU 4742, and the plant pathogen Fusarium oxysporum f. sp. cubense (FOC) in a tripartite system. Despite strong mutual antagonism between B. velezensis and T. guizhouense on solid medium, they synergistically promote plant growth in soil. To explore the molecular and chemical details of this BFI, we employed transcriptomics, HPLC-MS, scanning electron microscopy, and gene knockout approaches. Transcriptomic analysis revealed σB as a key regulator in B. velezensis rapid response to fungal stress. Further investigation showed that surfactin, the secondary metabolites of B. velezensis induced T22azaphilone in T. guizhouense, which enhanced fungal stress resistance through activation of reactive oxygen species (ROS) immunity. Moreover, we demonstrated that B. velezensis colonized fungal hyphae through biofilm formation. In this system, fusaric acid secreted by FOC delays B. velezensis growth, providing time and space for T. guizhouense growth, which subsequently degraded fusaric acid. This dynamic equilibrium ultimately led to effective pathogen suppression and degradation of its virulence factors, while both plant growth-promoting rhizobacteria synergistically enhanced plant growth. Our study reveals novel mechanisms of soil microbial community assembly and provides theoretical basis for developing microbiome-based biocontrol strategies.

Abstract ID: 2119 / Poster Number: 049.15P

Antagonistic Interactions: Impact of Bacillus subtilis Volatile Organic Compounds on Escherichia coli Viability and Virulence

Presenting author: Prof. King Lun Yeung, The Hong Kong University of Science and Technology, Hong Kong, Hong Kong

Co-authors: *Sittie Salik*

Microorganisms produce volatile organic compounds (VOCs) as metabolic byproducts, which can influence interactions with other microorganisms in their environment. These microbial VOCs (mVOCs) exhibit varying effects, including antagonistic properties against pathogenic bacteria. This study focuses on the mVOCs emitted by *Bacillus subtilis* (B. subtilis), a gram-positive bacterium known for its antimicrobial effects, particularly against the pathogenic gram-negative bacterium *Escherichia coli* (E. coli).

In this research, *E. coli* was exposed to the mVOCs released by *B. subtilis* under aerobic conditions for a specified duration. The viability of both bacterial strains was assessed using the plate count method, while proteomic analysis was conducted to investigate the influence of *B. subtilis* and its emitted VOCs on *E. coli*. Additionally, VOCs from both *B. subtilis* and *E. coli* were collected and identified using gas chromatography-mass spectrometry with thermal desorption (GC-MS/TDU).

The results demonstrated that *B. subtilis* exerts significant antagonistic effects on *E. coli*, resulting in the downregulation of several stress-related and virulence proteins in *E. coli*. These findings highlight the potential of *B. subtilis* as an effective antibacterial agent against pathogenic *E. coli*, suggesting its application in biocontrol strategies to mitigate bacterial infections.

Abstract ID: 777 / Poster Number: 334.16P

Antibacterial activity of secondary metabolites from the marine-derived Zooshikella sp. SI_B3-9

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Poultry flocks are susceptible to various bacterial diseases, including salmonellosis, colibacillosis, and clostridial infection. These diseases can lead to significant economic loss for poultry farm. For this reason, poultry farms have often resorted to the indiscriminate use of antibiotics as a means of mitigating productivity losses caused by diseases over the past decades. The emergence and spread of antibiotic-resistant bacteria have heightened the need for the development of new antibacterial agents. Thus, we conducted a screening to isolate marine bacteria with antibacterial activity from coastal sediments in Buan, South Korea. The isolated bacteria exhibited antibacterial activity against *C. perfringens*, *C. difficile*, *E. faecium*, and *S. aureus*. In particular, it showed potent antibacterial activity against *C. perfringens* of types A, D, and E. The strain was confirmed to belong to the genus *Zooshikella* based on the 16S rRNA gene sequences, which showed 99% similarity. It was designated as SI_B3-9. Whole-genome sequencing (WGS) analysis revealed that the SI_B3-9 strain contained nine plasmids within its 6.1M genome. The isolate was cultured in artificial seawater containing five mineral salts, 2.3% NaCl, and 0.4% yeast extract. Interestingly, this bacterium cannot grow in media without NaCl. This strain was found to produce red pigments as secondary metabolites.

These findings suggest that marine bacteria, which contain various functional materials from the ocean, will be a valuable source of antibacterial substances effective in controlling bacterial diseases in poultry.

Abstract ID: 1225 / Poster Number: 349.16P

Antibacterial and anti-inflammatory activity of Lactiplantibacillus plantarum BIA isolated from orange peel

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Co-authors: *Elisa Corazza, Asia Pizzi, Carola Parolin, Barbara Giordani, Angela Abruzzo, Federica Bigucci, Teresa Cerchiara, Barbara Luppi*

Introduction: The resident microbiota of the upper respiratory tract can prevent pathogens colonization on the mucosal surface, exerting a barrier effect and assuring protection against infection. Lactic acid bacteria (LAB) and their metabolites are extensively used as probiotics in both food and pharmaceutical fields for their antimicrobial and anti-inflammatory activities.

Objective: To explore the antibacterial and anti-inflammatory properties of the new strain Lactiplantibacillus plantarum BIA, isolated from organic orange peel, and to formulate a nasal spray, containing the live probiotic strain.

Method: L. plantarum BIA was isolated from orange peel and taxonomically characterized by 16S rRNA gene sequencing. The antibacterial activity of live cells and cell free supernatants was evaluated toward the most representative pathogens of the human respiratory tract. The anti-inflammatory potential was evaluated by Griess assay. BIA genome sequence was analyzed to exclude virulence factors. BIA was formulated in a freeze-dried matrix, containing prebiotics and cryoprotectants, which was in turn reconstituted with a polymer solution. The biological activity of BIA in the nasal spray was confirmed together with the microbiological and technological stability of the formulation.

Findings: L. plantarum BIA effectively inhibited the growth of pathogens and showed anti-inflammatory activity and a safe profile. BIA was lyophilized in a medium containing fructooligosaccharides, ascorbic acid and lactose and reconstituted with HPMC solution. This composition ensured the probiotic's viability for up to six months in its dried form and one week after reconstitution.

Conclusion: L. plantarum BIA and its formulations represent promising biotherapetic solutions for nasal infections and inflammation.

Abstract ID: 625 / Poster Number: 147.16P

Antibacterial effect of dihydropyrimidinone and dihydropyrimidinthione derivatives synthesized via Biginelli condensation reactions

Presenting author: Assoc. Prof. Magdalena Greczek-Stachura, University of the National Education Commission, Kraków, Poland

Co-authors: *Agnieszka Kania, Waldemar Tejchman, Anna Barbasz*

On the basis of literature Biginelli compounds have attracted considerable attention in organic and medicinal chemistry because of their pharmacological and therapeutic properties. Among others they are considered as potential calcium channel blockers, antiviral, antibacterial and anti-inflammatory compounds.

The aim of our study was to confirm the potential antimicrobial activity of selected Biginelli compounds.

Therefore, we conducted one-step synthesis of selected dihydropyrimidinone and dihydropyrimidinthione derivatives by three-component Biginelli condensation reactions starting from benzaldehyde derivatives, α- and β-diketones, and urea or thiourea, respectively. The chemical structures of the obtained heterocyclic derivatives were confirmed by IR, NMR and ES MS. The compounds were characterized by UV-VIS methods. Antibacterial activity was tested on standard bacterial strains: Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumoniae, using microdilution method or disk-diffusion and well-diffusion methods.

Cytotoxicity of the synthesized derivatives was studied on two human cell lines: U937 (promonocytes) and HL-60 (promyelocytes). Mitochondrial dehydrogenase activity (XTT) and lactate dehydrogenase activity (LDH) were determined.

All synthesized compounds showed antibacterial activity. Depending on the bacterial strain, the MIC value ranged from 250-500 μg/ml. The most sensitive to all tested derivatives was S. aureus.

Dose dependent cytotoxicity was observed. In the XTT assay, all compounds reduced cell viability at concentrations above 125 μg/ml. Based on the results, the median lethal dose LD50 was determined. The most toxic was the dihydropyrimidinthione derivative.

Comparing the activity of the tested compounds, it can be concluded that dihydropyrimidinthione derivative is the most effective in inhibiting bacterial growth.

Abstract ID: 3004 / Poster Number: 211.16P

Antibacterial Potential of a Novel Bacteriophage Infecting Aeromonas hydrophila

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Introduction: *Aeromonas hydrophila* had emerged as a significant animal and human pathogen. As antibiotic resistance continues to rise, bacteriophages remain one of the most prominent alternatives in bacterial growth control.

Objectives: In this study, we aimed to investigate the antibacterial potential of newly isolated lytic phage active against *A. hydrophila,* both alone and in combination with antibiotics.

Methods: Lytic efficacy of previously isolated and multiplied phage, designated as AhLy4, was assessed by determining the minimal inhibitory multiplicity of infection (MOI). The impact of the phage on bacterial ability to form biofilm was further examined by the colorimetric microtiter plate method. Finally, the effect of different concentrations of phage in combination with ciprofloxacin or gentamicin was evaluated using the checkerboard method and expressed through fractional inhibitory concentration indexes (FICIs).

Findings: AhLy4 phage inhibited *A. hydrophila* growth by 65% at a MOI=0.01. With MOI increasing up to 10, bacterial growth was inhibited by 95%. A MOI of 0.1 inhibited *A. hydrophila* biofilm formation by more than 90%. Checkerboard assay results implied that the combination of the tested phage with antibiotics achieved a synergistic effect in inhibiting bacterial growth, as the observed FICIs for the phage-antibiotic combinations were less than 0.5.

Conclusion: Obtained results indicate that isolated phage significantly affects bacterial growth, both alone and in combination with certain antibiotics, making this phage a promising candidate as a potential antibacterial agent. However, further phage characterisation and *in vivo* studies are essential to evaluate this phage as a possible therapeutic option.

Abstract ID: 2456 / Poster Number: 038.17P

Antibacterial, antibiofilm, and antivirulence mechanism of biosurfactants produced by Bacillus rugosus HH2 against Staphylococcus aureus

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Methicillin-resistant Staphylococcus aureus (MRSA) infections highlight the critical need for new antimicrobial agents to combat antibiotic resistance. Natural compounds with diverse chemical structures offer promising alternatives to traditional antibiotics. Among these, biosurfactants, surface-active molecules produced by microorganisms, have garnered significant attention for their antimicrobial properties. This study explores a novel strategy for employing biosurfactants produced by Bacillus rugosus HH2 as anti-MRSA agents. The biosurfactant demonstrated effective inhibition of MRSA growth, with a minimum inhibitory concentration range of 128–256 μg/mL. It also exhibited strong antibiofilm activity, successfully inhibiting biofilm formation and disrupting mature biofilms. Transcriptomic analysis revealed that biosurfactant treatment downregulated genes involved in cell attachment and quorum sensing in MRSA. Furthermore, molecular docking analysis indicated robust interactions between biosurfactants and key MRSA proteins (PBP2a and DNA gyrase), highlighting their potential to combat antibiotic resistance. The biocompatibility of the biosurfactant was validated through cytotoxicity and phytotoxicity assessments. In conclusion, this study underscores the potential of biosurfactants produced by B. rugosus HH2 as promising new antimicrobial candidates against MRSA.

Abstract ID: 720 / Poster Number: 074.17P

Antibiofilm and synergistic effect of Cinnamomum verum and Punica granatum extracts against multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa

Presenting author: Assoc. Prof. Luciane Oliveira, São Paulo State University, São Paulo, Brazil

Co-authors: *Thais Pereira, Patrícia Lima, Raquel Menezes, Lara Carvalho, Cristina Marcucci, Vanessa Meccatti-Domiciano, Amjad Abu-hasna, Cláudio Carvalho*

Introduction: Antibiotic resistance in pathogens such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* poses a global health threat, necessitating alternative therapeutic strategies.

Objective: To evaluate the antibacterial and antibiofilm efficacy of hydroethanolic extracts of *Cinnamomum verum* and *Punica granatum* against multidrug-resistant *A. baumannii* and *P. aeruginosa*.

Methods: Hydroethanolic extracts of *C. verum* and *P. granatum* were prepared and the bioactive compounds were determined via high-performance liquid chromatography (HPLC). The antimicrobial activity was analyzed using the broth microdilution test (CLSI M7-A9) to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against four clinical strains of multidrug-resistant *A. baumannii* and *P. aeruginosa*. The combination of the extracts was evaluated using the checkerboard technique and synergistic effects were analyzed on biofilms-forming. The biofilm viability was assessed using the MTT assay and data were statistically analyzed using ANOVA and Dunn's test ($p < 0.05$).

Findings: *C. verum* and *P. granatum* extract presented 0.935 and 0.853 mg/mL of flavonoids, respectively, and 2.069 and 1.406 mg/mL of phenols, respectively. The extracts showed potent bactericidal effects against all tested strains, with MBC as low as 3.3 mg/mL. Synergistic activity was observed particularly against clinical strains of *A. baumannii*, reducing the MBC of the *P. granatum* extract by 1220×. Both extracts demonstrated strong antibiofilm properties, significantly reduced *A. baumannii* and *P. aeruginosa* biofilms compared to the control group (untreated).

Conclusion: The *C. verum* and *P. granatum* extracts demonstrate antibacterial and antibiofilm activity against multidrug-resistant *A. baumannii* and *P. aeruginosa*, warranting further development for integrative treatments.

Abstract ID: 704 / Poster Number: 073.17P

Antibiofilm and synergistic effect of *Rosmarinus officinalis* and *Myrciaria cauliflora* against standard and clinical strains of *Enterococcus faecalis*

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Co-authors: *Mariane Fujimoto, Mariane Gimenez, Caroline Rocha, Raquel Menezes, Vanessa Meccatti-Domiciano, Maria Marcucci, Amjad Abu-Hasna, Luciane Oliveira*

Introduction: As microorganisms, like *Enterococcus faecalis*, increasingly resist chemical solutions and antibiotics is a resistant bacterium, medicinal plants with antibacterial properties have gained attention to finding an effective antimicrobial agent against this bacterium and others.

Objective: To evaluate the antibacterial and antibiofilm activity of the combination of *Rosmarinus officinalis* and *Myrciaria cauliflora* extracts against clinical strains of *E. faecalis*.

Method: Hydroethanolic extracts of *R. officinalis* and *M. cauliflora* were produced, and their antimicrobial activity was analyzed using the broth microdilution test (CLSI M7-A9) to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against one standard and three clinical strains of *E. faecalis*. The combination of the extracts was evaluated using the "checkerboard" technique, and synergistic and/or additive effects were analyzed on biofilms with treatment durations of 5 minutes and 24 hours. The biofilm viability was assessed using the MTT assay. Data were statistically analyzed using ANOVA and Dunn's test ($p < 0.05$).

Findings: The MBC of *R. officinalis* and *M. cauliflora* ranged from 0.5 to 1 mg/mL and 5 mg/mL, respectively. An additive effect was observed in the combination of the extracts, reducing the MBC of the *M. cauliflora* extract by 33×. Both isolated and combined extracts significantly reduced *E. faecalis* biofilms compared to the control group (untreated), and their effectiveness was statistically similar to the sodium hypochlorite group.

Conclusion: The *R. officinalis* and *M. cauliflora* extracts exhibited antibacterial and antibiofilm activity against *E. faecalis* and maybe used as alternative and/or adjunctive treatments for *E. faecalis* infections.

Abstract ID: 1216 / Poster Number: 338.15S

Anti-biofilm Potential of Crude Seaweed Extracts Against a Range of Bacterial Species Associated with Hospital Acquired Infections

Presenting author: Ms. Monica Murphy, Munster Technological University, Cork, Ireland

Co-authors: *Craig Murphy*

Introduction: Biofilms present a significant challenge in healthcare settings, as they facilitate bacterial persistence, antibiotic resistance and immune system evasion.

Objective: This study evaluated the antimicrobial and antibiofilm potential of compounds extracted from *Codium fragile* and *Fucus vesiculosus* seaweeds using methanol or water.

Method: Antimicrobial efficacy was determined using minimum inhibitory concentration (MIC) assays, while biofilm inhibition and eradication (MBIC and MBEC) were measured at 99, 90 and 50% inhibition using crystal violet assays. Total protein content was quantified via the BCA assay, while antioxidant potential and total phenolic content were determined using DPPH, FRAP, and Folin-Ciocalteu assays.

Findings: The extracts of *C. fragile* significantly inhibited growth of *S. aureus* achieving MIC_{90} and MIC_{50} against *E. faecalis, P. aeruginosa, A. baumannii, E. coli* and *K. aerogenes*. *F. vesiculosus* extracts were particularly effective against Gram-positive bacteria including MRSA. Methanol extracts of *C. fragile* exhibited broad-spectrum antibiofilm activity, while water extracts of both seaweeds inhibited Gram-positive bacterial biofilms. *F. vesiculosus* demonstrated greater potency, achieving $MBIC_{50}$ at 0.78% concentration compared to 6.25% for *C. fragile*. Methanol extracts of *F. vesiculosus* contained the highest protein (4.6 mg/mL) and phenolic content (72.7±0.19 gallic acid equivalent/g), correlating with robust antioxidant activity.

Conclusion: These findings suggest that bioactive compounds from *C. fragile* and *F. vesiculosus* may address the biofilm challenges in healthcare settings, such as chronic and persistent infections and relapse prevention. Further investigation is needed to optimise their efficacy against established biofilms.

Abstract ID: 1292 / Poster Number: 165.16P

Antibiofilm properties of polyphenols under static and dynamic conditions

Presenting author: Mrs. Rossella Mauro, Humanitas University, Pieve Emanuele, Italy

Co-authors: *Najla Hajji, Simone Domenico Guglielmetti, Mirko Marino, Roberto Rusconi*

Background and aims: Biofilms are aggregations of microorganisms that adhere to surfaces and are typically encased in a self-secreted extracellular polymeric matrix. Bacterial biofilms are estimated to be 1,000 times more resistant to antibiotics than their planktonic counterparts, posing a significant global health risk and economic burden. This study aims to explore the efficacy of natural polyphenol-rich extracts as antibiofilm agents, offering potential alternative strategies for the prevention or mitigation of biofilm-associated infections.

Methods: Clinically relevant bacterial strains, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, were cultured in respective growth media and incubated for 24 hours at 37 °C in 96-well plates with varying concentrations of polyphenols extracted from natural sources, to evaluate their effects on planktonic growth.

In addition, the surface-associated growth of *P. aeruginosa* under fluid stress was investigated in PDMS channels using flows rate from 1 to 5 µl/min at 37 °C. Surface colonization and biofilm formation were measured using phase-contrast and fluorescent microscopy (with the use of fluorescently labelled lectins) and quantified via image analysis.

Results: Natural polyphenol-rich extracts exhibited significant antibacterial properties, inhibiting the growth of certain bacterial strains under static conditions. In addition, these polyphenols demonstrated anti-adhesive properties and antibiofilm effects in a microfluidic system in the presence of fluid flow, as shown by *P. aeruginosa* after 20 hours at a concentration of 200 µg/ml, compared to the positive control.

Conclusions: These findings suggest that polyphenol-rich extracts have considerable potential as alternative strategies for the prevention and treatment of biofilm-associated infections.

Abstract ID: 2940 / Poster Number: 158.17P

Antibiotic and Biocide Co-Resistance in Gram-Negative Bacilli from a Wastewater Treatment Plant in Bucharest, Romania

Presenting author: Mr. Rareș Ionuț Dragomir, University of Bucharest, Bucharest, Romania

Co-authors: *Irina Gheorghe-Barbu, Marius Surleac, Liviu Rotaru, Leontina Bănică, Simona Paraschiv, Mariana Carmen Chifiriuc, Ilda Czobor Barbu*

Introduction: Wastewater treatment plants (WWTPs) are key reservoirs for antimicrobial resistance (AMR), facilitating the persistence and dissemination of resistance genes. The co-occurrence of antibiotic and biocide resistance mechanisms in Gram-negative bacilli is underexplored but holds significant environmental and public health implications.

Objective: This study investigated co-resistance profiles and the genetic basis of resistance in Gram-negative bacilli isolated from upstream and downstream samples of the Glina WWTP in Bucharest, Romania.

Method: Water samples were collected 200 meters upstream and downstream of the WWTP, filtered, and cultured on chromogenic media supplemented with antibiotics, biocides, or both. Antibiotic susceptibility and biocide tolerance were assessed using disk diffusion and MIC testing. Selected isolates were subjected to whole-genome sequencing.

Findings: Downstream isolates exhibited higher MICs for Didecyldimethylammonium chloride (DDAC), Methylisothiazolinone (MIT), indicating increased biocide tolerance. Genomic analyses revealed frequent co-localization of $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-48}}$, and *qnr* with biocide resistance genes like *qacEΔ1* and *emrE* on integrons and plasmids. Efflux pump genes (*acrB*, *oqxA*, *mdtA*) and heavy metal resistance genes (*pco*, *mer*) were widely distributed, contributing to cross-resistance. Class 1 integrons and IncF plasmids facilitated the clustering and transfer of resistance determinants. Media supplemented with carbapenems and biocides selected for isolates with multidrug resistance and detoxification pathways.

Conclusion: WWTPs serve as hotspots for the selection and spread of co-resistant Gram-negative bacilli. These findings underscore the need for integrated monitoring of antibiotics, biocides, and MGEs to curb the spread of multidrug-resistant bacteria.

Abstract ID: 2642 / Poster Number: 134.17P

Antibiotic Resistance and ESBL Producing Escherichia coli in Cured Cheeses

Presenting author: Alba Martínez-Laorden, PhD, University of La Rioja, Logrono, Spain

Co-authors: *Celia Arraiz-Fernández, Elena Gonzalez-Fandos*

Escherichia coli is a bacterium commonly found in the gut of animals and humans, and may contaminate food. Although most strains are harmless, some can cause disease and exhibit resistance to multiple antibiotics, as well as carry resistance genes. This represents a risk to both human and animal health.

The aim of this study was to evaluate the resistance and presence of genes in E. coli strains isolated from cured cheeses, and to determine whether these strains are producers of extended-spectrum beta-lactamases (ESBL).

Twelve E. coli strains isolated from different cured cheeses were tested using the Kirby-Bauer method to assess resistance to tetracyclines and sulfonamides, as well as to identify ESBL production. Furthermore, PCR techniques were used to detect the corresponding resistance genes.

Of the 12 strains tested, 5 carried genes associated with ESBL production. Likewise, 3 of the strains carried genes related to sulphonamides, and all of them showed resistance to at least one antibiotic of this family. Resistance to an antibiotic of the tetracycline family was confirmed in three of the five strains in which these genes were found to be present.

Finally, the detection of antibiotic-resistant and ESBL-producing E. coli strains represents a potential risk to human health. This highlights the need to implement thorough quality controls in the production of cured cheeses, especially considering that this food can be consumed raw. Adopting appropriate prevention measures is essential to reduce the risk of resistant infections and protect public health.

Abstract ID: 2054 / Poster Number: 111.17P

Antibiotic resistance genes in Enterococcus strains from a low-antibiotic dairy farm

Presenting author: Alessandra Mallardi, PhD, Institute of Agricultural Biology and Biotechnology, National Research Council, Lodi, Italy; University of Pavia, Pavia, Italy

Co-authors: *Hossain Delower, Stefano Morandi, Valentina Monistero, Bianca Castiglioni, Paolo Moroni, Paola Cremonesi*

Introduction: Antimicrobial resistance (AMR) represents a critical global health issue, with animal production systems significantly contributing to its spread. Among resistant bacteria, the high prevalence of resistant *Enterococcus* spp. to commonly used antibiotics has attracted increasing attention and posing risks to both animal and human health.

Objective: The aim of this study was to characterize the genotypic diversity and assess AMR profiles of *Enterococcus* spp isolates from a dairy farm with low antibiotic usage.

Method: A total of 131 *Enterococci* strains (63 *E. faecalis,* 31 *E. hirae,* 37 *E. malodoratus*) were isolated from the farm environmental, bulk tank and bedding samples. Genotyping was performed by Random Amplification of Polymorphic DNA (RAPD-PCR) with primers D11344, D8635 and M13 to identify different pattern. Additionally, the presence of genes responsible for tetracycline and erythromycin resistance and of the Tn916 transposon, known to facilitate the acquisition and transfer of tetracycline resistance, was screened in all isolates by PCR analysis.

Findings: RAPD-PCR revealed identical patterns among *E. faecalis* isolates, suggesting the circulation of a single strain. In contrast, *E. hirae* and *E. malodoratus* displayed a predominant profile and some variability.

E. faecalis showed the highest resistance levels (100% to tetracycline, 97% to erythromycin), whereas all *E. hirae* and *E. malodoratus* isolates carried tetracycline but not erythromycin resistance gene. The Tn916 transposon was detected in nearly half (44,4%) of *E. faecalis* isolates, indicating their potential for horizontal gene transfer, but further investigations will be performed to deepen the AMR knowledge.

Abstract ID: 1628 / Poster Number: 094.17P

Antibiotic Resistance in Wastewater: Detection, Quantification, and Risk Assessment

Presenting author: Tereza Stachurová, PhD, University of Ostrava, Ostrava, Czech Republic

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Wastewater treatment plants (WWTPs) are considered potential reservoir of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB). The aim of our study was to identify differences in the presence of ARB in wastewater samples from five WWTPs in the Czech Republic, and to monitor the impact of treatment processes on the removal of ARB. Another objective was to compare the relative abundances of resistance genes (blaTEM, blaNDM-1, blaKPC, blaOXA48, mecA, tetW, sul1, vanA) in the nitrification and sedimentation tanks of WWTPs.

Bacterial DNA was isolated from wastewater samples, and ARGs were quantified using real-time PCR. Resistant culturable bacterial strains were characterized by determination of titer (CFU/mL), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

The results showed that the highest relative abundances were observed for the blaTEM > tetW > blaNDM-1 > vanA > sul1 resistance genes, respectively. Isolated resistant strains exhibited a high degree of resistance to ampicillin, with 61.2% of the total isolates showing MIC > 32 mg/L and 78.8% showing MBC > 64 mg/L.

This study not only provided crucial evidence of high bacterial resistance to ampicillin in wastewater but also revealed a significant increase in the relative abundance of the blaTEM, tetW, blaNDM-1, vanA, and sul1 genes. These findings highlight an increasing risk of resistance spreading to other antibiotics. Particular attention should be paid to last-resort antibiotic resistance genes, such as blaNDM-1 and vanA, which pose a significant risk of treatment failure in bacterial infections, potentially leading to higher morbidity and mortality.

Abstract ID: 3442

Antibiotic resistance mutations drive bacterial evolution by fostering genetic recombination

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Co-authors: *Ana-Isabel Rodríguez-Rosado, José R. Valverde, Pablo García, Paula Ramiro-Martinez, Alvaro San Martin, José-Manuel Rodríguez-Martínez, Alvaro Pascual, Jesús Blázquez, Jerónimo Rodríguez-Beltrán*

Introduction: Antibiotic resistance (AR) is an inevitable consequence of bacterial evolution. The classical paradigm states that antibiotics exert a selective pressure that drives the rise of resistant bacteria. This selective pressure also favors bacteria that show an increased ability to evolve and gain new resistances. However, the existence of AR mutations that directly boost bacterial evolvability by increasing their ability to gain, transfer, or combine antibiotic resistance is still undescribed.

Objective: In this work, we aim to demonstrate that quinolone-resistant gyrA mutations can cause increases in the recombination rate in Escherichia coli. Moreover, we seek to understand the molecular causes and consequences of this phenomenon and wether or not it is conserved in E.coli genomes.

Methods: We employ a recombination measuring system based on a plasmid, and a supercoiling fluorescent reporter to decipher the effect of the AR mutations. By merging together different plasmid-encoded TEM β-lactamase alleles, we demonstrate the impact AR mutations could have in acquiring new resistances. In a bioinformatical approach, we infer recombination events from more than 4,000 E. coli high-quality genomes from public databases, and compare mutation-bearing genomes to their mutation-free counterparts.

Findings: Here, we report that clinically relevant quinolone-resistant gyrA mutations (i.e., S83L and D87G) cause a significant increase in the recombination rate in Escherichia coli (Figure 1a). Moreover, we demonstrate the molecular basis of the increase in recombination, which is mediated by changes in supercoiling levels (Figure 1b) that alter the accessibility of RecA to ssDNA. We also show that these enhanced recombination rates translate into an increased ability to evolve new resistances. Lastly, we demonstrate that strains with clinically relevant gyrA mutations display signs of enhanced recombination and increased presence of AR, demonstrating that gyrA-

mediated increase in recombination rates is pervasive across natural E. coli strains (Figure 1c).

Conclusion: Altogether, our results contribute to explaining the high prevalence of quinolone-resistance mutations and provide, for the first time, direct evidence of antibiotic-resistance mutations that produce a direct increase in evolvability, fostering AR evolution.

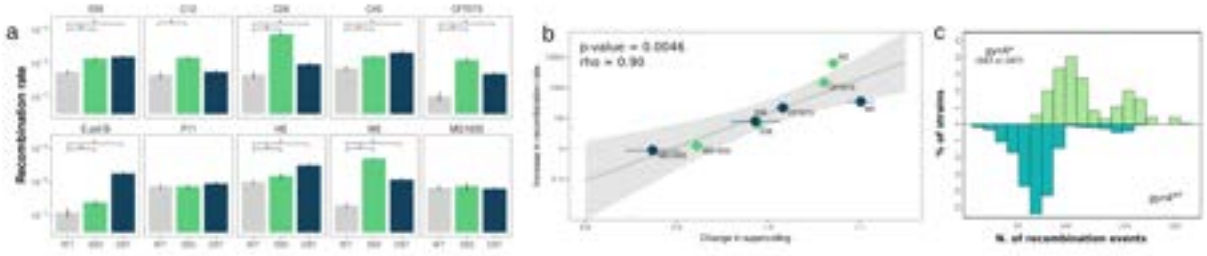


Figure 1: a) Recombination rates of mutants and the WT by strain. b) Spearman correlation between the change in supercoiling measured with pSUPR and the change in recombination rates of the mutants. c) Recombination events for *gyrA** and WT genomes

Abstract ID: 90 / Poster Number: 223.17P

Antibiotic resistance: comparison of various disinfection methods in combatting Multidrug- resistant pathogens in the food chain production

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Antibiotic resistance presents one of the most significant threats to global public health, food safety and societal development. The spread of antibiotic-resistant bacteria (ARB) and their associated resistance genes (ARG) is due to indiscriminate, excessive use and/or misuse of antibiotics in several sectors. New resistant pathogens can emerge from animal sources, and notably meat-processing facilities (e.g. slaughterhouses) have been identified as important reservoirs of antibiotic resistance. Consequently, many strategies have been developed by food supply chain stakeholders and researchers to limit the spread of antibiotic-resistant pathogens and their ARG within the meat production chain. While the global adoption of disinfection strategies was intended to control pathogens, the consequences of these methods on promoting the spread of ARG was determined by molecular techniques highlighting by thus the cross-resistance of disinfectants and antibiotics, which aggravated the problem of antimicrobial resistance.

In this study, we tested the effectiveness of new disinfection strategies, including the sustainable HLE disinfectant developed by our group, in comparison with “business as usual” strategies, UV treatment or combinations thereof. The aim was to evaluate the ability to control key pathogens and limit the spread of ARG in high-risk areas of slaughterhouses. Our results using culture-dependent methods showed a broad distribution of potential pathogens in all tested areas (sacrifice room, cold room and white room), while we also detected important shortcomings of business-as-usual methods. Furthermore, metagenomic analysis indicated the high effectiveness of HLE disinfectant alone or combined with business-as-usual strategies, eliminating pathogenic bacteria (e.g. Acinetobacter sp.) and also curbing the spread of ARG such as multidrug, aminoglycoside, tetracycline and betalactam encoding genes.

This new disinfection approach could be a valuable alternative to reduce the spread of antibiotic-resistant bacteria and their ARG in the food chain production and the environment.

Abstract ID: 2713 / Poster Number: 121.16P

Antibiotic-resistant bacteria decolonization through fecal microbiota transplantation facilitates de novo generation of multidrug-resistant organisms in mice

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Fecal microbiota transplantation (FMT) is an emerging approach with the potential to restore microbiota diversity and deplete antibiotic-resistant bacteria (ARB) such as antibiotic-resistant *Enterobacteriaceae* (ARE). However, it is not always effective in eliminating the ARB, and the factors driving FMT success are largely unknown. An unexplored factor is the ability of ARB, including ARE, to transfer antibiotic resistance genes (ARGs) to other microbiota members through plasmid conjugation, a process that could potentially be facilitated by this therapy. To comprehensively study ARGs dissemination within the gut microbiota, we have implemented a methodology involving a donor strain (*Escherichia coli*) carrying a multidrug-resistant conjugative plasmid. This plasmid contains a gene encoding a green fluorescent protein and a gene conferring resistance to chloramphenicol, both under the control of a promoter that is repressed in the donor but not in the recipient strains. Using flow cytometry, culturomics and high-throughput sequencing, we demonstrated ex vivo the transfer and persistence of the conjugative plasmid to multiple commensal bacteria, primarily those belonging to the *Enterobacteriaceae* family. Furthermore, using a mouse model simulating ARE colonization upon antibiotic treatment and subsequent microbiota restoration via FMT, we demonstrated that this therapy, albeit is effective at diminishing ARE levels, it promotes the generation of new multidrug-resistant (MDR) strains through plasmid conjugation. Our results suggest that the usage of FMT for ARE decolonization could have the negative side effect of generating novel MDR strains.

Abstract ID: 55 / Poster Number: 053.17P

Antibiotic-Resistant Viable Bacteria in Wastewater Sludge Reused in Agriculture: A Growing Concern for Resistance

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Wastewater and sludge from wastewater treatment plants (WWTPs) are known reservoirs for antibiotic-resistant bacteria (ARBs) and facilitate the transfer of antibiotic resistance genes (ARGs). Their reuse in agriculture, although beneficial for phosphorus recycling and cost-effective compared to disposal, poses a risk of spreading antibiotic resistance. In Spain, over 80% of sludge is reused in agriculture, heightening concerns about environmental contamination and the potential transmission of ARBs and ARGs to humans and animals.

This study analyzed 20 sludge samples from two WWTPs, treated by digestion or thermal hydrolysis, to detect carbapenem-resistant bacteria. Samples were cultured on CHROMagar mSuperCarba plates, and distinct colonies were further tested for antibiotic susceptibility using the disc diffusion method. The bacterial DNA was purified, and the 16S rRNA gene was sequenced for identification.

A total of 66 carbapenem-resistant bacteria were isolated, with 30 exhibiting multiresistance (resistant to two or more antibiotics). Most of these multiresistant strains were resistant to cefotaxime (CTX30) and cefotaxime with clavulanic acid (CTL40), with 97% of the isolates displaying both resistances. The identified bacteria predominantly belonged to the genera Pseudomonas (40%), Ochrobactrum (20%), and Acinetobacter (10%).

The study highlights the role of WWTPs as reservoirs for ARBs and ARGs, suggesting current treatments may be insufficient to eliminate these risks. While sludge reuse offers economic and environmental benefits, improved treatment technologies or stricter regulations are necessary to minimize the spread of antibiotic resistance, especially regarding contamination risks for raw-consumed vegetables.

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Abstract ID: 2545 / Poster Number: 387.15P

Antigenic evolution of seasonal influenza A viruses in Türkiye between 2017 and 2023

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Seasonal influenza A viruses (IAVs) pose a significant health threat, causing up to 650,000 respiratory deaths annually. Over the past century, IAVs have triggered four pandemics, with H3N2 and H1N1 subtypes establishing seasonal circulation in humans. The hemagglutinin (HA) glycoprotein, critical for viral entry and a primary vaccine target, contains major antigenic sites. While antigenic drift facilitates immune escape, certain substitutions may affect protein stability and intra-protein interactions, influencing viral fitness. This study investigated the phylogenetic origins of full-length HA genes of seasonal IAVs circulated in Izmir, third most populous city in Türkiye, between 2017-2023 using Bayesian analysis. Publicly available HA sequences from Türkiye on GISAID were included to analyze selection pressures using four different models available on Datamonkey and assess antigenic mismatches with vaccine strains for H1N1 and H3N2 viruses. The findings revealed four antigenic subclades for H1N1 and six for H3N2 in Izmir, demonstrating the co-circulation of antigenically distinct strains within the same season. While HA genes of both subtypes were predominantly under negative selection, the N260D substitution was consistently observed under positive selection across four models. Bioinformatics analysis suggested that this substitution might alter intra-protein dynamics in the vestigial esterase domain by forming a novel electrostatic bond. Additionally, H3N2 viruses exhibited more antigenic mismatches than H1N1, including a novel mismatch in the 2022–2023 season. This is the first study to comprehensively document the antigenic evolution of seasonal IAVs in Türkiye over five years. Regional monitoring of antigenic changes can improve vaccine strain selection and vaccination strategies.

Abstract ID: 510 / Poster Number: 407.16P

Antimicrobial and Anti-Inflammatory Silver Nanoparticles Coated with Chitosan and Hesperidin for Chronic Wound Healing Applications

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Co-authors: *Prashant Mishra*

Introduction: Silver nanoparticles (AgNPs) have been widely exploited for wound healing applications owing to their high antibacterial potential. However, the potential cytotoxicity of AgNPs limit their clinical applications.

Objective: In this study, a novel combination of AgNPs coated with chitosan (Chi) and hesperidin (Hsd), i.e Ag-Chi-Hsd NPs has been synthesized to synergistically enhance the antibacterial and anti-inflammatory action as well as the selectivity of AgNPs using chitosan and hesperidin while promoting their wound healing capabilities.

Methods: The antibacterial, anti-inflammatory, and wound healing potential of Ag-Chi-Hsd NPs have been evaluated using multiple techniques such as MIC and MBC determination, live-dead assay, anti-biofilm assay, time-kill assay, in vitro cytokine release assay, and in vitro scratch assay. The prepared Ag-Chi-Hsd NPs were characterized using UV-visible spectroscopy, FTIR spectroscopy, XRD, DLS, NTA, TEM and SAED. Physical characterizations of Ag-Chi-Hsd NPs showed a spherical shape with a size ranging between 20-30 nm and large positive zeta potential of +44 mV.

Findings: Ag-Chi-Hsd NPs exhibited remarkable antibacterial activity against both Gram-positive and Gram-negative pathogens commonly found at the wound sites, viz., S. aureus, B. subtilis, P. aeruginosa, and E. coli, respectively, with MIC of 5 µg/ml which was more potent in comparison with the bare AgNPs with the MIC of 7.5 and 15 µg/ml against Gram –ve as well as Gram +ve bacteria, respectively. It has also been observed that Ag-Chi-Hsd NPs eradicated about 90% of 24 hours and 48 hours old mature biofilms of the aforementioned bacteria. Ag-Chi-Hsd NPs showed excellent selectivity (up to 94) in its action when tested against the mammalian cells—NIH3T3 cells, RAW264.7 and THP-1. The nanoparticle formulation has also exhibited exceptional ability to lower the pro-inflammatory cytokines release such as—IL-6 and TNF-α—from the THP-1 cells stimulated with LPS by more than 90%. Moreover, in the vitro scratch assay, Ag-Chi-Hsd NPs have shown excellent wound healing potential with NIH3T3 cells by facilitating an almost complete closure of wound area by the end of day 2.

Conclusions: These results suggest that Ag-Chi-Hsd NPs are highly suitable candidates for wound healing applications, as they combine the advantages of AgNPs, chitosan and Hsd in a single nanosystem.

Abstract ID: 1870 / Poster Number: 100.17P

Antimicrobial effects of novel nanomaterials for medicine

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Materials newly developed for medical applications should meet specific requirements for antimicrobial properties and biocompatibility. Our objective was to assess the antimicrobial effects of polymer nanocomposite materials (polylactide films, polycaprolactone fibers) containing various types of nanofillers (silver/graphene oxide; silver, zinc oxide, hexadecylpyridinium or hexadecyltrimethylammonium cations, chlorhexidine, ciclopiroxolamine anchored on vermiculite) in direct contact tests, as well as in their degradation leachates.

The antimicrobial properties of the nanomaterials were detected by disk and well diffusion tests, the microdilution method, and colony-forming unit (CFU) counting on potentially pathogenic gram-negative bacteria (*Acinetobacter baumannii*, *Pseudomonas aeruginosa, Escherichia coli*, *Proteus mirabilis*, *Enterobacter aerogenes*), gram-positive bacteria (methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Streptococcus salivarius*), and yeast (*Candida albicans*). The inhibitory effect of the samples on biofilm formation was assessed using the Crystal violet biofilm assay. The biocompatibility of the nanomaterials was determined by *in vitro* MTT assay.

The study of polylactide nanomaterials showed that the composite filled with silver/vermiculite exhibited the best antimicrobial and biocompatible properties. Among the polycaprolactone nanomaterials, nanofibers containing zinc oxide/vermiculite/chlorhexidine demonstrated the highest inhibitory effect on the microbial growth and biofilm formation. The antimicrobial assessment, using a complex of detection assays, provided important information regarding the potential use of these nanomaterials in medicine.

Abstract ID: 3890 / Poster Number: 196.17P

Antimicrobial molecules from marine fungi against relevant pathogens

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Introduction: The need for new therapeutic approaches to combat the increasing number of resistant bacterial strains is a priority challange for public health world wide. Marine environment can be an extremely rich sources of bioactive compounds particularly in the field of secondary metabolites (SMs) production from different marine organisms.

Objectives: Marine fungi are emerging as a promising source of bioactive compounds. In this work, we focused on a culture collection of known, new, or neglected species of marine fungi, showing poly-extremophilic features. The selected fungi were screened to individuate those with possible high production of SMs. Targeting secondary metabolism genes allowed to individuate ten strains for the subsequent work steps. To stimulate the expression of silent secondary metabolite genes, according to the "one strain many compounds" strategy (OSMAC), the selected fungi were cultivated on various media under different chemico-physical growth conditions. In particular, we investigated antipseudomonal activities from culture extracts from *Penicillium rubens* (HP10), *Clonostachys rosea* (IG119), and a species belonging to the genus *Phaeosphaeriaceae* (PN33) against *Pseudomonas aeruginosa*.

Results: Biological evaluation of these strains confirmed their efficacy against *P. aeruginosa*. *P. rubens* exhibited a MIC of 0.07 mg/ml and an MBC of 0.15 mg/ml. For *Clonostachys rosea*, MIC values varied depending on the solvent used for culture extraction: 2.5 mg/ml with butanol and 1.25 mg/ml with chloroform. In the case of *Phaeosphaeriaceae spp*., a MIC of 2.5 mg/ml from chloroform extract was recorded.

Conclusions: Further experiments to evaluate the antibacterial, antibiofilm, antifungal, antiviral activity against emerging pathogens are underway. Isolation and purification of new SMs and determination of their chemical structures are in progress.

Abstract ID: 111 / Poster Number: 226.17F

Antimicrobial Peptide Combinations as a New Approach to Hinder Resistance Evolution in Staphylococcus aureus

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Co-authors: *Zvi Hayouka, Jonathan Friedman*

Introduction: Excessive antibiotic use has driven the spread of antimicrobial resistance (AMR), posing a major global health threat. Antimicrobial peptides (AMPs) are promising natural compounds that could replace conventional antibiotics; however, previous studies have shown that resistance to AMPs can also evolve.

Objective: This study investigates whether and which combinations of AMPs can hinder resistance evolution in *Staphylococcus aureus*, focusing on factors that influence efficacy and the role of AMP mode of action.

Methods: *S. aureus* bacteria were exposed to single AMPs and their combinations in a series of experimental evolution studies. We tracked resistance development and assessed cross-resistance and fitness costs as potential contributors to the effectiveness of combinations. Whole-genome sequencing was also conducted to identify resistance-conferring mutations and to determine whether these differed between single and combination treatments.

Findings: While resistance developed rapidly to some individual AMPs, few combinations significantly delayed resistance evolution. The efficacy of AMP combinations was independent of AMP mode of action; instead, combinations imposing a high fitness cost on resistant strains and limiting cross-resistance were especially effective. Whole-genome sequencing revealed mutations unique to certain AMPs, as well as two genes linked to general AMP resistance.

Conclusion: This study highlights AMP combinations as a promising strategy for managing AMR. By identifying genotypic and phenotypic factors that influence the AMP efficacy to delay resistance evolution, our findings contribute to the development of "resistance-proof" antimicrobial cocktails, expanding options for a sustainable antimicrobial treatment.

Abstract ID: 103

Antimicrobial peptide digital optimization enables the generation of bioinspired products

Presenting author: Prof. Octavio Franco, Universidade Catolica Dom Bosco/Universidade Catolica de Brasilia, Campo Grande, Brazil

Introduction: Antimicrobial peptides (AMPs) have attracted considerable attention because of their multiple and complex mechanisms of action toward resistant bacteria. However, reports have increasingly highlighted how bacteria can escape AMP administration.

Objective: In response to this challenge, we have developed a novel computational approach for designing novel AMPs.

Methods: Such an approach utilizes genetic and Joker algorithms, as well as an artificial intelligence strategy, to design bioinspired AMPs derived from bacteria, plants, and animals. This in silico strategy was associated with several methods for understanding the mechanism and function of such AMPs.

Findings: This approach has yielded different peptide classes, including those with unusually high cationic residues and hydrophobic counterparts. At least dozens of peptides emerged as prototype AMP among natural analogs screened for their activity against an engineered luminescent Pseudomonas strain. Peptides were further characterized in structure, activity, mechanism of action, and biotechnological potential for developing new compounds beneficial for human and animal health. Most novel peptides were unstructured in water and underwent a coil-to-helix transition in hydrophobic environments. This conformation was corroborated by NMR analysis in dodecylphosphocholine micelles, which revealed an α-helical structure. The generated AMPs caused a bactericidal effect at low micromolar concentrations on several resistant bacteria, causing membrane disruption without triggering depolarization but rather hyperpolarization. Finally, the large-scale production strategies used to prepare such peptides for the market were also discussed.

Conclusions: In summary, the present work presents an innovative computational approach to explore natural products to design short and potent peptide antibiotics that could be used against resistant bacteria, offering a promising solution to the global challenge of antibiotic resistance.

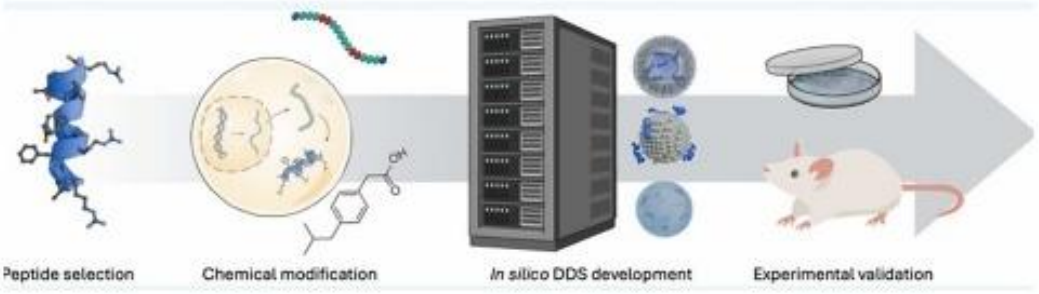


Figure 1: Approaches for antimicrobial peptide design and delivery. Overview of integrated approaches to improve the design and delivery of antimicrobial peptides.

Abstract ID: 3707 / Poster Number: 192.17P

Antimicrobial peptides producing endophytic fungi isolated from dominant plants across fynbos eco-region of South Africa

Presenting author: Dr. Kazeem Adekunle Alayande, North-West University, Potchefstroom, South Africa

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Fungi are very rich in easy-to-digest proteins and often produce both high- and low-molecular-weight bioactive compounds. While antimicrobial peptides are small protein fragments with proven efficacy against a wide range of pathogens, they are more stable and non-toxic. The study is designed to screen diverse endophytic fungi for novel antimicrobial peptides against multidrug-resistant pathogens. Leaf samples of dominant plants (Asteraceae) were collected along the fynbos ecoregion in the Western Cape and thereafter surface sterilised. A total of 45 fungal endophytes with distinct morphologically characterised mycelia were isolated and identified through PCR amplification of the ITS and 28S ribosomal subregion. The sequenced data was edited using Mesquite, aligned with MUSCLE algorithm, and maximum likelihood phylogeny reconstructed in RAxML-GUI v2.0 graphical interface. The tree was thereafter inferred using GTR and inverted gamma-distributed rate variation model with 1000 bootstrap replicates. The test pathogens include *Escherichia* coli, *Salmonella* Typhimurium, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, and screened for MDR traits following CLSI and EUCAST guidelines. Antimicrobial peptides were extracted from a 14-day hold mycelium culture in malt-yeast extract broth and tested using agar well diffusion and microplate dilution approaches to determine susceptibility and minimum inhibitory concentrations, respectively. Seven different strains of fungal endophytes produced bioactive peptides with varying degrees of antimicrobial effectiveness. The zones of inhibition ranged between 15 and 19.5 mm, while most of the extracted bioactive peptides exhibited minimum inhibitory concentrations that ranged between 1.25 and 0.625 mg/ml. The identified bioactive peptide-producing fungal endophytes include *Botryosphaeria* sp., *Dothideomycetes* sp., *Debaryomyces hansenii, Preussia* sp., and *Preussia africana*. Further study continues purification and characterisation of the peptides.

Abstract ID: 651

Antimicrobial Resistance and Genetic Diversity of Salmonella in Aquatic Ecosystems: A Thai Canal Study

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Salmonella is one of the leading pathogens responsible for acute gastroenteritis worldwide. While it is traditionally associated with foodborne illnesses, waterborne Salmonella infections pose a significant risk. The emergence of antimicrobial-resistant Salmonella further elevates public health concerns, with aquatic ecosystems, such as canals, playing a potential role in disseminating antimicrobial-resistant bacteria. In Bangkok, Thailand, canals are crucial for agricultural resources and daily water usage. However, these ecosystems remain underexplored in antimicrobial resistance research and surveillance despite their importance.

We analyzed 351 Salmonella isolates recovered from 6 different canals between 2016 and 2020 to investigate the characteristics, genetic diversity, genetic relationships, and antimicrobial resistance profiles of Salmonella enterica found in Bangkok canals using whole-genome sequencing and phenotypic analysis.

Our results revealed that Salmonella Agona was the predominant serotype, while S. Typhimurium and its monophasic variant were occasionally found. Seven novel sequence types (STs) were identified, including STs 11346, 11347, 11348, 11349, 11350, 11351, and 11352. Seven chromosomal-mediated gene mutations and 50 antimicrobial resistance genes were detected, with tet(A), blaTEM-1B, and qnrS1 as the top three most commonly detected resistance genes. In addition, tet(X4) was first identified in the Salmonella population in Thailand, and the colistin resistance gene, mcr-3.1, was also detected. In total, 39.0% of the strains were potentially multidrug-resistant, with the canal located in the high-density residential area having the highest proportion of MDR strains. The strains harbored double amino acid substitutions in GyrA and ParC, and a strain with GyrA substitutions and qnrS1 exhibited the strongest resistance to nalidixic acid and ciprofloxacin. Most ceftazidime, ceftriaxone, and cefotaxime-resistant strains (66.7%) possessed blaCTX-M-55. Over half (58.4%) of Salmonella strains were positive for plasmid replicon sequences, with Col(pHAD28) as the dominant plasmid replicon type. Phylogenetic analysis of Salmonella STs34 and 213 from canal water and database

strains depicted the possibility of circulating Salmonella STs34 and 213 between canal water and humans in Thailand and globally.

The findings from this study highlight the circulation of antimicrobial-resistant pathogens in urban aquatic ecosystems and underscore the importance of incorporating environmental sampling into comprehensive AMR surveillance programs according to the One Health approach.

Abstract ID: 412 / Poster Number: 062.17P

Antimicrobial Resistance and Genotypic Profiles of *Staphylococcus pseudintermedius* Isolated from Dogs in Montevideo, Uruguay

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Co-authors: *Elizabeth Lancaster*

Staphylococcus pseudintermedius is a common opportunistic pathogen in animals, particularly dogs, associated with skin, ear, urinary tract infections, and post-surgical wounds. It has also been identified as an emerging zoonosis, with the potential for transmission to humans.

This study aims to characterize the phenotypic and genotypic resistance profiles of *S. pseudintermedius* isolates from symptomatic and asymptomatic dogs in veterinary clinics in Montevideo, Uruguay.

A total of 133 isolates were analyzed and identified using PCR with species-specific primers. The phenotypic resistance profiles to multiple antimicrobials commonly used in veterinary practice were evaluated, and the associated resistance genes were detected through PCR.

MRSP was primarily isolated from symptomatic dogs (32.5%), with 89% of these strains carrying the *mecA* gene. The most detected chromosomal cassette type was V, followed by types III and IV. The highest resistance was observed to penicillin (80%), associated with the *blaZ* gene, followed by erythromycin (50%) and clindamycin (45%), with the *ermB*, *ermC*, and *msrA* genes. Clindamycin resistance was inducible (iMLSB: 11%), constitutive (cMLSB: 32%), macrolide-specific (MS: 7%), and lincosamide-specific (L: 5%). Lower resistance percentages were found for enrofloxacin (30%), tetracyclines (27%), and gentamicin (20%). Finally, 49.6% of the isolates were classified as MDR (multiple drug resistance).

These results highlight strains with diverse resistance genes in veterinary clinics, acting as reservoirs for transmission to humans, especially given the close contact between dogs and owners. This antimicrobial resistance, including MRSP, presents therapeutic challenges and emphasizes the critical need for a One Health approach.

Abstract ID: 2052 / Poster Number: 110.17P

Antimicrobial resistance in Escherichia coli isolates from cows with clinical mastitis

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Introduction: *Escherichia coli* is the main *Enterobacteriaceae* causing clinical mastitis. Bovine mastitis, characterized by inflammation of the mammary glands, has multiple etiologies and is difficult to control. This disease poses a significant challenge to the dairy industry, leading to substantial economic losses and potential public health risks, as the microorganisms involved can carry antimicrobial resistance genes. Antimicrobial resistance (AMR), as highlighted by the World Health Organization (WHO), is a global concern due to the rise of antibiotic-resistant bacteria, which compromises the efficacy of essential drugs for treating infections in humans and animals. **Objective:** The aim of this study was to genetically investigate the resistant to antimicrobial agents of 9 *E. coli* isolates resistant to antimicrobial agents, obtained from cases of bovine mastitis in ten high-production dairy farms located in the states of São Paulo and Minas Gerais, Brazil. **Method:** The DNA of isolates was extracted, and the Nextera XT Library Prep kit (Illumina, USA) was used for library preparation. The isolates were sequenced using the Illumina HiSeq 1500 equipment. **Findings:** Among the isolates obtained from cases of clinical mastitis, one isolate (11.11%) was identified as resistant to 5 classes of antimicrobials (aminoglycosides, beta-lactams, quinolones, sulfonamides, and tetracyclines). Among the remaining isolates, 33.3% showed resistance to quinolones, 22.22% to aminoglycosides, 22.22% to tetracyclines, 22.22% to extended-spectrum beta-lactams, and 11.11% to sulfonamides. **Conclusion:** This study reveals significant antimicrobial resistance in *Escherichia coli* isolates from bovine mastitis, emphasizing the need for surveillance and strategies to mitigate AMR risks to animal and public health.

Abstract ID: 3007 / Poster Number: 046.17P

Antimicrobial Resistance in Urban Wastewater: Insights from Operating Conditions and Bacterial Community Dynamics

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The new European Urban Wastewater Treatment directive (1) addresses concerns regarding the emission of contaminants of emerging concern (CEC), such as pharmaceutical compounds and antimicrobial resistance (AMR), challenging Urban Wastewater Treatment Plants (UWWTP) goals. This work aimed to investigate how different key operating conditions (Food/Microorganism ratio, coagulant and ozone doses) affect the final effluent, regarding total bacteria and AMR removal, as well as the composition of the bacterial communities.

Water samples were collected from three UWWTPs with different activated-sludge (AS) systems and six lines operated under different F/M and coagulant doses, and one UWWTP with different ozone doses. The samples were collected at the plants' inlet, after AS, after ozonation when applicable, and at the receiving waterbodies, upstream and downstream the UWWTP discharge. Microbiological analyses included total coliforms and *E. coli* enumeration, quantitative PCR of selected genes (e.g. 16S rRNA, *intl1*, *ermB*), bacterial community analysis by 16S rRNA gene metabarcoding, and isolates identification by MALDI-TOF.

Overall, wastewater treatment led to log-reductions from influent to final effluent of (log-units (gene copy/ml)) 0.5-2.7 for total bacteria (16S rRNA), 1.2-3.7 for *intl1* gene and 0-4.0 for *ermB* gene. Different operating conditions promoted mild changes on cultivable bacteria loads and AMR abundance, likely associated to shifts in bacterial populations. UWWTP discharges caused < 1.0 log-units increase in total bacterial abundance from upstream to downstream. Multidrug resistant isolates include *Citrobacter* spp., *Escherichia* spp. and *Klebsiella* spp. Bacterial community dynamics induced by operating conditions management can represent a promising approach to control AMR in UWWTPs.

Abstract ID: 3313 / Poster Number: 175.17P

Antimicrobial susceptibility profile and serotype distribution of pneumococcal blood culture isolates from Nepalese children

Presenting author: Mr. Madhav Chandra Gautam, Patan Academy of Health Sciences, Kathmandu, Nepal

Introduction: *Streptococcus pneumoniae* is a major cause of invasive bacterial disease in Nepalese children. We describe the serotype distribution and antimicrobial susceptibility patterns of *S. pneumoniae* isolates in the paediatric population since introducing the 10-valent pneumococcal conjugate vaccine (PCV10) into Nepal's immunization schedule in 2015.

Objective: To determine antibiotic susceptibility and serotype of *Streptococcus pneumoniae* isolated from blood culture of Nepalese children.

Methods: From January 2016 to December 2019, all pneumococcal blood culture isolates from children <14 years of age in Patan Hospital, Nepal, were tested for antimicrobial susceptibility by the Kirby Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Serotyping was performed by the Quellung method.

Findings: *S. pneumoniae* was isolated from blood cultures of 35 paediatric patients - out of which whom, 10 were PCV10 vaccinated. Of these isolates, 27/35 (77.1%) were PCV10 serotypes -the most common being serotype 1[22/35(62.9%)], 3/10(30%) among vaccinated children, whereas, 19/25(76%) were PCV10 serotypes among the unvaccinated. Most PCV10 isolates were from children <2-years-olds,25/27 (92.6%). The non-PCV10 serotypes were 4/10 (10A, 2,3,38) among vaccinated children and 4/25(2,19A,6C,41) among the unvaccinated. All isolates were susceptible to penicillin, and 33/35 (94.3%) were susceptible to chloramphenicol. Resistance was common to erythromycin 13/35 (37.1%).

Conclusion: PCV10 serotypes (particularly serotype 1) continue to be predominant among paediatric pneumococcal blood culture isolates, however lower in vaccinated children. All isolates remain susceptible to penicillin. Ongoing monitoring of pneumococcal serotype distribution following the introduction of PCV10 and the antibiotic susceptibility pattern of the isolates is essential.

Abstract ID: 3452 / Poster Number: 180.17P

Antimicrobial susceptibility to non-beta-lactam drugs in methicillin-resistant Staphylococcus pseudintermedius (MRSP) isolated from dogs in Brazil

Presenting author: Assoc. Prof. Marita Vedovelli Cardozo, Sao Paulo State University, Jaboticabal, Brazil

Co-authors: *Mareliza Possa de Menezes, Natalia Pereira, Leandro Augusto Mariano Silva*

Introduction: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) strains are resistant to all beta-lactam antibiotics, with the exception of ceftaroline, and frequently exhibit resistance to multiple non-beta-lactam antimicrobial classes, significantly limiting therapeutic options.

Methods: This prospective study aimed to assess the susceptibility of MRSP isolates from canine clinical samples in Brazil to non-beta-lactam drugs. Twenty-eight isolates were confirmed as MRSP via PCR targeting the *mecA* and *spsL* genes. Antimicrobial susceptibility testing was performed using the broth microdilution or disk diffusion methods, following CLSI guidelines.

Findings: All isolates were susceptible to daptomycin (24/24), linezolid (24/24), and nitrofurantoin (10/10), while all exhibited resistance to penicillin G (25/25) and trimethoprim-sulfamethoxazole (28/28). All but one isolates (96%) were susceptible to amikacin (27/28) and vancomycin (25/26). Additionally, high resistance rates were observed for enrofloxacin (96% [27/28]), marbofloxacin (96% [26/27]), ciprofloxacin (93% [26/28]), levofloxacin (89% [25/28]), clindamycin (88% [21/24]), rifampin (86% [24/28]), and gentamicin (79% [22/28]).

Conclusion: Our findings revealed high rates of resistance to commonly used non-beta-lactam antimicrobials in veterinary patients. Vancomycin, gentamicin, rifampin, daptomycin, linezolid, and ciprofloxacin are classified as critically important drugs for human medicine. Of these, only vancomycin, daptomycin, and linezolid—drugs typically not used in veterinary medicine in Brazil—demonstrated high susceptibility rates. The high resistance rates to fluoroquinolones are concerning, as these drugs are important options for treating serious infections in humans and other animals. Interestingly, amikacin appears to be a non-critically important drug suitable for use in MRSP infections in dogs.

Abstract ID: 3591 / Poster Number: 186.17P

Application of EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) in Coagulase-Negative Staphylococci Isolated from Blood Cultures

Presenting author: Ms. Zeynep Tekbaş, Eskişehir Osmangazi Üniversitesi, Eskişehir, Turkey

Co-authors: *Duygu Öcal, M. Rıdvan Tayşi*

Introduction and Aim: Early and accurate antimicrobial therapy is crucial for sepsis treatment success. However, the rising prevalence of resistant pathogens complicates the identification of the correct infectious agent and limits treatment options. This study aims to evaluate the effectiveness of the EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) method in rapidly and accurately assessing coagulase-negative staphylococci (CNS) isolated from blood cultures.

Methods: From January to December 2024, 72 blood culture samples with CNS growth were sent to the Microbiology Laboratory of Ankara University Faculty of Medicine Cebeci Research and Application Hospital for antibiotic susceptibility testing using the EUCAST RAST method. Antibiotic zone measurements were taken at 4, 6, 8, 16-24, and 24 hours, and compared with re-tested suspensions prepared from 24-hour passage cultures at a 0.5 McFarland density. SPSS 23.0 software was used for data analysis to identify significant changes. RAST results were evaluated based on EUCAST standards, particularly for *Staphylococcus aureus*.

Results: Among the 72 microorganisms, 36.11% were *Staphylococcus epidermidis*, 33.33% were *Staphylococcus hominis*, 13.89% were *Staphylococcus capitis*, 13.89% were *Staphylococcus haemolyticus*, and 2.78% were *Staphylococcus warneri*. At 4 and 6 hours, no growth or zone diameter changes were observed, while some growth was noted at 8 hours. No significant changes were observed between 16-24 hours, but after a 24-hour passage, slight increases in zone diameters were noted for antibiotics such as trimethoprim-sulfamethoxazole and tobramycin. One-way ANOVA analysis showed significant differences between time points ($p < 0.001$).

Discussion and Conclusion: Rapid antibiogram results obtained using the RAST method enable early selection of appropriate antibiotics, enhancing treatment success. For more accurate antibiotic susceptibility testing, additional data, particularly regarding zone diameters for CNS species, is recommended. In conclusion, reliable rapid antibiogram

tests are crucial for improving treatment outcomes, especially in urgent situations like sepsis.

Abstract ID: 3233 / Poster Number: 390.17P

Application of green and smart sensors to visual monitoring soft cheese quality: a multi-analytical approach to validate the sensors effectiveness

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Co-authors: *Muhammad Rehan Khan, Luca Zignego, Lisa Rita Magnaghi, Ester Trigueros, Lucia Vannini, Marcello Alinovi, Massimiliano Rinaldi, Raffaela Biesuz, Elena Bancalari*

Food waste has become a critical global issue, posing significant economic, social, and environmental challenges. Among the strategies to address this issue, innovative smart packaging solutions, such as intelligent food packaging, have shown significant potential. This study aimed to validate a bioplastic colorimetric sensor array (BCSA) as a novel tool for real-time freshness monitoring of highly perishable soft cheeses. To achieve this, the degradation mechanisms of stracchino cheese were investigated under refrigerated conditions (4°C) and thermal abuse (8°C) through microbiological, chemical, and physical analyses conducted over the storage period.

The study identified key spoilage markers, including microbial populations, volatile organic compounds (VOCs), and physical quality attributes. Microbiological assessments revealed that lactic acid bacteria and *Enterobacteriaceae* were the dominant species, with microbial loads nearing spoilage thresholds within 10 days of storage. VOC analysis detected critical spoilage markers, such as hexanoic acid, butanoic acid, and acetic acid, strongly linked to off-odor development. Physical analyses indicated notable textural changes, including softening and moisture loss, as spoilage progressed.

Principal Component Analysis (PCA) and data fusion techniques were employed to integrate microbiological, chemical, and physical indicators, providing a comprehensive understanding of spoilage dynamics. The validation of the BCSA demonstrated its ability to effectively track the onset of spoilage in real-time. These findings underscore the importance of integrated approaches to improve shelf-life prediction and drive innovations in packaging technologies. The proposed solution has the potential to reduce food waste, enhance consumer safety, and promote sustainable food systems.

Abstract ID: 935 / Poster Number: 078.17P

Application of machine learning to characterise antibiotic mode of action through metabolic fingerprints

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Co-authors: *Christoph Bieniossek, David Dylus, Ueli Von Ah, Holger Wache, Ezra Valido*

The rapid emergence of antimicrobial resistance (AMR) presents a critical global health concern, highlighting the urgent need for novel and effective antibiotics. Phenotypic screening often remains the preferred approach in antibiotic drug discovery, with target identification being an important step. Different methods have been developed to elucidate the antibiotic Mode of Action (MoA), with each of them having their own limitations. Here we describe a novel approach to identify the antibiotic MoA via the metabolic activity of bacterial cells upon compound treatment. Based on the hypothesis that changes in bacterial carbon metabolism are indicative of the antibiotic MoA, this study aimed to complement the research gap by accurately predicting the MoA of known antibiotics effective against *Escherichia coli* using machine learning (ML) models. The ML models were trained on phenotypic data describing changes in carbon metabolism by *Escherichia coli* over time in presence and absence of drugs. Three ML algorithms – Artificial Neural Networks (ANN), Extreme Gradient Boosting (XGBoost), and Random Forest (RF) – were applied to classify the antibiotic MoA, which showed an overall accuracy of 93.23%, 90.58% and 85.54%, respectively. These results demonstrate the potential of this method as a powerful tool in antibiotic discovery. Moreover, data quality and pre-processing were highlighted as critical for model performance.

Abstract ID: 1875 / Poster Number: 370.17P

Application of microbial inoculants for pesticide degradation during industrial composting of plant waste

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During the composting process of plant waste, liquid leachates are generated as a by-product of the aerobic decomposition of the organic fraction. Although vegetable compost is an organic amendment with recognized benefits for soil and plants, pesticides used in intensive agriculture can end up concentrating both in the composts and in the leachates generated during the process, thus reducing their excellent agronomic properties.

Therefore, the objective of this work was the search for a pesticide-degrading microbial consortium and its subsequent validation in composting processes of plant waste on an industrial scale.

To this end, the microbial community of plant leachates was used as a starter culture and subjected to a sequential enrichment experiment using flonicamide, a pesticide widely used in intensive agriculture. Microbial strains were selected based on their ability to grow on minimal salt medium supplemented with the pesticide. The selected strains were used in a laboratory-scale degradation assay, in which bacterial cell counting using specific culture media and the determination of flonicamide degradation were carried out. Finally, once the efficacy of the strains to degrade flonicamide was confirmed, they were inoculated in industrial-scale plant waste composting process, and the overall microbiota and flonicamide degradation during the process were monitored. The results obtained confirmed that plant compost leachates are an appropriate source for bioprospecting of pesticide degrading strains. Furthermore, the results of the work will reveal the feasibility and efficacy of the proposed methodology for pesticide bioremediation during the composting process.

Abstract ID: 4075 / Poster Number: 365.16P

Application of Nanopore Sequencing in the Wastewater Treatment Plant Process

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Nanopore sequencing a form of high-throughput parallel sequencing, has emerged as a cost-effective and rapid technique that is gaining prominence in contemporary research. This technique can also be of great value for microbial analyses in wastewater treatment plants. In particular, understanding the diversity and abundance of technologically undesirable bacterial species in activated sludge is crucial for optimising treatment processes aimed at their removal or reduction. Specifically, the detection of Candidatus *Microthrix parvicella* and *Microthrix subdominans* is of particular importance, as these microorganisms contribute to wastewater foaming and are indistinguishable through conventional microscopic analysis. Accurate differentiation between these species is essential for the effective application of aluminium coagulants, as *Microthrix parvicella* exhibits resistance to this treatment.

In this stidy, we analysed two samples of activated sludge. For DNA isolation, a total of six different isolation methods were tested and compared in terms of isolated DNA concentration purity and complexity of sample preparation, and efficiency to isolate various microbial taxa. First, activated sludge metagenomic DNA was sequenced with a ligation sequencing kit. Metagenomic sequencing data facilitate not only the detection of all single microbial species, but also the reconstruction of a metagenome-assembled genome or the detection of other genes of interest, such as, for example, antibiotic resistance genes. To enhance the precision of target species detection, 16S rRNA gene amplicon sequencing was performed using a barcoding kit. The presence of target microorganisms was confirmed through both primary bioinformatics analysis (EPI2ME software) and advanced bioinformatics pipelines.

Abstract ID: 3402 / Poster Number: 411.16P

Application of New Genomic Techniques in Bifidobacterium animalis subsp. lactis: Eliminating Tetracycline Resistance While Preserving Probiotic Properties

Presenting author: Dr. Marianna Bozzetti, Università Cattolica del Sacro Cuore, Cremona, Italy

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Introduction: Probiotics are live microorganisms that provide health benefits when administered in sufficient quantities. Nevertheless, concerns persist regarding their safety, particularly the potential for transferring of antibiotic resistance genes to the gut microbiota. Tetracycline resistance, mediated by the *tetW* gene, has often been identified in *Bifidobacterium* spp. The proximity of *tetW* to mobile genetic elements poses a potential risk of horizontal gene transfer to other bacteria. Recent advancement in new genomic techniques (NGT), in particular CRISPR-Cas technology, offer a promising solution to mitigate these risks by inactivating resistance genes.

Objective: This study aims to evaluate whether a targeted modification in *tetW* gene can affect the functional and probiotic characteristics of a commercial strain of *Bifidobacterium animalis* subsp. *lactis*.

Methods: A mutant strain of *B. animalis* subsp. *lactis* BLC01 was generated inserting three stop codons in the *tetW* gene using the endogenous CRISPR-Cas system. Whole-genome sequencing was performed to confirm the absence of off-target modifications. The impact of this editing was assessed by determining the tetracycline sensitivity of the mutant strain using a microplate-based Minimum Inhibitory Concentration assay. The functional and probiotic properties of the wild-type and genome-edited strains were compared through several assays. These included tolerance to different pH values (2, 3, 4 and 7), resistance to bile salts concentrations (0, 0.5 and 2%), and survival in simulated gastric and intestinal fluids. Further tests, including the ability of the strains to aggregate and co-aggregate with *Escherichia coli* and *Salmonella enterica*, adhesion to the Caco-2 and HT-29 cell lines, and *in vitro* digestion using the INFOGEST method, were performed.

Findings: Genome sequencing confirmed successful modification of the *tetW* gene with a single off-target change leading to a Threonin→ Prolin substitution at position 280 of the Prolipoprotein diacylglyceryl transferase enzyme. The MIC assay demonstrated that the mutant strain's tetracycline resistance was reduced to levels below the threshold

defined by EFSA guidelines, unlike the wild-type strain. Both strains demonstrated robust resistance to acidity, showing no significant differences after three hours at pH 2 and 3. Similarly, no statistically significant differences were observed in bile salt resistance between the two strains after two hours. The exposure to simulated intestinal fluid resulted in a notable reduction in viability for both strains after 30 minutes, but no significant differences were observed in their behaviour at 60, 90, or 120 minutes. The adhesion capacity to the Caco-2 cell line was similar for both the wild-type strain and the mutant, when compared to the control strain, *Lacticaseibacillus rhamnosus* GG. Furthermore, the mutant strain's adhesion capacity was approximately 15 times higher than of GG, while the wild-type strain's adhesion capacity was 10 times higher than the control.

Conclusions: The Δ*tetW* mutant strain developed in this study applying NGT retains the probiotic properties of the wild-type strain while effectively mitigating the risk of tetracycline resistance transfer. This strain shows great promise for its use in food supplements or functional foods, contributing to improved consumer safety without compromising functional efficacy.

Abstract ID: 2796 / Poster Number: 372.16P

Application of phage endolysins to combat bacterial spoilage of wine

Presenting author: Dr. Cas Mosterd, University of Bordeaux, Bordeaux, France

Co-authors: *Olivier Claisse, Claire Le Hénaff*

15.9 billion litres of wine are produced in the European Union alone, making grapes one of the most valuable crops. Converting grapes into wine relies on two rounds of fermentation. First an alcoholic fermentation turning sugars into alcohol, followed by malolactic acid fermentation by lactic acid bacteria. A few LAB species associated with wine, especially Oenococcus oeni, contribute positively to wine sensory characteristics, but other members of the LAB community, such as Lentilactobacillus spp. and Pediococcus spp., can produce undesirable compounds. Measures are in place to control spoilers, most of them making use of chemical or physical treatment of the wine. The demand for more natural alternatives by consumers and environmental factors made us examine the potential of phage endolysins to be applied in wine as a biological control for undesired bacteria. We have extracted the endolysin sequences from both public and private sequences of lytic and temperate phages infecting O. oeni, Lentilactobacillus spp and Pediococcus and a range of acetic acid producing bacteria associated with wine spoilage. Using an in silico approach, the endolysins have been classified and candidates were selected for downstream experiments. These will include overexpression and purification of the endolysins, after which they will be applied in experiments in vitro and in vino to test their activity against individual bacterial strains and a reconstituted wine microbiota.

Abstract ID: 9 / Poster Number: 296.16S

Application of the zinc-binding protein psoriasin as an antifungal food preservative

Presenting author: Dr. Maayan Gal, Tel Aviv University, Tel Aviv, Israel

Introduction: The increasing global population makes microbial food spoilage a significant economic and environmental concern. Current food preservatives are chemicals that are associated with various health risks and often have limited effectiveness. Moreover, consumers and the food industry are turning towards "clean label" food products. The challenge lies in the scarcity of food-safe materials with sufficient anti-microbial activity, which brings the need for biological and effective food preservatives.

Objective: The protein psoriasin, known as S100-A7, is a zinc-binding protein naturally secreted in the oral cavity with an effective antimicrobial activity. This makes it a potentially effective and safe protein-based food preservative. Our goal herein is to study the effect of the protein on microbial growth in food and evaluate its application as a biological food preservative.

Methods: The protein was recombinantly expressed in E. coli and purified to homogeneity. Its in-vitro dose-response effect against various bacterial and fungal strains was evaluated. Then, the protein effect on microbial growth in different food matrices and the effect of various di-valent ions on its activity were evaluated.

Findings: The application of S100-A7 on food shows promising antimicrobial activity. At a concentration higher than 50 mM, the protein completely abolishes in-food fungal growth in both solid matrices and liquid medium. Owing to the protein thermal stability its application on bread pre-baking effectively inhibits microbial growth post-baking. The addition of zinc in-vitro and in-food regains microbial growth, concluding the mechanism by which the protein competes with the microorganism's essential metal nutrients.

Conclusions: This research can potentially develop psoriasin as a safe and effective natural food preservative, addressing consumer demand for healthier food options and reducing food waste. Protein engineering tools could further be applied to impart selected activity profiles such as lipophilicity and thermal stability, further improving its range of applications.



Figure 1: The effect of psoriasin on microbial growth on bread. The bread was baked with psoriasin (left) or PBS (right). The image shows the bread 3-weeks after baking.

Abstract ID: 1405 / Poster Number: 288.17P

As to Saccharomyces cerevisiae flocculation

Presenting author: Mr. Hashimoto Seito, Toyo Graduate School, Asaka, Japan

Co-authors: *Kojima Kaho, Mizuki Toru, Miura Takeshi*

Yeast is a microorganism that produces ethanol from sugar through alcoholic fermentation. It has been widely used in foods and beverages from the past to the present. Since flocculation, a characteristic of yeast, is caused by the binding of mannose and lectin-like proteins on the cell surface layer, it is believed that the presence of mannose in the culture medium inhibits flocculation. Furthermore, there is a reported case in which a gene involved in flocculation of Saccharomyces cerevisiae (Flo1) and a gene involved in flocculation of S. pastorianus (Lg-Flo1) were introduced into a non-agglutinating yeast, and the yeast showed flocculation.

In this study, we aimed to confirm flocculence in brewing strains used for beer and sake (beer yeast: S. cerevisiae TUM 210 and S. cerevisiae NBRC 104019T, sake yeast: S. cerevisiae Kyokai No. 7) and isolated yeast from pear(S. cerevisiae MSP 1, MSP 2). Only in all brewing strains, flocculation was observed in the presence of sugars such as glucose, maltose and mannose. On the other hand, lectin like protein, which has been reported to be calcium ion-dependent in flocculation, was found to be calcium ion-independent in the brewing strains and isolated strains. These results suggest that the mode of flocculation in all strains may be different from what is known.

We are currently working to clarify the conditions for flocculation and the genetical analysis about existence of Flo gene seriese by PCR in yeast.

Abstract ID: 1623

Assembly Curator: rapid and interactive consensus assembly generation for bacterial genomes

Presenting author: Dr. Thomas Roder, University of Bern, Bern, Switzerland; Swiss Institute of Bioinformatics, Lausanne, Switzerland

Co-authors: *Rémy Bruggmann*

Introduction: Long-read sequencing technologies enable the generation of near-complete bacterial genome assemblies. However, no assembler is perfect – issues like duplicated or missing plasmids, spurious contigs, and failures to circularize sequences remain common. Achieving optimal results still requires manual consensus generation.

Objective: While tools like Trycycler simplify this process, they are time-consuming and demand command-line expertise. With the ability to sequence hundreds of datasets quickly and affordably, there is a growing need for faster, more accessible solutions.

Method: Here, we present Assembly Curator, a platform that imports multiple assemblies and clusters contigs, and then facilitates interactive comparison and selection through a user-friendly graphical interface.

Conclusion: This streamlined workflow allows users to generate high-quality hybrid assemblies more efficiently, delivering high-quality results with greater speed and ease.

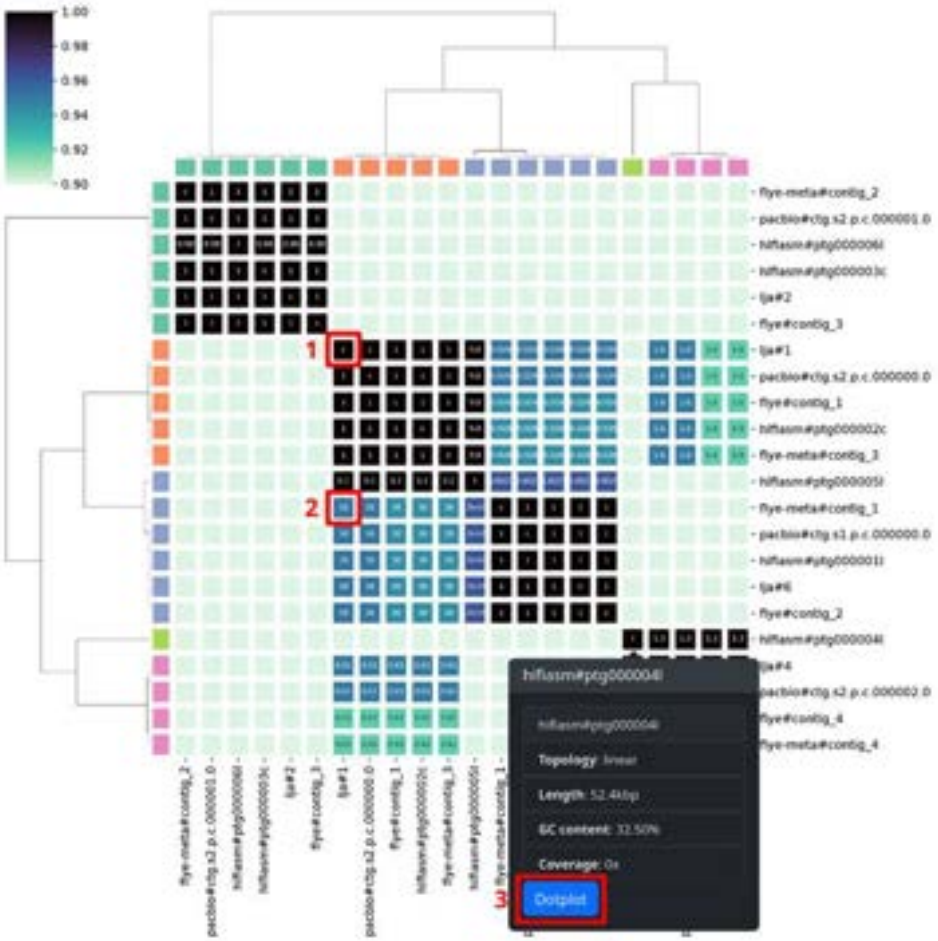


Figure 1: *Screenshot showing one step of Assembly Curator. This is an interactive clustermap with dendrograms displaying all contigs produced by different assemblers. The color scale indicates contig similarity, calculated using pyskani. Diagonal labels specify whether a contig is circular or linear (e.g., **1** "lja#1" is circular). Clicking on the diagonal selects a contig. The off-diagonal numbers (e.g., **2**) represent the size factor between two contigs. Hovering the mouse over cells reveals additional information. Clicking on Dotplot in the context menu (**3**) generates a dotplot on the fly using biowasm/minimap2 and Plotly.*

Abstract ID: 4037 / Poster Number: 098.16P

Assessing fidelity of vertical transmission: mother to twin transmission dynamics

Presenting author: Sara Fumagalli, PhD, University of Milano-Bicocca, Milano, Italy

Co-authors: *Pamela Ferretti, Maria Carmen Collado, Nicola Segata, Antonia Bruno, Mireia Valles-Colomer*

Introduction: Microbial vertical transmission is a fundamental evolutionary process shaping early neonatal colonization. Despite exposure to a diverse maternal microbiota, only a selective fraction successfully establishes in the newborn, profoundly influencing immune development and long-term health. Yet, the forces driving this transmission remain largely unresolved: does deterministic selection dictate microbial transmission, or do stochastic processes govern colonization? Twin models offer a unique opportunity to assess the consistency of vertical transmission. However, no studies have systematically explored the stochasticity of maternal microbial transmission or explored microbial exchange between twin pairs at the strain level.

Objectives: This study aims to investigate the selective patterns and consistency of maternal microbiome transmission at the strain level in twins. Specifically, the main objectives are a) determine whether microbial transmission follows structured dynamics or is primarily influenced by stochastic processes; b) assess transmission consistency in early life using a strain-sharing approach and explore potential microbial exchange between twin pairs.

Method: This study analyzed 168 metagenomic samples from 19 families, including 18 mother-twin pairs from Italy, Spain, and Sweden. Samples included stool, oral, breast milk, skin, and vaginal, collected from birth to two years. Strain profiling was performed using StrainPhlAn4, with strain-sharing analysis based on normalized phylogenetic distances and species-specific strain identity thresholds. A strain-sharing index quantified microbial transmission. SGB (species-level genome bin) transmission patterns were assessed for twin acquisition (Fisher's Exact Test), while pairwise strain-sharing was compared using Kruskal-Wallis with post-hoc Dunn's tests (Benjamini-Hochberg correction).

Findings: A total of 40 SGBs were transmitted from the mother to at least one twin, with 20 shared by both and 16 detected at the same time point. The strain-sharing index revealed a strong correlation between mother-infant 1 and mother-infant 2 (Pearson =

0.94, p = 0.02), indicating highly consistent maternal transmission. A differential transmission pattern was observed among SGBs, with some species consistently acquired by both twins, while others were differentially transmitted (Fisher's Exact Test, p =1.26e-3), suggesting species-specific transmission dynamics. Twins from the same family exhibited a significantly higher number of shared strains compared to unrelated twins (Kruskal-Wallis, Benjamini-Hochberg correction, p = 2.36e-105, p = 5.31e-46, p = 1.07e-82).

Conclusion: This study provides novel insights into maternal microbial transmission, revealing a structured transmission pattern with species-specific variation and association with delivery mode, breastfeeding, and zygosity. Understanding these transmission dynamics is essential for identifying factors that promote optimal microbial seeding and establishment. Future work will investigate twin-to-twin microbial exchange to clarify whether strains are independently or secondarily transmitted between siblings.

Abstract ID: 1330 / Poster Number: 204.17P

Assessing Soil Microbial Diversity Using Traditional Culture, Enrichment, and iChip Methods for Antibiotic Discovery

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Co-authors: *Juliette Morice, Sabrina Gueulle, Eliette Riboulet-Bisson, Cécile Muller-Pujol, Florie Descriac*

Introduction: In 2019, antimicrobial resistance was associated with 4.95 million deaths worldwide[1]. To fight this threat, a continued discovery of new antibacterials is urgently needed. Historically, microorganisms have been a significant source of antibacterial compounds with 67% of FDA-approved antibacterials being microbial secondary metabolites or their derivatives[2]. Moreover, it is estimated that only 1% of microbes can be cultivated under laboratory conditions[3]. Consequently, accessing new microbial diversity holds great promise for discovering novel antibacterial compounds.

Objectives: In this context, we employed different cultivation approaches to access a wide range of microorganisms. Following this, we conducted a screening to evaluate their antibacterial activity and are currently purifying bioactive compounds to discover potentially new antibacterials.

Method (Figure 1): Ten soil samples were collected in Normandy, France. *In situ* pre-culture using iChip[4], heat pre-treatment, prolonged incubation times, and a panel of culture media were combined with direct plating to create a microbial collection. Isolates were screened for antibacterial activity through the deferred antagonism and agar-well diffusion assays against WHO-priority pathogens[5] and *Micrococcus luteus*. Isolates were identified using 16S rRNA sequencing and/or MALDI-TOF mass spectrometry. Some isolates were submitted to a one strain-many compounds (OSMAC) protocol to induce the expression of cryptic biosynthetic gene clusters[6].

Findings: Our microbial collection comprises 416 isolates belonging to 68 genera[7] (Figure 1). Eleven isolates appear to be potential new bacterial species, and 40 are associated with species described after 2017. Among the collection, 103 isolates exhibited antibacterial activity against at least one reference strain (Figure 1). Some of them belong to well-known antibiotic producers such as *Streptomyces, Micromonospora, Pseudomonas,* and *Bacillus*, while others originate from less common genera, including *Chryseobacterium, Rahnella,* and *Delftia*. Some initially non-active rare *Actinomycetota*, especially *Nonomuraea* spp., showed strong antibacterial activities after induction via OSMAC.

Conclusion: The combined use of different cultivation methods provides access to a wide diversity of microorganisms, especially those less frequently isolated. The bioactive compounds responsible for the observed antibacterial activities are currently being purified. Our efforts are focused on the most phylogenetically relevant microorganisms (e.g. *Nonomuraea*[8]), which may produce novel antibacterial metabolites.

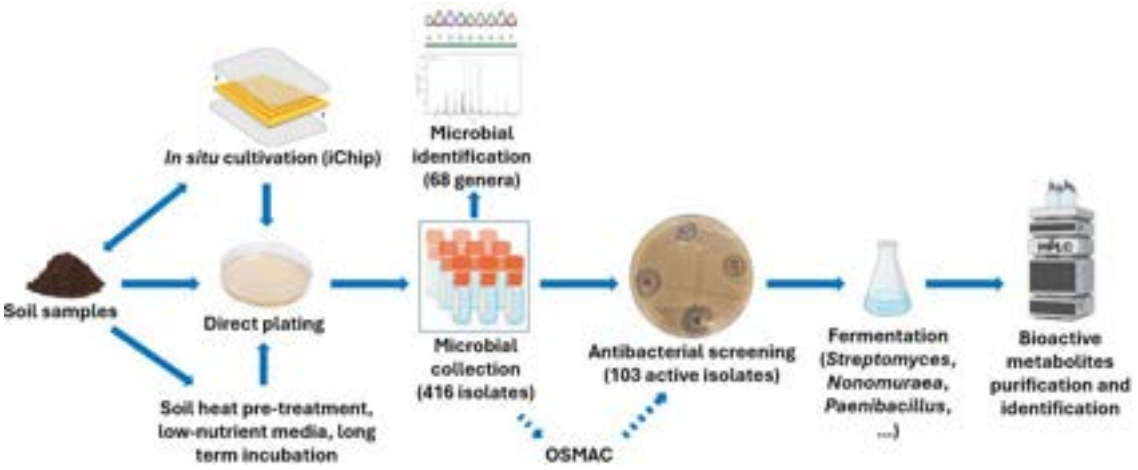


Figure 1: Overview of the methodological approach and results of the study.

Abstract ID: 3643 / Poster Number: 415.16S

Assessing soil microbiome preservation strategies for application in sustainable agriculture

Presenting author: Mr. Federico Sbarra, University of Turin, Turin, Italy; ENEA, Rome, Italy

Co-authors: *Marco Garello, Filippo Sevi, Eleonora Colantoni, Benedetto Aracrì, Silvia Tabacchioni, Andrea Visca, Manuela Costanzo, Luciana Di Gregorio, Antonella Del Fiore, Francesco Aloi, Giovanna Cristina Varese, Luca Simone Cocolin, Ilario Ferrocino, Davide Carmelo Spadaro, Annamaria Bevivino*

Introduction: Preserving intact soil microbiomes in as close a state as possible to that originally present in field maintaining the composition and functional potential of its microbiome is therefore a primary concern for the development and application of microbiome-based solutions.

Objective: In the present work we investigated how different sample preservation techniques can affect the soil rhizosphere microbial communities by combining culture-based methods, metagenomic investigation and analysis of the functional profile of soil microbial communities.

Methods: Three different storage conditions were considered: freezing at -80°C, lyophilization and refrigeration at 4°C in dark conditions. Samples were analysed at 0, 6 and 12 months from sampling. The analysis of culturable fraction was performed by means of the total microbial count, r-k strategists and EP index. The functional diversity was investigated using the Biolog-ECOPlates™ system. DNA extraction was performed for the subsequent 16S and ITS metagenomic investigation followed by bioinformatic analysis. Soil rhizosphere samples were collected from strawberry, grapevine and kiwifruit plants grown in two field types.

Finding: Functional-level metabolic profiling of microbial communities based on Biolog EcoPlates highlighted widespread carbon sources utilisation in samples with high population diversity confirmed by metagenomic analysis, such as kiwifruit and strawberry, when compared to poor species abundance soils, as grapevine. The functional analysis performed at 6 and 12 months after sampling revealed that the storage of samples at -80°C is suitable for preserving intact soil microbiomes. On the other hand, bioinformatics analyses indicate that, based on taxon and soil parameters, different storage protocols have a different suitability for microbiome storage. In particular, for grapevine, storage at -80°C resulted in the least amount of differences

compared too the time zero control for fungi of both the low and high disease incident soil. For the bacteria communities, storage at 4°C worked best for low incidence soil, while storage at both 4°C and -80°C worked equally well for high incidence soil. For healthy kiwivine soil, 4°C storage appeared to be the best protocol for fungi and bacteria, while in diseased kiwivine soil 4°C and -80°C storage worked equally well for fungi and -80°C was best for bacteria. For strawberry, -80°C storage resulted in the least amount of change compared to the time zero control, both for bacteria and fungi, in the control soil and in the solarized soil. Finally, a total of 271 bacterial strains and 12 fungal strains were isolated and analysed for plant growth promoting activity and 162 strains, characterized by MALDI-TOF MS, were selected as promising Plant Growth Promoting Microbes for the set-up of microbiome-based solution for sustainable agriculture.

Conclusion: In conclusion, omics-based approaches and metabolic fingerprinting provide snapshots of microbial diversity, metabolic activities and functional potential of soil microbial communities. Within isolates showing PGP traits, microbial consortia set-up will be carried out to exploit natural inhabitants of soil communities (NatComs) as biofertilizer to enhance crop productivity.

Abstract ID: 2659 / Poster Number: 364.15F

Assessing Soil-Rhizosphere-Plant Microbiome Interactions and their Impact on Animal Gut Health

Presenting author: Dr. Marcel Baer, University of Bonn, Bonn, Germany

Co-authors: *Fen Zhang, Xiaoming He, Mareike Baer, Peng Yu*

Soil, as the largest reservoir of microbial diversity, influences ecosystem function and the health of plants and animals. This study aims to unravel the dynamics of the soil-rhizosphere-plant axis and identify its causal effect on the animal gut microbiome and fitness.

We investigated 36 lettuce (*Lactuca sativa*) genotypes under four nitrogen input conditions with four biological replicates, generating in total 1,168 diverse samples originated from different compartments i.e. bulk soil, rhizosphere, root and leaf tissues. Microbial communities were characterized using high-throughput PacBio sequencing of full-length 16S rRNA gene to estimate how plant genetic variation and nitrogen input influence microbiomes across different domains. A cultivation-based approach was implemented to isolate microorganisms, which will be used *in vitro* to assess their impact on system health.

In a second part, the influence of these leaf-associated microbiomes on the animal gut microbiome is evaluated. Leaf samples, processed to simulate digestion (acid and enzymatic hydrolysis, dialysis), were incorporated into gut microbial models. Sterilized and non-sterilized samples were compared for microbial taxa changes using real-time quantitative PCR.

This project explores potential connections within the system health concept by investigating how nutrient input and plant genetic variation may influence microbial communities. It considers the roles of cooperation and competition within these microbiomes and their possible implications for environmental sustainability and animal health. By integrating soil and gut microbiomes, this study aligns with the 'One Health' framework, highlighting interconnected health benefits across ecosystems through nutrient compounds.

Abstract ID: 599 / Poster Number: 188.15P

Assessing the role of gastrointestinal fluids tolerance in Listeria pathogenicity and virulence

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Co-authors: *Juan José Quereda Torres, Carla Palacios Gorba, Inmaculada López Almela*

Listeria genus includes zoonotic pathogenic L. monocytogenes and non-pathogenic L. innocua. L. monocytogenes population is divided into four lineages. Lineage I is often associated with clinical cases and is considered hypervirulent. Lineage II, usually isolated from food-related samples, is described as hypovirulent. Here, we focused on the effects of gastrointestinal (GI) fluids lineage I and II of L. monocytogenes and on L. innocua. The objectives of the study are: (1) To evaluate if pathogenicity is linked to a higher resistance to GI fluids and (2) To evaluate if a higher resistance to GI fluids confers lineage I its hypervirulence. To do so, we inoculated 18 Listeria strains in simulated GI fluids to compare their growth ability. The principal results showed that there are no significant differences between the growth of hypervirulent and hypovirulent L. monocytogenes and non-pathogenic L. innocua.

In conclusion, lineage I strains did not seem to have a higher resistance to GI fluids than lineage II and L. innocua strains. Thus, pathogenicity and could not be associated with the ability to grow in the presence of GI fluids.

Abstract ID: 3177

Assessment of biotic and abiotic mechanisms involved in mineral alteration of deep-sea hydrothermal chimney during a decrease in activity using gas-lift bioreactor approach

Presenting author: Dr. Maxime Fuster, Univ Brest, CNRS, IFREMER, EMR 6002 BIOMEX, BEEP laboratory, F-29290 Plouzané, France

Co-authors: *Alix Rossi, Lena Ailliot, Johanne Aube, Erwan Roussel, Anne Godfroy, Marie-Anne Cambon, Karine Alain, Olivier Rouxel, Sophie Mieszkin*

Deep-sea hydrothermal vent systems are complex ecosystems subjected to numerous physicochemical constraints, including chemical gradients, temperature gradients, high pressures, anoxic areas and presence of toxic chemicals. These environments are also significant sources of increasingly sought-after mineral resources. Hydrothermal systems provide all the necessary components for chemosynthesis, enabling life to thrive in the absence of light. While the activity of hydrothermal vents is primarily defined by fluid emissions and temperature anomalies, limited information is available in the literature regarding the biological and chemical processes occurring in hydrothermal chimneys during periods of reduced activity, such as declining temperatures.

To bridge this knowledge gap and contribute to the protection of these ecosystems, the primary objective of this study is to elucidate the biotic and abiotic mechanisms involved in mineral weathering at hydrothermal vents using an autecological approach based on the use of a gas-lift bioreactor mimicking conditions of hydrothermal activity. Using a combination of microbiological, molecular, mineralogical and geochemical analyses, we aim to unravel the roles of these mechanisms in mineral alteration during a decline in hydrothermal activity and to demonstrate their significance in the functioning of biogeochemical cycles.

As part of this study, an active hydrothermal chimney and associated fluids (sampled at two distinct temperatures: 90°C and 30°C) were collected from the TAG hydrothermal field on the Mid-Atlantic Ridge during the BICOSE 3 oceanographic expedition. Onboard experiments were conducted using two gas-lift bioreactors, maintained under controlled conditions (80°C in anoxic conditions and 20°C in microaerophilic conditions) as described by Godfroy *et al.* (2006). The collected chimney material was divided between the two bioreactors, which were supplied with crushed chimney material and hydrothermal fluids for 30 days. Liquid and solid phase samples were collected periodically from each bioreactor to monitor changes in cell density, microbial diversity (both taxonomic and functional), mineral composition, and chemical elements over

time. Preliminary results reveal the dissolution of certain minerals, the release of toxic compounds and significant differences in microbial community composition during the decrease of activity. Additionally, our data suggest a turnover of microbial communities over time in systems with stable activity conditions. These findings indicate that microbial communities are closely linked to hydrothermal activity levels and the availability of mineral resources over time.

Our results underscore the critical role of hydrothermal vent systems, regardless of their activity state, in regulating the main biogeochemical cycles in the oceans. They also provide evidence that these ecosystems will be vulnerable to and impacted by potential exploitation of deep-sea mineral resources. This work contributes to a broader scientific initiative (LIFEDEEPER, ANR France 2030), which aims to emphasize the importance of conserving low-temperature hydrothermal sites and protecting these fragile ecosystems.

Abstract ID: 847 / Poster Number: 011.16P

Assessment of microbiological hazards in raw goat milk and milk products

Presenting author: *Assoc. Prof. Jolanta Rola*, National Veterinary Research Institute, Pulawy, Poland

Co-authors: *Dominika Debczak*

Goat milk and milk products consitute important place in the human's diet as they are good source of proteins, fats, carbohydrates, vitamins and minerals. In addition to all the benefits of consuming goat milk and its products, they are also a very good medium for the growth of many microorganisms, among others pathogenic bacteria and their toxins.

The obtained research results will allow to determine the risk of occurrence of these pathogens in raw goat's milk and products made from goat's milk. Additionally, phenotypic and genotypic characterization of the isolated strains was also obtained.

A total of 404 samples, including 297 samples of raw milk, 78 samples of cheese and 29 samples of milk powder were used in this study. The samples were analysed for the presence of *Listeria monocytogenes, Salmonella* spp.*, Campylobacter* spp. and *Escherichia coli* O157 by enzyme linked fluorescent assay (ELFA) method. The species identification and characterization of isolated strains was performed by biochemical tests, PCR and real-time PCR.

L. monocytogenes was detected in 6,4 % of raw milk samples and 5,1 % cheese samples. The percentage of *E.coli* O157 and *Campylobacter* spp. positive raw milk samples was 3.7% and 1%, respectively. *Salmonella* spp. was not isolated from any of the tested samples.

The results obtained in this study indicate the presence of foodborne pathogens, mainly *Listeria monocytogenes*, but also *Camylobacter jejuni* and *Escherichia coli* O157. The consumption of raw goat milk and goat milk products has been growing and this may pose a risk to health of consumers.

Abstract ID: 3066 / Poster Number: 006.15P

Assessment of PFAS-degrading microorganisms and microbial diversity in contaminated sites of Northen Italy

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Co-authors: *Filippo Vaccari, Gabriele Bellotti, Ghada Arbi, Antonio Masi, Ilaria Battisti, Anna Rita Trentin, Giancarlo Renella, Luigi Lucini Lucini, Nicoleta Suciu, Edoardo Puglisi*

Introduction: The widespread contamination of soil and groundwater by per- and polyfluoroalkyl substances (PFAS) presents a major environmental challenge due to their persistence and toxicological risks. Despite promising in vitro studies, the mechanisms and efficiency of PFAS biodegradation remain an ongoing challenge.

Objective: This research aims to address the challenge of PFAS contamination through two main objectives. First, we focus on isolating and identifying microorganisms capable of degrading PFAS from industrial contaminated sites. The second objective investigates the microbial diversity present in soils, water and plants sampled from heavily contaminated sites in northern Italy.

Methods: To achieve this, a sequential enrichment approach was employed, targeting two PFAS compounds: perfluorooctanoic acid (PFOA), and hextafluorobutyric acid (HFBA). This approach was followed by molecular and conventional microbiological techniques, to identify bacterial strains with the capacity of PFAS degradation. Additionally, High throughput 16S amplicon sequencing was employed to profile microbial populations in soil, water and plants, providing insight into diversity and characteristics of microorganisms in PFAS contaminated areas. Enzymatic activities expressed by these microbial communities were also assessed.

Findings: Promising bacterial strains capable of degrading PFOA and HFBA are expected to be isolated, and microbial diversity analyses are anticipated to reveal distinct communities associated with PFAS degradation. Enzymatic activities will further confirm the roles of these communities in the PFAS biodegradation.

Conclusion: This research will provide new insight into PFAS biodegradation and may contribute to the development of sustainable bioremediation strategies for PFAS contaminated areas helping to mitigate the environmental impact of these pollutants.

Abstract ID: 1659 / Poster Number: 096.17P

Assessment of the potential of 'biotic' nutritional additives to restrict in vitro growth of Staphylococcus aureus

Presenting author: Dr. Helen Smith, Alltech, Dunboyne, Ireland

Co-authors: *Richard Murphy*

Nutritional supplements promoting animal health and well-being have become progressively more popular through the increased use of prebiotics and/or probiotics. These and other biotics such as postbiotics, synbiotics and even paraprobiotics have a role to play in supporting intestinal function and the gut microbiome. With the rise in antimicrobial resistance (AMR) and limitations on the use of antibiotics, a greater focus has been placed on the potential of novel feed ingredients to combat the prevalence and transmission of AMR. In this study we evaluate the antimicrobial potential of eleven prebiotic, probiotic, postbiotic, as well as synbiotic preparations toward antimicrobial resistant Staphylococcus aureus.

These 'biotic' nutritional additives were cultured overnight in MRS media (0.1%, w/v) in various combinations. Cell-free supernatant (CFS) was collected following overnight incubation at 37°C (4000rpm, 0.22µm filter). Antimicrobial activity was tested by examining overnight growth rate using a Varioskan LUX Multimode Microplate Reader (OD @595nm). Statistical analysis was performed using Minitab (version 20.4). One-way ANOVA with Dunnett's multiple comparison was carried out to test for significant differences compared to bacteria control, at a 95% confidence level. Differential impacts were noted with differing 'biotic' preparations. Nine preparations significantly restricted the growth of S. aureus by 28 – 76% ($p < 0.05$). Overall, it was noted that the probiotic, postbiotic, and synbiotic preparations that were assessed had varying abilities to inhibit growth of resistant S. aureus. The use of functional dietary components, such as 'biotic' nutritional additives, could represent an innovative approach to combat antimicrobial resistance.

Abstract ID: 4008 / Poster Number: 404.16P

Assimilation of sucrose and its derivates by Rhodospirillum rubrum and Rhodobacter capsulatus

Presenting author: Mrs. Manon Gilson, University of Mons, Mons, Belgium

Co-authors: *Guillaume Bayon-Vicente, Ruddy Wattiez, Baptiste Leroy*

Introduction: The circular economy has gained increasing attention in recent years. Particularly, the upcycling of liquid by-products of industrial processes rich in organic matter to produce high added-value compounds is promising. Purple non-sulphur bacteria (PNSB) are good candidates for the development of such circular bioprocesses due to their metabolic versatility. The growth of PNSB on co-products has already been tested, notably on molasses, the by-product of sugar production which mainly contains sucrose. However, the sugar assimilation metabolism has been poorly investigated in PNSB.

Objectives: In this work, we analysed the growth of two strains of PNSB with sucrose as a main carbon source under phototrophic conditions, either in pure culture or in co-culture.

Method: Bacterial strains *Rs. rubrum* S1H and *Rh. capsulatus* were cultivated separately or in co-cultures under photoheterotrophic and anoxygenic conditions. The carbon source of the culture medium was sucrose, glucose or fructose at 120 mM carbon equivalent in all experiments. Depending on the experiment, the medium has previously been autoclaved (causing partial hydrolysis of sucrose into fructose and glucose) or filtered for sterilization. Cultures were placed in an incubator at 30°C and 170 rpm agitation with a light intensity set at 50 µmol photons m^{-2} s^{-1}.

Findings: Cultures of *Rs. rubrum* in a sucrose-containing medium showed that this strain is unable to assimilate sucrose. However, it was able to assimilate fructose and, at a much lower rate, glucose sequentially, with fructose being first assimilated (**Fig. 1A**). Concerning *Rh. capsulatus*, it was observed that it is capable of assimilating sucrose, fructose and glucose (**data not shown**). In co-cultures, bacterial growth was higher than in pure cultures (**Fig. 1B**). Surprisingly, *Rs. rubrum* was still present at the end of the experiment, either in presence of sucrose only or in presence of sucrose, fructose and glucose (**Fig. 1C**). Based on these results, we hypothesised that *Rh. capsulatus* releases organic molecules into the extracellular environment, which would then be assimilated by *Rs. rubrum* as a carbon source.

Conclusion: In pure cultures, *Rs. rubrum* and *Rh. capsulatus* showed different capacities for sugar assimilation. When co-cultivated, we observed a trophic link between the two strains with *Rs. rubrum* growing photoheterotrophically on the fermentation products of *Rh. capsulatus*.

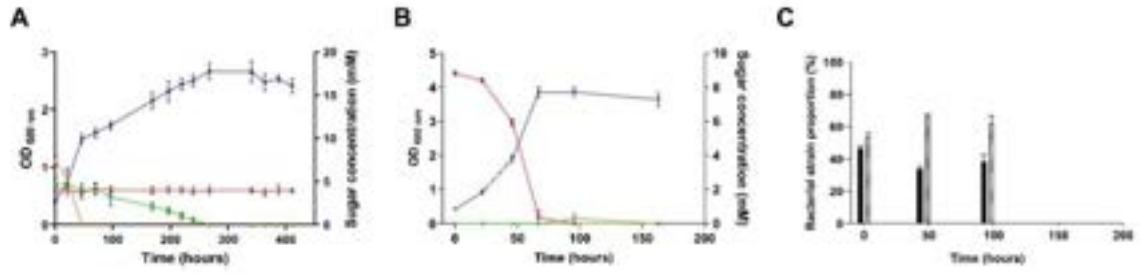


Fig. 1. | Monitoring of growth (blue line) of *Rhodospirillum rubrum* **(A)** and a co-culture of *Rhodospirillum rubrum* and *Rhodobacter capsulatus* **(B)** cultivated in a medium containing sucrose and its derivates, previously autoclaved **(A**) or in a medium containing sucrose and previously filtered **(B).** Red, green and orange lines represent the evolution of sucrose, glucose and fructose concentration, respectively. Bar plots represent the proportion of *Rs. rubrum* (black bars) and *Rh. capsulatus* (hatched bars) at different time points in co-cultures in a medium containing sucrose and previously filtered **(C)**. *N* = 5.

Abstract ID: 3642 / Poster Number: 243.16P

Association of enteric microbiota with intestinal health in broiler chickens

Presenting author: Mrs. Marili Vasilogianni, Royal Veterinary College, London, United Kingdom

Co-authors: *Melanie Hay, Devin Stuart, Rachel Hawken, Jonathan Williams, Dong Xia, Androniki Psifidi, Damer Blake*

Poor intestinal health is a major consideration for the poultry industry. Rapidly growing broiler chickens, which have acquired their modern-day growth rates as a result of intense genetic selection, are increasingly faced with intestinal health challenges after the ban of antimicrobial use as growth promoters. Intestinal dysbiosis and the contribution of the intestinal microbiome to inflammatory intestinal conditions in chickens remains poorly understood. Our study aims to analyse the intestinal and faecal microbiome profiles of broilers growing under commercial conditions and associate their composition with intestinal health related phenotypes. These phenotypes include post-mortem macroscopic observations of the intestine, leg joint health grading scores and translocation of bacteria to the liver. For this study, a homogeneous group of pedigree broilers were reared in the same environment under standard commercial conditions for a period of five weeks. At the end of the study period, 44 chickens were subjected to postmortem examinations for Intestinal Dysbacteriosis, Femoral Head Necrosis (FHN) and Tibial Dyschondroplasia (TD), and liver tissue was collected to assess translocation of bacteria from the gut. Caecal content and faecal samples were also collected and used to analyse the microbiome composition of each chicken through 16S rDNA amplicon and Oxford Nanopore metagenomic sequencing. Our results demonstrate an association between microbial composition and host intestinal health outcomes. Characterising faecal microbiomes offers potential for identification of bacterial groups as intestinal health biomarkers.

Abstract ID: 1782 / Poster Number: 216.15P

Atmospheric trace gases power cross-biome dispersal of thermophiles

Presenting author: Mr. Lucas Barbieri Oliveri, Monash University, Clayton, Australia

Co-authors: *Tess Hutchinson, Thanavit Jirapanjawat, Chris Greening, Pok Man Leung*

While the importance of dispersal is well established in the context of microbial ecology, the mechanisms allowing microorganisms to meet their maintenance energy requirements during long-range dispersal across unfavourable environments remain elusive. This is especially true of geothermal environments, traditionally regarded as ecologically isolated due to the perceived physicochemical constraints on their inhabitants' metabolisms. Through culture-dependent and -independent techniques, we propose oxidation of trace gases from the atmosphere is a widely employed metabolic strategy powering dispersal of thermophilic microorganisms. Gas chromatography showed obligately thermophilic archaeal (*Metallosphaera sedula*) and bacterial (*Thermomicrobium roseum*) representatives are capable of oxidising Hydrogen (H_2) and Carbon Monoxide (CO) at atmospheric concentrations during survival in sub-optimal temperatures as low as 10°C. Quantitative proteomics confirmed high levels of expression of the high-affinity [NiFe]-hydrogenases and CO dehydrogenases responsible for the oxidation are sustained at these temperatures. Atmospheric levels of H_2 enhanced long-term survival of carbon-starved cells at 25°C. Metagenomic-based metabolic profiling of hundreds of publicly available samples showed geothermal environments are enriched in the high-affinity enzymes responsible for trace gas oxidation. Trace gas oxidisers were found in 1.5-fold higher abundances when compared to temperate environments, which suggest the trait is advantageous for thermophiles. Furthermore, hundreds of metagenome-assembled genomes were recovered during our research, making it possible to assess the occupancy of and phylogenetic relationships between community members present in geographically distant geothermal sites. Our results offer an energetic approach to the study of microbial dispersal, and have the potential to influence geothermal biogeography at large.

Abstract ID: 799 / Poster Number: 272.17P

Authentication of Saccharomyces cerevisiae var. boulardii in probiotic-enriched matrices with High-Resolution Melting Analysis

Presenting author: Dr. Monika Borkowska, Poznan University of Life Sciences, Poznan, Poland

Co-authors: *Michał Kułakowski, Kamila Myszka*

The only probiotic yeast with clinically proven health-promoting effects is Saccharomyces cerevisiae var. boulardii (Sb). The increasing number of dietary supplements and functional food products labelled as probiotics prompts the search for effective methods to verify their microbial composition.

The objective of the presented study was to design a genetic analysis for Sb variety authentication.

qPCR-HRM was used, as it offers remarkable sensitivity for detecting polymorphisms and enables the simultaneous identification of closely related microorganisms. The effectiveness of the designed primer pair set was examined. The primers were created to amplify heterogeneous regions in rDNA sequences and genes associated with the physiological divergence of Sb.

18SrRNA and ITS sequences proved to be the best for species Saccharomyces cerevisiae (Sc) identification using qPCR-HRM. HO and RPB2 amplicons were the most effective in differentiating Sb from other Sc strains. The region amplification test of HXT9 and MAL11 allowed for additional verification of the probiotic properties of Sc strains. Presented qPCR-HRM analysis effectively differentiated Sc strains in probiotic supplements. Sb was identified in the microbial mixtures using intraspecies primer pairs. The RPB2 sequence showed the highest intraspecies differentiation power. The established qPCR-HRM analysis enabled the identification of Sb in the microbial mix with the predominance of Sb, corresponding to the required dose of health-promoting microorganisms for the food labelled as probiotics.

Conclusions: 1. The qPCR-HRM analysis successfully differentiated Sc probiotic strains. 2. The application of the selected RPB2 sequence and the predominant presence of Sb in complex matrices were essential for qPCR-HRM-based identification.

Abstract ID: 578 / Poster Number: 068.17P

Awareness and Potential Utilization of Bacteriophages for Therapeutic Purposes Among Doctors in Saudi Arabia

Presenting author: Dr. Mohammed Alqasmi, College of Applied Medical Sciences, Shaqra University, Shaqra, Saudi Arabia

Antimicrobial resistance (AMR) presents a significant global health challenge, highlighting the need for alternative therapeutic strategies beyond conventional antibiotics. Phage therapy offers a promising alternative due to its high specificity for bacteria, ability to access complex infection sites, reduced off-target effects, and synergistic benefits when used with antibiotics. This study aims to evaluate the awareness and potential adoption of phage therapy among doctors in Saudi Arabia, with the goal of guiding future research and clinical integration of phage therapy in the Kingdom. An anonymous online survey was distributed via email by the Saudi Commission for Health Specialties (SCFHS) and further promoted through local networks of clinicians. The inclusion criteria include active doctors who are working in Saudi Arabia and registered with the SCFHS. The survey yielded 102 valid responses from over 20 specialties and subspecialties. Respondents expressed significant concern about AMR's impact on their clinical decisions, and demonstrated a moderate level of prior knowledge about phage therapy. For phage therapy development, Staphylococcus species, particularly methicillin-resistant S. aureus (MRSA), were identified as the highest-priority pathogens, followed by Pseudomonas, Klebsiella, Escherichia coli, Mycobacterium, and Streptococcus species. In contrast, infections in immunocompromised patients, diabetic foot infections, blood infections, and infective endocarditis were considered key targets clinical conditions. Despite the limited reported awareness of alternative treatments for AMR, participants showed optimism regarding the future implementation of phage therapy. Expanding research, development, and access to phage therapy could offer substantial clinical and economic benefits for both patients and healthcare systems.

Abstract ID: 2375 / Poster Number: 123.17P

Azithromycin-driven development of collateral sensitivity to meropenem in XDR/PDR Klebsiella pneumoniae hospital isolates

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Co-authors: *Olena Moshynets, Viktoria Potochilova, Olga Iungin, Danylo Puzikov*

Introduction: Antibiotic resistance is spreading rapidly among inpatients, contributing to the development of severe complications and prolonging hospitalization. An increasing number of reserve antibiotics are ineffective against the common pathogen *K. pneumoniae* (KP), and their high cost and shortage worsen treatment. At the same time, some older antibiotics may show unexpected effects against multidrug-resistant KP strains.

Objective: To investigate azithromycin (AZM) effects on the resistance phenotype of some multidrug-resistant KP isolates.

Methods: Four multidrug-resistant KP isolates, specifically, UHI 329, 1090, 1633, 1667 were adapted to 5 mg/ml AZM in MH broth through a sequential daily passaging in planktonic and biofilm forms. After adaptation, antibiotic resistance phenotypes of three randomly selected colonies were measured and compared with wild-type isolates.

Findings: As a result of adaptation, all cultures lost their sensitivity to AZM, but the analysis of growth retardation zones revealed an increase in sensitivity to certain β-lactams (piperacillin-tazobactam, ceftazidime-avibactam, meropenem and imipenem), which can be due to changes in the efficiency of efflux pumps. The susceptibility to meropenem and imipenem increased particularly well; for meropenem, strains moved from the R to S or I category (KP UHI 1667). Interestingly, a more pronounced increase occurred in biofilm isolates. The susceptibility to fluoroquinolones (ciprofloxacin) and aminoglycosides (tobramycin) did not change.

Conclusions: Thus, prolonged exposure of multidrug-resistant strains of KP to AZM resulted in resistance to AZM itself, but increased their sensitivity to certain β-lactams, primarily meropenem. This creates prospects for combination therapy for highly multidrug-resistant KP infections.

Abstract ID: 4064 / Poster Number: 070.15P

Azole resistance in yeast from borehole water in Mahikeng, South Africa: Diversity, Public Health Risks, and the need for continuous monitoring

Presenting author: Dr. Deidre Van Wyk, North-West University, Potchefstroom, South Africa

This study investigates the presence and azole antifungal resistance profiles of yeast isolated from borehole water in rural Mahikeng, South Africa. Over two years (2023–2024), water samples were collected from 15 boreholes across three villages. Yeast isolates were identified using biochemical assays and 26S rRNA sequencing, revealing increased diversity in 2024, with 40 isolates compared to 35 in 2023. The dominant species, *Rhodotorula mucilaginosa*, remained prevalent across both years. Antifungal susceptibility testing showed high azole resistance levels, with fluconazole resistance increasing from 80% in 2023 to 85% in 2024, while miconazole and itraconazole also exhibited reduced efficacy. Physiochemical parameters were within South African water quality standards but fluctuated across years, potentially influencing yeast distribution. The presence of opportunistic pathogens such as *Cryptococcus* and *Candida* highlights potential public health risks, particularly for immunocompromised individuals. The study underscores the need for continuous groundwater monitoring and inclusion of yeast in routine water quality assessments. Findings provide critical insights into the environmental distribution of antifungal-resistant yeasts and their implications for One Health frameworks. Further research is essential to mitigate risks associated with resistant yeasts in water sources and develop effective antifungal management strategies.

Abstract ID: 854 / Poster Number: 277.17P

Bacillus mycoides as a potential biocontrol agent for Fusarium suppression

Presenting author: Ms. Galina Jevđenović, University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia, Belgrade, Serbia

Co-authors: *Magdalena Knežević, Jelena Maksimović, Marina Jovković, Marina Dervišević, Aneta Buntić*

Introduction: Suppression of fungal pathogens that are responsible for massive crop losses, with *Fusarium* species as the most prevalent ones, represents a huge challenge in sustainable agriculture. Application of bacterial inoculants is an effective alternative to chemicals as it poses no health risks and has an insignificant impact on the environment.

Objective: The application of new bacterial strain *Bacillus mycoides* BHC 3.2 for suppression of *Fusarium poae*, *Fusarium graminearum*, *Fusarium proliferatum* and *Fusarium oxysporum* was the objective of this research.

Method: Antifungal activity of isolates was tested *in vitro* on PDA medium using dual culture method and inhibition of fungal growth was calculated based on the formula: Inhibition(%)=[(Fungal control (mm) - Treatment (mm))/Fungal control (mm)] x 100. Production of siderophores and indole-3-acetic acid (IAA) was determined by growing strain on Chrome Azurol S (CAS) Blue agar and in tryptophan (2 mg/mL) enriched nutrient broth (NB) by using Salkowski reagent, respectively.

Findings: Among tested fungal pathogens, strain BHC 3.2 showed the strongest antagonistic effect against *F. poae* with inhibition over 45%. BHC 3.2 also showed antagonistic effect against *F. graminearum, F. proliferatum* and *F. oxysporum* with inhibition lower than 35%. The production of siderophores was not recorded for BHC3.2, while the production of IAA was 3.69 μg/mL.

Conclusion: Previous studies showed that *B. mycoides* could be used for reducing the severity of *Fusarium* infections in crops. Results of this research highlight the feasibility of utilizing *Bacillus mycoides* BHC 3.2 in formulating biofertilizers, thereby offering environmentally friendly strategy for plant pathogens control.

Abstract ID: 3551

Bacillus subtilis ensures high spore quality in competition with *Salmonella Typhimurium* via the SigB-dependent pathway

Presenting author: Prof. Ines Mandic Mulec, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

Co-authors: *Eli Podnar, Tjaša Danevčič, Barbara Kraigher, Bram Lories, Marko Petek, Špela Baebler, Hans Steenackers, Kristina Gruden*

Introduction: Microbial interactions are driving forces in microbial ecology and play a crucial role in the development and management of biotechnological and biocontrol applications. Several studies have shown that B. subtilis upregulates sporulation in the presence of other bacteria, mainly for its protection against competitors.

Objective: Here we investigate the interactions between the probiotic strain Bacillus subtilis PS-216 and the pathogen Salmonella Typhimurium SL1344 under sporulation promoting conditions to determine recognition cues and consequences of this competition.

Methods: Bacteria grown as mono or coculture in static biofilm model system and in nutrient limited conditions. Their competition is followed by colony counts, cell sorting, fluorescent microscopy and testing of specific mutants, reporter fusions and finally evaluated by transcriptomics.

Results: We show here that sporulation of B. subtilis is impaired when it competes with S. Typhimurium in a nutrient-depleted medium, while fitness of salmonella is not jeopardized. The sporulation impairment in *B. subtilis* is mediated by the SigB-dependent general stress response, as the ΔsigB mutant remains blind to manipulative cues from S. Typhimurium. Furthermore, we show that decreased sporulation frequency in B. subtilis depends on cell-cell contact between the two species involving the type VI secretion system (T6SS) of salmonella. Finally, transcriptomic analyses show dramatic differential expression of genes in coculture.

Conclusion: Results reveal a novel response of B. subtilis to interspecific competition and nutrient limitation, in which it favours growth over sporulation at an earlier stage. While sporulation has been considered as the primary defence mechanism of B. subtilis, here we show that co-culture with S. Typhimurium stimulates B. subtilis to adopt a strategy to impair sporulation and via SigB-dependent response trades spore quantity for higher spore quality in presence of salmonella.

Abstract ID: 2597 / Poster Number: 442.15P

Bacteria genome rewriting by the developed genome editing HoSeI method

Presenting author: Prof. Kaneyoshi Yamamoto, Hosei university, Tokyo, Japan

Bacteria genome editing is generally achieved by cutting and repairing at specific loci on the genome. Our HoSeI (Homologous Sequence Integration) method based on CRISPR-Cas and λRed is *Escherichia coli* genome editing technology at the single nucleotide level without genetic marker, enabling us to edit DNA sequences at several loci on the genome. The PCR-based construction of an sgRNA expression plasmid, called a genome cutter with CRISPR-Cas, prepared a set of plasmid cutting at more than 1,000 specific loci on *E. coli* genome. An ds DNA, called a genome editor with λRed, is prepared by hybridization of a pair of complementary synthetic oligonucleotides. The designed genome editor changed codon including stop codon on an open reading frame to shorten genetic information and substitute site-specific amino acid residue. Additionally the introduction of two FRT sequences deleted 4 kp DNA sequence on *E. coli* genome by Flp. Repetition of HoSeI method isolated 5-loci editing, 30-loci editing, and 34-loci editing *E. coli*. Our efforts developed HoSeI method enable to rewrite *E. coli* genome sequence by multi-editing on its genome without genetic marker, meaning creation of non-genetically modified and artificial bacteria.

Abstract ID: 868

Bacteria Living in Biofilms in Biological Fluids: a Paradigm Shift in Diagnostics?

Presenting author: Prof. Lorenzo Drago, UOC Laboratory of Clinical Medicine with Specialized Areas, IRCCS MultiMedica, Milan, Italy; Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

Co-authors: *Fabiana Giarritiello,*

Introduction: Bacterial biofilms, complex aggregates of microorganisms embedded in a self-produced extracellular matrix, are often associated with chronic and recurrent infections. While traditionally studied on surfaces, emerging evidence highlights their presence in biological fluids such as synovial fluid, urine, or blood. These non-attached biofilm aggregates present unique diagnostic challenges, as their protective matrix shields bacteria from antibiotics and conventional detection methods.

Objectives: This study aims to underscore the diagnostic implications of bacterial biofilms in biological fluids and propose a paradigm shift in microbiological practices. Specifically, it highlights the need for innovative strategies to detect and study biofilms in fluid environments, advocating for their inclusion in clinical workflows.

Methods: Biological fluid samples, including synovial fluid, urine, etc, were obtained from patients treated at the MultiMedica Laboratory of Microbiology, Milan. Samples were cultured on specific agar media to isolate bacterial colonies. Observations from these cultures, combined with existing literature, were used to analyze the role of biofilm aggregates in diagnostic accuracy.

Findings: Biofilm aggregates were identified in all analyzed fluid types, with significant clinical implications for chronic infections. Current diagnostic methods, designed for planktonic bacteria, exhibit reduced sensitivity in detecting biofilm-associated microorganisms. The biofilm state influences bacterial metabolism, virulence, and antibiotic resistance, complicating therapeutic strategies.

Conclusion: Biofilms in fluids represent an overlooked but critical factor in diagnostics and treatment planning. By incorporating biofilm-specific detection methods into routine diagnostic protocols, we can achieve more accurate pathogen identification and improve patient outcomes. This shift is essential for addressing the challenges posed by biofilm-associated infections.

Abstract ID: 3958 / Poster Number: 069.15P

Bacteria's Swiss Army Knife for Plastic Biodegradation

Presenting author: Ms. Shakira Mustari, University of Turku, Turku, Finland

Co-authors: *Margalida Fontcuberta-Rigo, Suni Mathew, Aude Iris-Sanchez, Clara Brunet, Masaya Yamamoto, Marjo Helander, Kari Saikkonen, Miho Nakamura, Pere Puigbò*

The constant physical and chemical erosion of plastic materials produces microplastics and nanoplastics that find their way into food-web of aquatic and terrestrial ecosystems. Furthermore, biological processes are also involved for plastics to be degraded naturally in the wild. Our research team aims to understand the role of biofilms for plastic biodegradation in natural environments. Within this framework, we have characterized bacterial biofilms of macro-plastic biotopes from two geographical locations in Spain and Finland.

Plastic samples were randomly collected by applying environmental sampling technique from a sandy beach environment and analyzed individually. After removing non-attached biofilms from the samples, an in vitro biofilm culture method was applied to culture biofilms separately. Subsequently, bacterial monocultures were obtained using the streak-plate method, and amplicon sequencing to obtain 16S rRNA for phylogenetic characterization and Gram Staining for further morphological analysis. Moreover, Individual plastic pellets were also analyzed with a Fourier-Transform Infrared Spectroscopy (FTIR) to identify the type of plastic particle. The isolates were mapped onto a database of plastic-degrading enzymes in archaea and bacteria, to determine their biodegradation capacities, and functional and domain annotations were performed with the KEGG and InterPro databases.

This experimental study on microbial biofilms of the plastisphere, in combination with bioinformatics eco-evolutionary analyses, will contribute to the understanding of the environmental adaptations of plastic-degrading enzymes in prokaryotes.

Abstract ID: 3008 / Poster Number: 223.16P

Bacterial amyloids and their effect on the permeability of intestinal and brain blood barriers

Presenting author: Ainara Aginaga-Etxamendi, PhD, Instituto de Agrobiotecnología (IdAB, CSIC), Mutilva, Spain

Co-authors: *Miriam Serrano, Jaione Valle, Macarena Sanchez, Alejando Toledo-Arana*

The gut-brain axis is a bidirectional pathway between the gastrointestinal tract and the central nervous system. A dysbiosis of the gut microbiota can affect the function of intestinal and blood-brain barriers, allowing microbiota-derived products to enter the central nervous system and exert undesirable effects. The discovery of bacterial amyloids in the gut microbiota, including the Esp protein of *Enterococcus faecalis*, has sparked interest due to their potential connection with neurogenerative diseases. Our group has demonstrated that Esp amyloids induce the aggregation of human α-synuclein, which is a key pathological feature of Parkinson's disease.

Here, we investigated whether the Esp amyloids from *E. faecalis* affected intestinal and brain blood barrier permeability. Using cellular models of the intestinal barrier based on Caco-2 epithelial cells cultured on Transwell-plates, besides brain endothelial cells for the blood-brain barrier, we determined that bacterial amyloids induced changes in the integrity of the cellular barriers. Immunofluorescence imaging of zonulin-1, claudin and occludin-1, along with the permeability assay using FICT-dextran, revealed disruptions in the intercellular junction of monolayers cells treated with Esp amyloids. Moreover, Western Blot results showed lower levels of the tight junction proteins in treated cells.

These results demonstrated that Esp amyloids can compromise the integrity of the intestinal and blood-brain barriers, facilitating the spread of amyloids into the central nervous system. Our results contributed to deciphering the mechanisms by which amyloids from the microbiota may contribute to the development of neurological diseases and suggest amyloids could serve as a therapeutic target for treatment of neurogenerative diseases.

Abstract ID: 3516 / Poster Number: 414.17P

Bacterial C-F bond cleavage in action: searching for new defluorinases on the path from fluoroacetate to short-chain PFAS dehalogenation

Presenting author: Mr. Enrico Bocconetti, GMGM, UMR 7156 CNRS, University of Strasbourg, Strasbourg, France

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Per- and polyfluoroalkyl substances (PFAS) are a heterogeneous group of synthetic, often toxic, xenobiotic compounds extensively used in all types of applications. Due to their chemical stability, PFAS are ubiquitous contaminants that progressively accumulate in the environment [1]. Microbial degradation of PFAS represents a promising alternative to address PFAS contamination, complementing physical and chemical remediation strategies. Despite increasing evidence of C–F bond cleavage in PFAS molecules by living organisms, the underlying mechanisms and frequency of this process are still not well characterized. At the same time, the effects of PFAS on microbial communities remain poorly understood, limiting not only our ability to fully harness their potential for bioremediation strategies and a comprehensive understanding of the environmental toxicity of these chemicals.

Short-chain PFAS are particularly challenging due to their low adsorptive potential on adsorbent matrices used in remediation processes such as activated charcoal, and their mobility in the environment [1]. We seek to identify microorganisms capable of degrading short-chain PFAS and to characterize and optimize the corresponding defluorination enzymes, making use of a novel aptamer biosensor for fluoride detection [2]. This work aims to: a) investigate bacterial responses to fluorinated compounds; b) assess the effects of PFAS on microbial community composition to facilitate the detection of PFAS contamination and support remediation of PFAS-polluted sites.

The fluoroacetate-degrading strain *Caballeronia* sp. S22 was investigated as a model system of microbial defluorination [3]. This strain was shown to degrade several mono- and difluorinated short-chain compounds, to grow using “presumably xenobiotic” fluorinated substrates as its sole carbon source, and to tolerate high concentrations (>100 mM) of short-chain fluorinated compounds and fluoride. Genomic analysis tentatively identified a unique cluster of genes potentially involved in the metabolism of fluorinated compounds [3] along with other genes encoding putative defluorinases.

Some of these proteins were heterogously produced and exhibited defluorination activity.

In parallel, incubation experiments of PFAS-contaminated soil and groundwater samples with short-chain fluorinated compounds were set up to explore bacterial defluorination in microbial communities. Cultivable microorganisms with defluorinase activity were isolated, including from taxa not previously associated with defluorination. Ongoing experiments are expected to provide insights on the impact of different PFAS on the composition of microbial communities and to help identify specific bacterial taxa as bioindicators of PFAS exposure.

Abstract ID: 2987 / Poster Number: 265.15P

Bacterial Community and Antibiotic Resistance in an Aquaponic System Farming Fish and Lettuce

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Co-authors: *Verónica Camelo, Maria Dolores Molina, Cristina Pablos, Javier Marugan, Olga C. Nunes, Célia M. Manaia*

Aquaponics, integrated production of fish and vegetables, is a sustainable farming method, particularly in water-scarce areas. These systems, rely on water recirculation between fish and vegetable tanks through a biofilter, making water quality crucial for productivity and food safety.

This study assessed a small-scale tilapia and lettuce aquaponics system (in operation for 20 weeks), for bacterial abundance, relevant microbiological contamination and possible circulation of bacteria between fish and vegetables. Samples were collected overtime from fish gut&gills, lettuce roots, water tanks and biofilter. The microbiota was desorbed by sonication, which water and water from tanks was filtered (0.22 µm) for total DNA and bacterial cultures isolation. Total DNA was examined by quantitative PCR (16S rRNA, intI1 gene) and 16S rRNA metabarcoding. Cultures were identified by MALDI-TOF.

Total bacterial abundance (16S rRNA gene) ranged between 2.6-2.9 log-units/mL in lettuce and fish tanks. Total bacteria desorbed from biofilter rocks reached 6.6 log-units/g. Initially below detection limits, the gene intI1 reached 3.7 log-units/g of rock and 1.2 and 0.9 log-units/mL in fish and lettuce tanks, respectively. While bacteria increased in both fish (5.6 log-units/mL to 7.0 log-units/mL) and lettuce roots (4.8 to 7.0 log-units/mL), intI1 decreased in fish (3.7 to 3.0 log-units/mL) and increased (2.6 to 4.0 log-units/mL) in lettuce. Colonized by different bacterial species, some such as *Serratia fonticola* were shared by lettuce roots and fish at the end of the experience. Biofilter, fish and lettuce seem to represent barriers for the proliferation of unwanted microorganisms, although some may circulate in the system.

Abstract ID: 2688 / Poster Number: 374.15F

Bacterial community colonizing polypropylene during the composting process

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Plastic waste is a global problem, particularly polypropylene (PP) trellising guides widely used in intensive horticulture, poses a significant environmental challenge. These materials are often discarded with plant residues at the end of the growing season, complicating the typical treatment through composting. However, the composting process itself provides a dynamic and diverse ecosystem that could facilitate the colonisation of highly resistant materials like as plastics. Therefore, this study focused on analysing the microbial population associated with precolonised (PP-precolonised) and new (PP-new) polypropylene trellising guides during the composting process. Samples of both PP types were collected from a composting pile of horticultural plant residues. Metabarcoding sequencing was employed to compare taxonomic groups between the samples at the beginning and end of the composting period. Cultivar analysis was also conducted to isolate and identify bacteria with broader plastic-degrading enzymatic profiles. Results of relative abundance at the Order level revealed differences between PP-new and PP-precolonised samples. In PP-new samples, Micrococcales (26.78%) and Rhodobacterales (15.06%) were initially codominant, while Pseudomonadales (29.31%) and Bacillales (19.09%) became dominant at the end of the process. In contrast, PP-precolonised samples showed more consistent dominance, with Micrococcales (19.95%) and Bacillales (27.09%) remaining prominent throughout the composting period. A total of 13 bacterial isolated were identified, highlighting the *Bacillus* and *Priestia* genera, exhibiting a broad range of plastic-degrading enzymes. These findings suggest that the composting environment may indeed facilitate the development of microbial communities capable of colonizing and potentially degrading plastic materials.

Abstract ID: 470 / Poster Number: 186.15P

Bacterial contamination of surgical gloves during clean orthopedic surgery

Presenting author: Prof. Gyu Min Kong, Haeundae Paik Hospital, College of Medicine, Inje University, Busan, South Korea

Co-authors: *Jee Young Lee*

Introduction: Surgical procedures, often assumed to be sterile, are surprisingly prone to bacterial exposure. A prior study reported a high rate of positive intraoperative back table swab cultures, suggesting potential hand contamination among surgical participants.

Objective: The objective of this study is to investigate the frequency of hand contamination among surgical participants and appropriate handling methods based on previous findings.

Methods: This study included 35 surgeries conducted from April 2023 to June 2023 for closed fractures or noninfectious arthrosis. The gloves of the surgeon, first assistant, and scrub nurse were examined for bacterial contamination at different stages: draping, unpacking and transferring implants to the scrub nurse, and at the end of the surgical procedures.

Findings: Contamination was observed on the gloves of the surgeon and assistant at the end of the procedure in 4 (11.4%) and 5 (14.3%) of the 35 cases, respectively. The gloves of scrub nurses were contaminated in 5 cases after receiving artificial inserts and in 1 case at the end of the procedure. Staphylococcus hominis was the most common contaminating bacteria, with eight cases, including two that overlapped with Corynebacterium contamination.

Conclusion: During operations, glove contamination increases for the surgeon and assistant near the end of the operation, and scrub nurses are at a higher risk of contamination when opening the packaging and handing over the artificial insert. Therefore, changing gloves at specific perioperative periods is recommended regardless of glove contamination, and extra caution should be exercised when opening the packaging and handing over artificial inserts.

Abstract ID: 1218 / Poster Number: 238.17P

Bacterial degradation of graphene oxide

Presenting author: Katia Touahri, PhD, University of Strasbourg, Strasbourg, France

Co-authors: *Catherine Grosdemange-Billiard, Didier Lièvremont*

Background: Graphene is one single layer from the thousands composing graphite. Due to unique properties, the use of graphene materials became widespread, raising the question of their fate in the environment. Graphene oxide (GO) was selected as a model for this study.

Objectives: We aim to screen bacterial strains previously isolated from graphite ores for their ability to degrade GO.

Methods: Synthetic culture media were used to obtain a comprehensive view of the bacterial diversity. The isolates were identified by 16S rRNA gene sequence analysis.

Bacterial isolates belonging to genera known for the degradation of polyaromatic hydrocarbons (PAHs) were cultured in presence of GO for weeks without a carbon source. The integrity of GO was monitored using raman spectroscopy and transmission electron microscopy.

Results: We determined the culturable microbial diversity of graphite ores. The majority of the isolated bacterial species belonged to Proteobacteria and Actinobacteria and many of the genera identified had at least one representative strain able to degrade PAHs according to literature.

Furthermore, among the 17 genera tested, 6 isolates belonging to the 3 genera Arthrobacter, Pseudarthrobacter and Paenarthrobacter were able to fragment GO after 3 months of incubation. They belong to the phylum Actinobacteria.

Conclusions: This work represents the first study to uncover the microbial diversity of graphene ores and to demonstrate the ability of environmental bacterial isolates to fragment graphene oxide. Thus, they represent potential candidates that could play key roles in the bioremediation of emerging graphene-based materials.

Abstract ID: 1067

Bacterial degradation of the toxic halogenated solvent dichloromethane in aquifer microcosms: impact of disturbances in physico-chemical parameters

Presenting author: Dr. Emilie Muller, CNRS, Strasbourg, France

Co-authors: *Carmen Lázaro Sánchez, Gwenael Imfeld, Stéphane Vuilleumier*

The ability of a microbial population to degrade a pollutant *in situ* also depends on the physico-chemical parameters it is exposed to. Dichloromethane (DCM, CH_2Cl_2) is a toxic industrial solvent, frequently detected in groundwater and biodegradable under both oxic and anoxic conditions. Previous work with laboratory-scale aquifers demonstrated that DCM biodegradation was more pronounced under fluctuating water level conditions. We hypothesized that matrix porosity, which strongly impacts water content and redox conditions in aquifers, affects DCM bioremediation in natural environments. To test this, we studied the effect of matrix granulometry, water content and oxygen concentration, and the response of a drastic change in oxygen concentration on DCM biodegradation kinetics and bacterial community composition.

Laboratory microcosms with beads of 2, 0.5 or 0.1 mm of diameter were set up with contaminated industrial groundwater showing DCM biodegradation. Experiments involved addition of 7 successive pulses of 1 mM DCM at different water content, i.e. water saturation, water holding capacity (WHC) or ½ WHC, and under different oxygen conditions, i.e. stable oxic or anoxic conditions, or disturbed with a sudden switch in oxygen content.

The obtained results showed that water content act as a first selection barrier, since the community did not degrade DCM when water content was very low. In addition, the onset of the activity was faster at higher water contents. When water content was not a limiting factor, oxygen concentration was the main driving force behind both the activity and composition of the microbial community, with anoxic conditions being the main limiting factor for DCM biodegradation and taxonomic richness. Furthermore, an effect of water content on community composition was observed, suggesting that, under anoxic conditions, the community had a more deterministic dynamic. The matrix granulometry also influenced the DCM-degrading community activity and composition, but mainly through its control over water content. In particular, finer grain sizes facilitated the maintenance of the activity of the DCM degrading microbial community at the lowest tested water content (i.e., ½ WHC). Finally, the effect of a disturbance in oxygen content

differed depending on the initial conditions, and ranged from susceptibility to disturbance to the emergence of the DCM degradation function.

Taken together, our results suggest that granulometry informs the effect of water content on DCM biodegradation and bacterial community composition. In contrast, the effect of oxygen content on DCM biodegradation was independent of grain size.

Abstract ID: 1741 / Poster Number: 044.15P

Bacterial dynamics in a tropical water reservoir during high and low water levels

Presenting author: Rocio J Alcántara-Hernández, PhD, Instituto de Geología, Universidad Nacional Autónoma de México, Mexico City, Mexico

Co-authors: *Ana MJ Reyes-Hernández, Mariel Barjau-Aguilar, Jorge A Ramírez-Zierold, Carolina Mateos-Salmón, Gloria Vilaclara, Martín Merino-Ibarra*

Water reservoirs are aquatic key systems that guarantee water availability in cities around the world; yet, these systems are in constant change, especially during the last years, where pollution and drought have affected them in a significant way. The objective of this study was to observe the changes in the bacterial composition and community structure in a tropical water reservoir during three phases: 1) a high-water-level, 2) a transition status and 3) the lowest water level reported in the last 12 years. A 16S rRNA metabarcoding aproach was used to survey a total of 94 water samples taken at different moments and considering a vertical profile in the water column, further often measuared limnological parameters were determined. The most relevant change in the bacterial composition during the low water level status was the increased abundance of Cyanobacteria, with up to 82% of relative abundance when the resevoir was at a 26% of its capacity due to the drought. Further, this low level water was also associated with a high input of nutrients N and P by rivers and local sewage, estimated in 566 ton-N per year and 75 ton-P per year. This low level period then showed lower bacterial diversity compared to the high-water-level status, when the reservoir reached 100% of its maximum capacity. Therefore, these results show the impact of droght and pollution over the bacterial communities of an aquatic ecosystem vital for human development.

Abstract ID: 1963

Bacterial extracellular vesicle vaccine platform within One health

Presenting author: Prof. Hanne Winther-Larsen, University of Oslo, Oslo, Norway

Co-authors: *Verena Mertes, Dirk Linke, Athanasios Saragliadis, Elisa Mascherin*

Introduction: Extracellular vesicles (EVs) are nano-sized membrane enclosed entities that are secreted from the bacterial body. Their release is associated with a range of phenotypes including cell-cell communication, host-pathogen interactions and antimicrobial resistance. EVs also elicit an immune response after immunization of a host making them an interesting vaccine candidate.

We have investigated the use of EV as a potential vaccine against several bacterial pathogens creating problems in farmed animals including fish. Immunization with EV revealed protection against some diseases, but not in others where we hypothesized that the EV was part of the microbial pathogenesis.

Objectives: To circumvent possible negative side effects of using natural EVs, we have explored the possibility to use recombinant EV (rEV) produced in non-pathogenic in *E. coli* expressing antigens from other infectious agents (Figure 1).

Methods: We have expressed antigens from the fish bacterial species *Yersinia ruckeri* in *E. coli*. The rEV was isolated using tangential flow and ultracentrifugation before analysis by transmission electron microscopy (TEM) and nano-particle tracking analysis (NTA). Antigen expression was confirmed using SDS-PAGE and proteomics analysis, while their potential toxicity was tested on fish cell lines.

Findings: We could isolate rEV form E. coli using tangential flow and ultracentrifugation which was confirmed by TEM analysis. rEV particle size was from 120 – 180 nm pending on *E. coli* strain used and the expressed antigen. The number of antigen expressed seem to effect the *E. coli* viability. The rEVs did not elicit any toxicity when exposed to fish cells in culture.

Conclusion: These results show that *E. coli* has the potential to be utilized as a vector for production of EVs expressing antigens from other bacterial species. These rEVs will hopefully circumvent the negative side effects of native EVs isolated from the pathogenic bacteria when used as vaccines and may enhance the repertoire of EVs based vaccines within animal health.

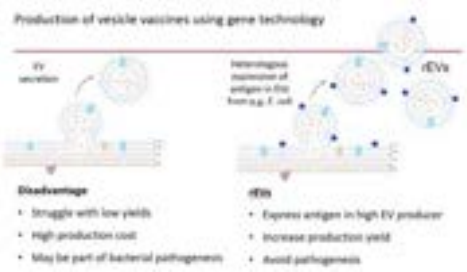


Figure 1. Secretion of extracellular vesicles (EVs) from the bacterial membrane and capture of the major disadvantages using native vesicles versus recombinant vesicles (rEVs) for vaccine development in animal health. The expression of the recombinant antigen is shown as dark blue stars.

Abstract ID: 1777 / Poster Number: 006.16S

Bacterial fermentation dominates over respiration in oxic soil during hypoxia

Presenting author: Mr. Thanh Nguyen-Dinh, Monash University, Melbourne, Australia

Co-authors: *Tess Hutchinson, Wei Wen Wong, Pok Man Leung, Rachael Lappan, Perran Cook, Chris Greening*

Fermentation and respiration are two key metabolic processes driving energy production and nutrient cycling in soil ecosystems. In the soil, conditions frequently shift between oxia and anoxia, forcing microbes to be metabolically flexible. For instance, certain bacteria, such as *Mycobacterium smegmatis*, can switch between fermentation and respiration to maintain redox homeostasis and ATP production under these changing conditions. However, the community-level dynamics of these processes during oxic-anoxic transitions remain largely unexplored. Here, we investigated how soil communities from diverse ecosystems (i.e. forest, garden, and wetland soils) in Victoria, Australia maintain their metabolism during these transitions. Our results showed following anoxia, microbial communities in oxic and bioturbated soils from garden and forest sites excreted acetate (**Fig.1D,E**) and predominantly produced hydrogen (H_2), even when nitrate was provided as a terminal electron acceptor (**Fig.1A,B**). This suggests that fermentation is more prevalent than nitrate respiration in these soils. Additionally, using differential isotope labelling, we observed rerouting of central carbon metabolism through the Embden-Meyerhof-Parnas fermentation pathway in these soils. By contrast, microbial communities in wetland soils, which frequently experience anoxic conditions, produced minimal H_2 or volatile fatty acids (**Fig.1C, F**), implying there was more coupling between fermentation and respiration. These findings will be corroborated by profiling community composition, functional capacity and microbial activity during oxic-anoxic transitions using metagenomic and metatranscriptomic sequencing. Altogether, our results suggest that microbes adapted to variable oxygen regimes maintain metabolism preferentially through fermentation which appears to be largely uncoupled from anaerobic respiration during transient anoxic conditions.

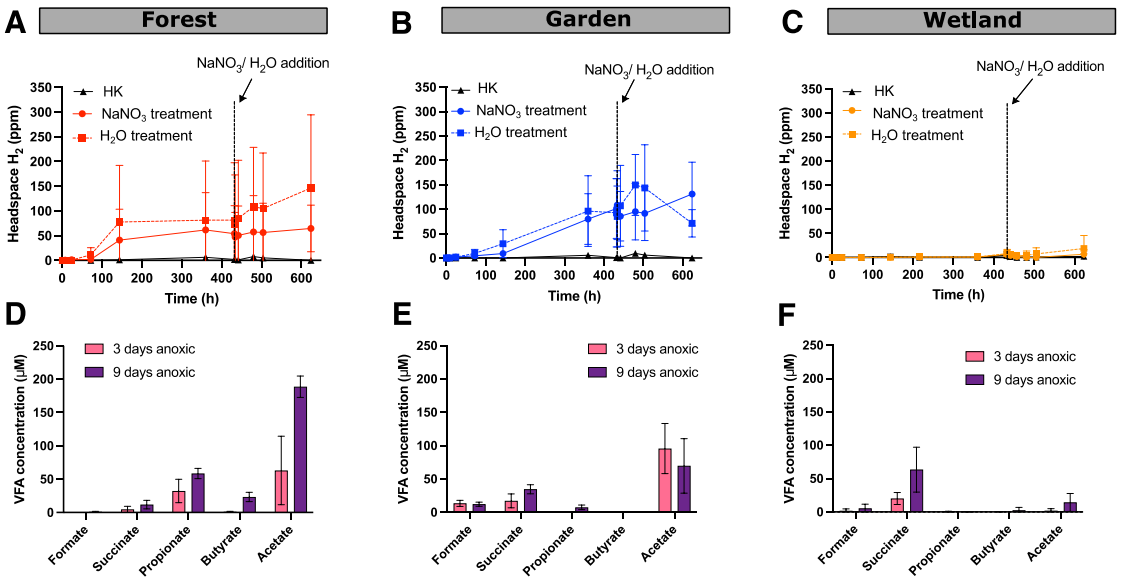


Figure 1. Fermentation and respiration dynamics in forest, garden and wetland soils. (**A-C)** Soil slurry experiments detecting changes in H_2 concentrations during anoxic incubations. Slurries were purged with helium to create anoxic conditions. The dotted line shows the time when 1mM $NaNO_3$ or water was added as the terminal electron acceptor. The centre values show means and error bars show standard deviations from 3 independent slurries. (**D-E**) Measurement of volatile fatty acids in slurry supernatant (n=3); error bars show the standard deviation.

Abstract ID: 355

Bacterial iron uptake system as a delivery route for gene-silencing peptide nucleic acid (PNA) conjugates

Presenting author: Mateusz Wdowiak, PhD, University of Warsaw, Warsaw, Poland

Co-authors: *Uladzislava Tsylents, Monika Wojciechowska, Joanna Trylska*

Introduction: Drug-resistant bacteria are predicted to cause 100 million infections and 10 million deaths annually by 2050, with billions USD in associated costs. This crisis demands novel antibacterial strategies. Antisense therapy, particularly using peptide nucleic acids (PNAs), offers a specific and safe approach. PNAs specifically inhibit bacterial gene expression and show improved stability compared to natural nucleic acids. However, PNA delivery into bacterial cells remains the bottleneck.

Objective: Since iron is essential for bacterial growth, we based the delivery of PNAs into *Escherichia coli* cells on the natural iron uptake mechanism – siderophores – iron chelators produced by bacteria to retrieve iron from the environment. To uptake ferric iron chelated by siderophores, Gram-negative bacteria use a selective TonB-dependent transport (TBDT) system composed of receptors in the outer and inner membranes and periplasm. To overcome the issue of PNA delivery, we developed PNA conjugates with siderophore mimics.

Methods: Inspired by natural siderophores, we synthesized several hydroxamate siderophores, including moanachelin-type variants containing glycine and alanine side chains (M_{Gly} and M_{Ala}). Using click-chemistry, we conjugated these siderophore mimics to a PNA sequence targeting the mRNA of a plasmid-encoded red fluorescent protein (RFP) reporter gene. To evaluate these mimics as PNA carriers, we verified their ability to inhibit *rfp* gene expression using microplate readings and fluorescent confocal microscopy assays combined with live-dead fluorescent staining.

Findings: We observed significant silencing of RFP expression in *E. coli* cells treated with siderophore-PNA conjugates. While the scrambled mismatched anti-*rfp* PNA sequence showed approximately 30-40% gene expression inhibition (measured as a reduction in fluorescence intensity), the complementary anti-*rfp* PNA conjugates achieved 60-80% fluorescence silencing. Notably, the M_{Gly} conjugate showed higher fluorescence silencing (~80%) than the M_{Ala} conjugate (~60%). Additionally, using *E. coli* mutants lacking the genes encoding particular TBDT receptors (including the transmembrane

FepA receptor), we determined that the hydroxamate pathway is primarily responsible for the uptake of our siderophore conjugates.

Conclusions: Using the model reporter gene, we demonstrated that siderophore-PNA conjugates specifically and efficiently silence bacterial genes. In future applications, this approach could be adapted to target essential genes for selective bacterial elimination.

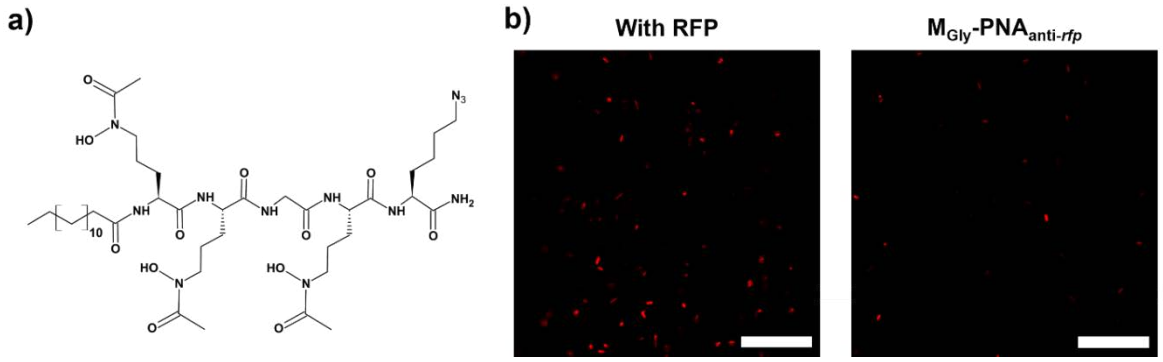


Figure 1: a) Structure of M_{Gly} siderophore. b) Inhibition of *rfp* gene expression by M_{Gly}-$PNA_{anti\text{–}rfp}$ conjugate, shown as decreased fluorescence intensity, compared to untreated *E. coli* Δ*fur* RFP bacteria. Scale bar: 20 µm.

Abstract ID: 650 / Poster Number: 286.16F

Bacterial luciferase as a reporter allowing non-destructive monitoring of intracellular NADPH levels in Bacillus subtilis

Presenting author: Prof. Ken-ichi Yoshida, Kobe University, Kobe, Japan

Introduction: A cell factory of Bacillus subtilis allows the conversion of myo-inositol into scyllo-inositol, a therapeutic agent for Alzheimer's disease. This conversion comprises two reactions catalyzed by two distinct inositol dehydrogenases, IolG and IolW. The IolW-mediated reaction requires the intracellular regeneration of NADPH, and there appears to be a limit to the endogenous supply of NADPH.

Objective: The primary mechanism of NADPH regeneration in this bacterium remains unclear. We aimed to develop a reporter that non-destructively measures intracellular NADPH levels.

Method: The gdh gene of B. subtilis encodes a sporulation-specific glucose dehydrogenase that can regenerate NADPH. gdh was expressed constitutively in the scyllo-inositol cell factory. In addition, a bacterial luciferase of Photorhabdus luminescens was simultaniously introduced.

Findings: Upon expression of gdh, intracellular NADPH levels elevated, increasing the conversion of inositol. Moreover, the bacterial luciferase became more luminescent in cells in liquid culture and colonies on agar plates.

Conclusion: The bacterial luciferase can, therefore, be applied to screen for mutations in genes involved in NADPH regeneration in B. subtilis to elucidate the primary mechanism of NADPH regeneration, which will allow us to manipulate metabolism to enhance NADPH regeneration, promoting efficient production of various value-added chemicals, including scyllo-inositol.

Abstract ID: 3270

Bacterial microcompartments are intracellular powerhouses that can host native and heterologous metabolic pathways in Escherichia coli

Presenting author: Dr. Denis Jallet, Toulouse Biotechnology Institute, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France

Co-authors: *Doran Gregnanin, Vanessa Soldan, Stéphanie Balor, Evelyne Deery, Martin Warren, Pierre Millard, Stéphanie Heux*

Bacterial microcompartments (BMCs) are self-assembling protein megacomplexes composed of a semipermeable shell that encapsulates an enzymatic core. BMCs function as intracellular nanoreactors by spatially isolating specific pathways within the cytosol, sequestering toxic or volatile intermediates, and enabling metabolic channelling effects. Although approximately 20% of sequenced bacterial genomes contain operons encoding putative BMCs, few have been thoroughly characterized.

Our group uses *E. coli* as a model organism to address two main questions related to BMCs:

(i) How do native BMCs contribute to fitness? Ethanolamine (C_2H_7NO) is an abundant substrate in the gut environment. Most *E. coli* strains natively possess an *ethanolamine utilization* (*eut*) operon that contains all the genetic elements required to produce Eut BMCs. We are investigating the function of these Eut BMCs in both laboratory strains and commensal isolates.

(ii) How can the biotechnological potential of BMCs be harnessed? BMCs offer straightforward tools for synthetic biology, enabling the implementation of orthogonal metabolic networks. Introducing heterologous BMC types into *E. coli* could expand the range of available substrates and facilitate the production of novel biological compounds. Currently, we are expressing a heterologous *1,2-propanediol utilization* (*pdu*) operon to exploit the metabolic promiscuity of Pdu BMCs *in cellula*.

We developed an interdisciplinary approach to address these questions, incorporating synthetic biology techniques (MoClo, genome editing), microscopy (fluorescence microscopy, electron cryotomography), analytical chemistry (^{1}H-NMR, LC-MS), systems biology (proteomics, ^{13}C-fluxomics), and modelling (flux balance analysis, isotopic models). Using it, we demonstrated that *E. coli* natively produces well-formed (Figure 1)

and functional Eut BMCs. These Eut BMCs act as orthogonal cytosolic modules, enabling ethanolamine catabolism with minimal proteome reorganization. Specifically, Eut BMCs convert ethanolamine into carbon and nitrogen sources usable for biomass production while also generating significant metabolic overflows. In parallel, we successfully produced well-formed and functional heterologous Pdu BMCs. Beyond their preferred substrate, 1,2-propanediol, we found that Pdu BMCs can metabolize a variety of diols and triols. This promiscuous metabolism generates compounds of biotechnological interest, including several novel bioplastic building blocks.

Overall, our work provides a quantitative molecular and functional understanding of both native and heterologous BMCs in *E. coli*. Future applications will include the production of novel BMC types, including many with still unknown functions, for both fundamental and biotechnological purposes.

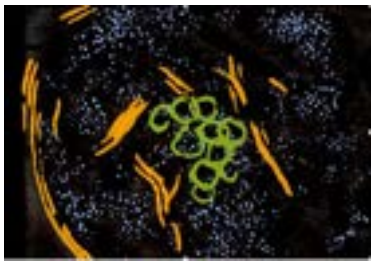


Figure 1: Electron cryotomography followed by 3D segmentation showing the cellular membranes (orange), ribosomes (blue) and native Eut BMCs in E. coli K-12 W3110.

Abstract ID: 4141

Bacterial multi-cellular computing using intercellular DNA communication

Presenting author: Manish Kushwaha, Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France

Co-authors: *Abhinav Pujar, Amit Pathania, Corbin Hopper, Amir Pandi, Cristian Ruiz Calderón, Matthias Függer, Thomas Nowak*

Coordinated actions of cells in microbial communities and multicellular organisms enable them to perform complex tasks that may be too difficult for single cells. This has inspired biological engineers to build cellular consortia for larger circuits with improved functionalities while implementing communication systems for coordination among cells. Here, we investigate the signalling dynamics of synthetic DNA messaging and couple it with CRISPR interference to build distributed circuits performing logic gate operations in multicellular bacterial consortia (doi: 10.1093/nar/gkae1256). We find that physiological states of cells, as well as resource competition between them, shapes communication outcomes. Leveraging the easy programmability of DNA messages, we build several orthogonal signals and demonstrate that intercellular CRISPRi (i-CRISPRi) regulates gene expression across cells. Finally, we multiplex the i-CRISPRi system to implement several multicellular logic gates that involve up to seven cells and take up to three inputs simultaneously, with single- and dual-rail encoding. The communication system presented here lays the groundwork for implementing complex biological circuits in engineered bacterial communities, using DNA as communication signal.

Abstract ID: 2230 / Poster Number: 205.17P

Bacterial resistance evolution on antimicrobial surfaces – Mechanistic insights from a standardizable method

Presenting author: Dr. Niclas Nordholt, Bundesanstalt für Materialforschung und -prüfung, Berlin, Germany

Co-authors: *Selina Schmidt, Viola Boenke, Ina Stephan, Claudia Sündermann, Susann Wiltner, Frank Schreiber*

Introduction: Antimicrobial surfaces and coatings (AMCs) are important to prevent the spread of pathogens, especially in hygiene-sensitive areas. However, the evolution and selection of bacterial resistance to AMCs may threaten their efficacy in the long term. In addition, resistance evolution to AMCs may pose the risk for the development of cross-resistance to antibiotics. The assessment of unacceptable resistance risks during the authorization of AMCs is hampered by the lack of standardized test methods that quantify the adaptability of exposed bacteria to AMCs.

Objectives:

- To develop a standardizable method to determine resistance evolution of bacteria on AMCs (*ALEE-AMC*);
- To assess performance and robustness of ALEE-AMC in a ring trial;
- To uncover the mechanisms underlying evolution of resistance to a metallic copper AMC;
- To use ALEE-AMC to assess the evolution of resistance on a novel, nano-particle-based AMC.

Methods: ALEE-AMC was developed based on an international standard to determine the efficacy of antimicrobial surfaces (ISO 22196). In the ALEE-AMC test, adaptive laboratory evolution is conducted by repeated cycles of AMC exposure and re-growth of surviving cells, selecting for increased survival, followed by isolation of evolved clones. Metallic copper was used as a reference AMC and *Escherichia coli* as a model microorganism in the ring trial. Evolved *E. coli* populations from the ring trial partners were subjected to phenotypic (antimicrobial susceptibility testing, ISO 22196) and genotypic (whole genome sequencing) characterization. ALEE-AMC will be used to assess the evolution of resistance on a novel, nano-particle-based AMC currently under development.

Findings: The results of the ALEE-AMC ring trial show that repeated exposure to a metallic copper AMC can reproducibly select for reduced copper susceptibility in individual evolutionary lineages across ring trial participants. However, failure to adapt in individual lineages was also observed in all trials. Isolated evolved *E. coli* clones exhibited increased survival upon exposure to copper surfaces. Adaptation to copper did not induce cross-resistance to antibiotics because the antibiotic susceptibility of copper-adapted clones did not increase above the clinical breakpoint. Whole genome sequencing of the evolved *E. coli* revealed a high diversity of mutations, including mutations in genes involved in survival to antibiotics. These results indicate the existence of multiple, underexplored evolutionary pathways towards increased survival of antimicrobial copper surfaces.

Conclusion: ALEE-AMC offers a standardizable platform to assess the risk of resistance development towards novel and existing AMCs, including nano-particle-based and metallic copper AMCs. Specifically, using ALEE-AMC provided insights into evolvable survival mechanisms to copper AMCs and its consequences for antimicrobial resistance.

Abstract ID: 3001 / Poster Number: 266.15P

Bacteriocin genes asset shapes Lactobacillus crispatus environmental adaptation

Presenting author: Giulia Santarelli, PhD, Università Cattolica del Sacro Cuore, Roma, Italy

Co-authors: *Roberto Rosato, Maurizio Sanguinetti, Delogu Giovanni, Flavio De Maio*

In healthy reproductive-aged women, the vaginal microbiota is predominantly composed of *Lactobacillus* species, particularly *L. crispatus*, which plays a crucial role in maintaining vaginal homeostasis by preventing the overgrowth of harmful pathogens. This study investigates the genomic relationships and bacteriocin diversity among *L. crispatus* strains to understand the mechanisms driving strain-specific adaptation and microbial competitiveness.

A total of 95 L. crispatus genome sequences were analyzed, including bacteriocin gene detection using BAGEL4 and a core genome multilocus sequence typing (cgMLST) approach to assess genetic diversity. Multiple alignments of bacteriocin genes identified single nucleotide polymorphisms (SNPs) and indels, with conservation evaluated through the ratio of non-synonymous to synonymous substitutions (dN/dS).

The cgMLST analysis revealed significant genetic variation among *L. crispatus* strains, highlighting differences in bacteriocin gene presence and diversification. Animal-derived strains clustered separately from human-derived strains, indicating host-specific evolutionary divergence. The most common bacteriocin, *Enterolysin A*, was present in 87 of the 91 genomes analyzed. Other identified bacteriocins included *Helveticin J*, *SakT alpha*, *Penocin A*, *LAPs*, and *Bovicin 255*, with *Enterolysin A* and *Helveticin J* found in higher copy numbers, suggesting their key roles in bacterial fitness. Mutational analysis indicated that bacteriocin genes are generally conserved, reflecting negative selection pressure.

Our results suggest that *L. crispatus* strain-specific competitiveness is influenced by a core set of conserved bacteriocin genes, while additional cloud genes contribute to niche-specific adaptation. The combination of these genes likely explains *L. crispatus*'s role in maintaining a healthy vaginal microbiota.

Abstract ID: 2105 / Poster Number: 063.16P

Bacteriophage addition to a synthetic bacterial community impacts colonisation resistance

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Co-authors: *R. Paul Ross, Colin Hill*

Background: The commensal gut microbiota provides colonisation resistance against many orally acquired pathogens such as *Listeria monocytogenes*, and disruption of the microbiota by antibiotics reduces resistance to infection. While bacterial community composition has been studied in the context of colonisation resistance, it is also important to consider the impact that highly abundant gut bacteriophages (phages) can have on this phenomenon.

Objectives: To isolate and characterise novel bacteriophages against both pathogens and members of a defined synthetic gut bacterial community to develop a framework to study phage-host dynamics in the context of foodborne infection.

Methods: We isolated and purified novel phages that target *L. monocytogenes* as well as individual members of the Oligo-Mouse-Microbiota (OMM12) bacterial community. We characterised these phages using electron microscopy, host-range assays, and bioinformatic approaches. Next, we assessed the impact of adding bacteriophages to the OMM12 synthetic bacterial community *in vitro* using 16S rRNA amplicon sequencing, bacterial plate counts and plaque assays.

Results: Five novel phages that target *L. monocytogenes* and *Enterococcus faecalis* were isolated and characterised from environmental samples. *In vitro* experiments show that the addition of phages infecting *E. faecalis* impairs colonisation resistance to *L. monocytogenes*. A two-log increase in *Listeria* CFUs was observed in the phage-disrupted community relative to the control. Additionally, phage dynamics and the emergence of phage resistance were characterised in a time course experiment. Uncovering these dynamics and isolating phages that target foodborne pathogens may reveal additional methods by which phages can impact the microbiome and be leveraged as therapeutics.

Abstract ID: 3369

Bacteriophage-based bioremediation to control microbial contaminants in wastewater

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Background: Wastewater is a well-known environmental reservoir for antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs). Conventional wastewater treatment plants (WWTPs) are effective at reducing the overall bacterial load through secondary clarifications. However, like many other bacteria, ARB and their pool of ARGs can still be detected along with antibiotic residues in the treated effluent. These problematic biological contaminants can spread into the environment and the water chain through water reuse. Constructed wetlands are an economic alternative to WWTPs, especially in low- and middle-income countries such as the Republic of Moldova, where mortality rates associated with antimicrobial resistance (AMR) are high. Phage-based biocontrol is an emerging method that has been gaining traction for selectively suppressing pathogens and ARB from wastewater effluents. Bacteriophages (phages) are viruses which can infect and replicate inside a bacterial host, leading to bacterial cell death and the release of the phage progeny.

Objectives: This project aimed to evaluate the effectiveness of a phage cocktail in eliminating ARB from a pilot-scale constructed wetland, whilst also assessing the self-purification capacity of the wetland for removing ARB, ARGs and antibiotic residues.

Methods: Phages were isolated from wastewater and were genotypically and phenotypically characterised. A read-mapping approach was used to quantify the transduction ability of each phage. High-titre stocks of a selected subset of phages were prepared in a bioreactor and subsequently concentrated and purified using anion-exchange chromatography. The pilot-scale constructed wetland was acclimated over 15 days before the inoculation of the phage cocktail, operating with a hydraulic retention time of 24 hours. Chemical oxygen demand (COD) and nitrogen removal was monitored throughout the study, with water samples collected at various time points for metagenomic and viromic analyses of the microbial communities.

Findings: Over 90 phages targeting ESKAPE pathogens were isolated. Host range analysis, conducted against a panel of patient-derived ESKAPE isolates from the *Timofei Moșneaga* Republican Clinical Hospital in the Republic of Moldova, revealed that most phages had a narrow host range. Six of the phages had a broader host range, infecting multiple strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*. Five of the six phages were identified as belonging to known genera; however, one phage was classified within an uncharacterised genus in the *Molineuxvirinae* subfamily. These phages were shown to survive and remain infectious under the operating conditions of the constructed wetland, including low temperatures and UV exposure. Long- and short-read sequencing enabled the generation of complete genomes for each phage and clinical bacterial isolates, which facilitated the assessment of their transduction ability, found to be low.

Conclusion: Phages may prevent AMR spread from wastewater to surface water, leveraging their bacteria-killing specificity in combination with constructed wetland technology to eradicate ARB. However, scaling up from lab to pilot scale presents challenges, and further optimisation is needed. If successful, this technology can hold significant potential to control waterborne AMR release in low- and middle-income countries.

Abstract ID: 3091 / Poster Number: 300.15P

Bacteriophages versus Staphylococci. How phages deal with biofilm formation by these bacteria

Presenting author: Dr. Edyta Kaczorek-Łukowska, University of Warmia and Mazury , Olsztyn , Poland

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Introduction: *Staphylococcus aureus* is one of the aetiological agents of mastitis in cattle. Although the problem of *S. aureus* in dairy cattle has been known for years, a perfect preventive therapy or treatment has not yet been developed. Unfortunately, even with targeted antibiotic therapy, in most cases there is no therapeutic effect and the disease recurs. This is usually the result of a chronic infection where the pathogens have already formed a biofilm in the mammary gland. For this reason, alternatives to antibiotics are increasingly being explored for the treatment of chronic infections. One such option is bacteriophage therapy. Phages are viruses that specifically infect bacteria. Their main advantage over antibiotics is that they are highly specific. The aim of this study was to see how the bacteriophage cocktail developed affects the biofilm-forming ability of field strains of *Staphylococcus aureus*.

Methods In our study, we used *Staphylococcus aureus* strains (n=28) isolated from dairy cattle. The biofilm forming ability of these strains was determined by crystal violet staining in 96-well plates, the expression of *icaD*, *eno* genes was determined by the 2-ΔΔCt method, and microscopic evaluation was performed by fluorescence and scanning electron microscopy.

Findings: A significant reduction in biofilm formation was observed by both crystal violet staining and microscopy.

Conclusions: Our research has shown that the developed cocktail is effective in preventing biofilm formation in the staphylococci studied. This opens up prospects for the future use of this cocktail in the treatment of staphylococcal infections in dairy cattle.

Abstract ID: 260 / Poster Number: 349.17P

Behavior of Non-Saccharomyces Yeasts Microencapsulated by Spray Drying: Viability and Growth Assessment

Presenting author: Ms. Suzana Moreira, FEDERAL UNIVERSITY OF LAVRAS, LAVRAS, Brazil

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The drying process offers a better alternative for preserving epiphytic coffee yeasts. This study evaluated the cell viability of three non-Saccharomyces microencapsulated yeasts during storage and assessed their growth in a coffee-based medium. *Pichia kluyveri* CCMA0237 (PK), *Wickerhamomyces anomalus* CCMA1640 (WA), and *Hanseniaspora uvarum* CCMA0236 (HU) were microencapsulated by spray drying. Whey powder (WP) was used as the wall material. The drying air inlet temperatures were 85°C (WA), 90°C (HU), and 88°C (PK), with WP concentrations of 24.8% (WA), 30.0% (HU), and 18.1% (PK). The cell viability was monitored for three months at 25°C and 7°C. Fresh and microencapsulated yeast growth rates and generation times were evaluated in a coffee peel and pulp media (CPM). After 90 days of storage at 7°C, WA, HU, and PK showed a reduction in the population of 13.04%, 17.42%, and 25.02%. Conversely, at 25°C the reduction was 28.81%, 26.98%, and 33.39%, respectively. Regarding yeast growth in the CPM medium, PK was the only yeast that did not show differences between specific growth rate (0.26h -1 and 0.33h -1) and generation time (2.13h and 2.69h) between the fresh and microencapsulated states. Microencapsulated WA and HU showed a reduction of 38.46% and 52% in specific growth rate and an increase of 68.91% and 105% in generation time, respectively. The results suggest that while microencapsulated PK was more sensitive to storage, it showed better growth in the CPM medium. Future research should be conducted to evaluate the behavior of these yeasts in coffee fermentations with self-induced anaerobiosis.

Keywords: Whey powder, Pichia kluyveri, Wickerhamomyces anomalus, Hanseniaspora uvarum

Abstract ID: 2946 / Poster Number: 411.17P

Benchmarking PacBio full-length 16S rRNA gene amplicon sequencing analysis for characterizing food microbiota

Presenting author: Dr. Mareike Katharina Baer, University of Bonn, Bonn, Germany

Co-authors: *Lisa Höppe, Melina Döring, Sonja Wiegert, Lisa Schmitt, André Lipski*

Characterizing food microbiomes is important to evaluate the presence of pathogens, spoilage organisms, or important aroma producers. Molecular approaches based on 16S rRNA gene amplicon sequencing are increasingly used. Many studies focus on short-read amplicon sequencing, covering only around 500 bp of the 1,500 bp 16S rRNA gene. They allow taxonomic classification only down to family of genus level. However, correct species level identification is of utmost importance in the food sector, as many genera comprise both pathogenic and apathogenic species.

The objective of this study was to assess the suitability of PacBio amplicon sequencing of the full-length 16S rRNA genes for characterizing food microbiomes. We created mock communities of isolates representative of milking machine biofilms and meat, mimicking relevant characteristics of real food samples. These were presence of various species belonging to the same genus, presence of yet undescribed species, as well as over- and underrepresentation of certain species. We aimed to assess the impact of DNA extraction, PCR amplification of 16S rRNA genes, sequencing, and bioinformatic analysis on the observed microbial composition. Finally, the findings of the benchmarking study were used to characterize the microbiota of raw milk.

First, we tested the combined impact of DNA extraction, PCR amplification of 16S rRNA genes and sequencing starting from whole-cell mock communities. Then, we used mixtures of pure culture DNA to assess the impact of PCR and sequencing. Starting from PCR products of pure cultures, we assessed the impact of sequencing alone. We created a custom RefSeq database, which was used for analysing mock communities and raw milk.

We found that PCR amplification of 16S rRNA genes produced deviations between preset and observed species abundances, while sequencing alone nearly preserved the preset abundances (Fig. 1). The effect can in part be attributed to preferred amplification of mol% G+C poor genes. All species present within the mock communities were retrieved and correctly identified using our custom database. This applied also to taxonomically closely related species, e.g. of the genus *Pseudomonas*.

Our findings underline the importance of using mock communities with known species composition to benchmark DNA extraction, sequencing, and bioinformatic analysis procedures. We proved the suitability of long-read 16S rRNA gene amplicon sequencing for analysing the microbiota of raw milk.

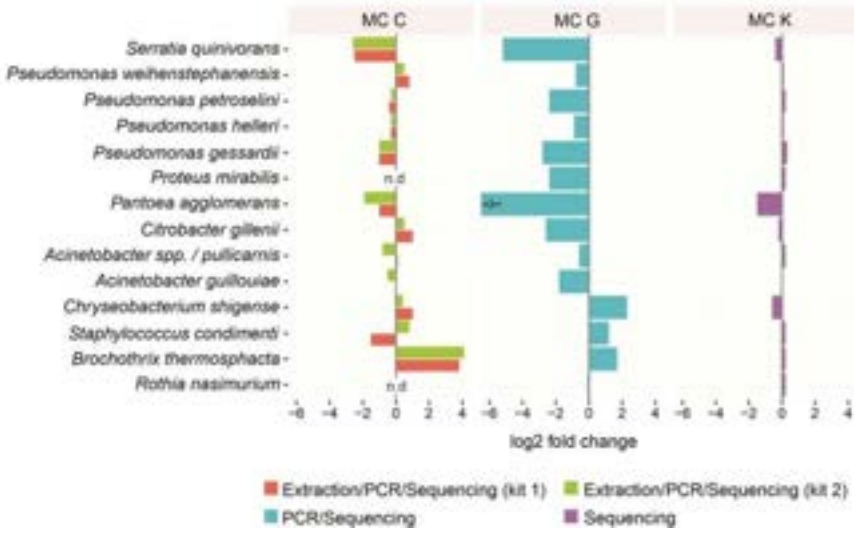


Figure 1: Impact of DNA extraction, PCR amplification of 16S rRNA genes, and sequencing (combined and alone) on the observed abundance of each species within mock communites (MC).

Abstract ID: 1451 / Poster Number: 433.16P

Benefit-sharing from genetic sequence data: what researchers need to do in practice

Presenting author: Ms. Melania Muñoz-García, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Co-authors: *Amber Hartman Scholz*

Do I need to share benefits from sequence data under the Convention on Biological Diversity (CBD)? Digital Sequence Information (DSI), a term broadly referring to genetic sequence data and other biological data types, is central to microbiological research and genetic analysis. Since 2016, the CBD has been deliberating on whether and how to share benefits from the scientific and commercial use of publicly available DSI. Although requiring individual permits for thousands of sequences is impractical and would hinder research and development, provider countries receive nothing from data used towards commercial outcomes. Recognizing these challenges, the DSI Scientific Network has actively participated in CBD negotiations, and called for a multilateral mechanism that preserves open access to DSI while ensuring benefit-sharing.

At COP16 in November 2024, the CBD adopted new rules for sharing benefits from publicly available DSI. We will translate the complex language of these negotiations into simple terms to explain their practical implications for scientific researchers.

What does it mean in practice? Our analysis of the DSI decision addresses real-life questions from researchers, clarifying what remains unchanged, such as open access to DSI, and detailing the new policy requirements. For example, all users must share non-monetary benefits (e.g., open publications, open data, and capacity building), while only commercial users are expected to contribute financially to the global fund.

What is next? We advocate for harmonized rules across four different UN fora that are currently developing new rules for DSI. Aligning modalities across UN fora would preserve open access, ensure data interoperability across databases, enhance benefit-sharing, and foster research and innovation.

Abstract ID: 1561

Beyond Antibiotics: Investigating Aurodox as an Antivirulence Treatment for Shiga-toxin-Producing E. coli Infections

Presenting author: Dr. Rebecca McHugh, University of Glasgow, Glasgow, United Kingdom

Co-authors: *Andrew Roe*

Introduction: Shiga Toxin-producing *E. coli* (STEC) is an acute pathogen of the small intestine which is responsible for foodborne outbreaks of bloody diarrhoea. STEC infections are often associated with high morbidity and mortality rates due to the production of Shiga toxins which can initiate Haemolytic Uremic Syndrome (HUS) which is a major cause of acute renal failure in children. Unlike most bacterial infections, EHEC cannot be treated with traditional antibiotics as they can lead to increased production of Shiga toxin, resulting in poorer patient outcomes.

Aims/Objectives: In these studies, we aimed to investigate aurodox- a natural product of *Streptomyces goldininiensis*- as a potential anti-virulence therapy for the treatment of STEC.

Methods/Findings

To understand its mechanism of action and assess the suitability of this molecule for repurposing as an anti-virulence compound, a multidisciplinary approach to understanding aurodox was used. Whole transcriptome analysis, cell infection and GFP-reporter assays were used to demonstrate that aurodox transcriptionally downregulates the expression of the Type III Secretion System (T3SS)- an essential colonisation factor in STEC. We have also observed these effects across other enteric pathogens carrying a homologous T3SS such as Enteropathogenic *Escherichia coli* (EPEC) and *Salmonella* species. Furthermore, we have used a combination of Vero cell cytotoxicity assays and GFP-reporter assays to determine that, unlike many traditional antibiotics, aurodox does not induce the bacterial SOS response, and, consequently, does not induce the production of Shiga toxin. Finally, we have established a *Citrobacter rodentium* + Stx murine model and proven the efficacy of aurodox treatment against Shiga-toxin-positive infections and the effect of the compound on the gut microbiome.

Conclusion: Aurodox inhibits the T3SS of multiple including STEC. Aurodox protects mice against the effect of Shiga toxin in both the intestines and the kidneys and therefore is an excellent candidate antivirulence therapy for the treatment of STEC.

Abstract ID: 2168 / Poster Number: 369.15F

Beyond the host: Bioinformatic detection of Symbiotic Microbiota in Public Nudibranch Genomes

Presenting author: Ms. Oleanna Guerra-Font, Universitat de Valencia, Valencia, Spain; I2SysBio, Paterna, Spain; CSIC, Valencia, Spain

Co-authors: *Vicente Arnau Llombart, Mária Džunková*

Nudibranchs are soft-bodied marine animals that use secondary metabolites for self-defense, produced either by the nudibranch itself or by its symbiotic bacteria. Their producer can be identified through DNA sequencing. While 30 draft genomes of nudibranchs are available in public databases, only three genera of symbionts have been identified. Our previous metagenomic studies indicated the presence of symbiotic bacteria in high proportions on the nudibranch mantle. We hypothesize that the draft genome assembly of nudibranch genomes may contain traces of their bacterial symbionts.

The main objectives were twofold: firstly, to identify previously unnoticed sequences of bacterial symbionts in publicly available nudibranch genomes, and secondly, to develop bioinformatic pipelines to distinguish symbionts from transient bacteria, with potential applications to any symbiotic system.

Our approach is based on genomic signatures, specifically k-mer distributions, which are useful for identifying the phylogenetic origin of sequences. In this method, the frequency of each k-length word in a sequence is used to generate a unique signature for each organism. K-mers have proven valuable for identifying the phylogenetic origin of sequences previously believed to be exclusively nudibranchs.

We integrated our k-mer program with other tools, such as MetaPhlan, Kraken2, and Barrnap, into a comprehensive pipeline. A detailed analysis of 30 nudibranch genomes from the RefSeq database revealed bacterial DNA in all draft assemblies, leading to the discovery of previously uncharacterized bacterial symbionts.

This workflow is applicable to draft assemblies of animal species with a high proportion of symbiotic bacteria, offering a valuable tool for future symbiotic research.

Abstract ID: 3988

Beyond the Matrix: Rethinking Antibiotic Tolerance in CF Biofilms Using 3D Models

Presenting author: Mrs. Goodness Osondu-chuka, BOKU University, VIENNA, Austria

Co-authors: *Stephan Schandl, Aleksandr Ovsianikov, Erik Reimhult*

While antimicrobial therapies have been intensively developed, chronic lung infection remains an impossible disease to treat, especially in Cystic Fibrosis (CF) patients. No clear explanation exists for the extreme tolerance of CF biofilms to antibiotics. We address how and why these biofilms are impossible to eradicate by studying the influence of the biofilm matrix and developing a platform to simplify and speed up such studies. We have developed 3D biofilm models to mimic lung conditions in CF patients. Commercial alginate was chemically modified to mimic the degree of acetylation of bacterial alginate, mixed with eDNA and P. aeruginosa, and used as bioink to print 3D biofilms. Minimum inhibitory and bactericidal concentrations of Tobramycin, Ciprofloxacin, Meropenem, Colistin, and Aztreonam were determined for the PAO1 strain, followed by kill-curve assays to evaluate antibiotic efficacy. Live-dead fluorescence microscopy and (cryo)-scanning electron microscopy assessed the impact of antibiotics on bacterial viability and biofilm structure. A penetration model explored interactions between alginate (±eDNA) and antibiotics. P. aeruginosa thrived in the printed bacterial alginate, forming microcolonies similar in appearance to in vivo biofilms. Antibiotic assays indicates that acetylation reduces antibiotic effectiveness when compared to non-acetylated alginate. However, eDNA appears to counteract this reduction in efficacy, possibly by stabilizing biofilm structure. The addition of eDNA enhances antibiotic efficacy by reducing CFU/mL to lower levels compared to non-acetylated and acetylated alginate conditions. We further demonstrated that modified alginate and eDNA act as barriers to antibiotic penetration. However, penetration rates improve over time, reaching almost 100% for most antibiotics by 8-20 hours, regardless of the matrix type (with or without eDNA). These results challenge the conventional understanding of biofilm tolerance mechanisms focused on biofilm matrix barrier properties. These findings will open the door to new therapeutic strategies that targets beyond the matrix barrier by concentrating on disrupting bacterial stress responses, quorum sensing and metabolic adaptations within the biofilm environment as the likely mechanisms for CF biofilm antibiotics tolerance.

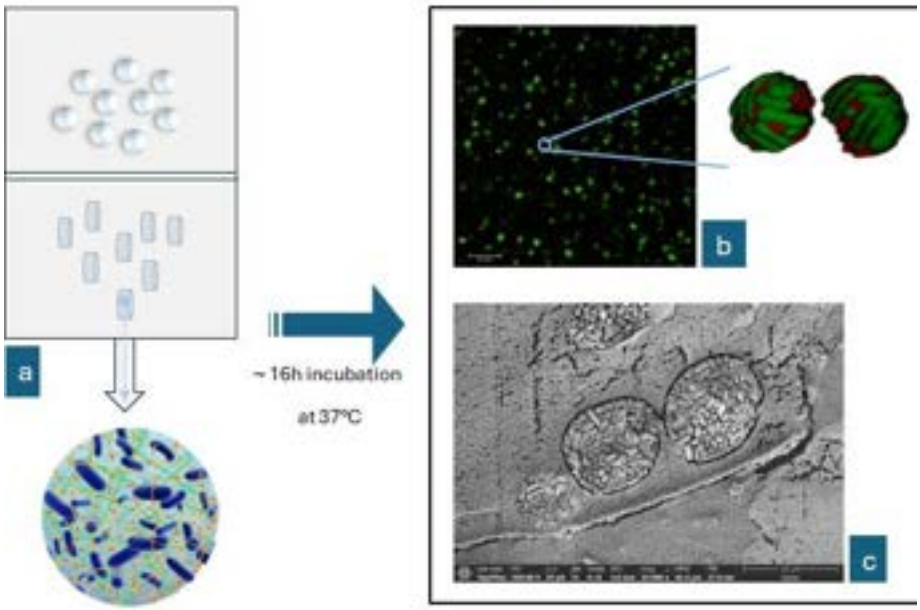


Figure 1: (a) CF-biofilm model (b) CLSM image of *P. aeruginosa* growth pattern in CF-biofilm model. (c) SEM micrograph of P. *aeruginosa* growth pattern in CF-biofilm model

Abstract ID: 2219 / Poster Number: 209.16P

Bile acids as the next generation of postbiotics: 7-KLCA promotes beneficial bacterial species and improves metabolic health

Presenting author: Dr. Abigail Basson, University of Reading, Reading, United Kingdom

Co-authors: *Dannielle Kydd-Sinclair, Kimberly Watson, Glenn Gisbson*

Introduction: Bile acids play crucial roles in physiological processes, including lipid synthesis, bile acid metabolism, and modulation of the gut microbiome. Research highlights their significance in metabolic health through complex signalling systems. Primary bile acids, synthesized in the liver from cholesterol, are converted into primary bile salts before being transported to the gallbladder and secreted into the digestive tract. ~95% are recycled via enterohepatic circulation, while the remaining enters the colon, where gut bacteria convert them into secondary bile acids, acting as signalling molecules. These interactions contribute to glucose and lipid metabolism, influencing the gut microbiome.

Objectives: 7-KLCA, a secondary bile acid, has previously shown potential therapeutic effects on the gut microbiome and metabolic health.

Methods: Fermentation methodologies coupled with cell culture studies were utilised to understand changes in gut microbes and bacterial metabolites, alongside changes to gene expression.

Findings: Fermentation studies demonstrate that 7-KLCA increases beneficial microbes such as *Bifidobacterium* spp., and butyrate-producing bacteria, alongside elevated SCFA levels. These effects differed across the large intestine, supporting positive microbiome shifts. 7-KLCA was able to regulate gene expression linked to healthier metabolic phenotypes, possibly mitigating high-glucose environments by downregulating genes associated with metabolic disorders.

Conclusion: Results of these studies indicate that 7-KLCA can positively influence gut microbial health and metabolic processes. 7-KLCA is a promising candidate for clinical trials, with ethical approval granted for an RCT, as a postbiotic intervention for prediabetes. Positive microbiome, metabolome, and metabolic outcomes could pave the way for its use in treating prediabetes, T2DM, and MetS.

Abstract ID: 600 / Poster Number: 285.16F

Binding site motif based identification of receptor function

Presenting author: Dr. Elizabet Monteagudo-Cascales, Spanish Council for Scientific Research- CSIC, Granada, Spain

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Introduction: The limited information about signals recognized by receptor sensor domains (SDs) is currently a major limitation in microbiology. As SDs show a high degree of sequence divergence, their ligand specificity can rarely be extrapolated from characterized domains by overall sequence similarity. We report a novel approach that permits large scale signal identification based on SD signal binding site sequence motifs.

Objective: Large-scale identification of receptor signals.

Methods: Bioinformatics, X-ray crystallography, differential scanning fluorimetry, microcalorimetric binding studies of individual SDs, site-directed mutagenesis, biosensor-based c-di-GMP in vivo quantification.

Findings: dCache domains are the predominant family of extracellular bacterial SDs. Using 3D structures of signal-bound dCache domains, we defined binding site sequence motifs which were subsequently used to retrieve matching sequences from databases. This approach resulted in the definition of SD families specific for amino acids, biogenic amines, purines and formate. In all cases, microcalorimetric titrations of selected family members confirmed their ligand specificity. In total, signals recognized by about 31,000 receptors throughout the Tree of Life were identified, many of which from important bacterial pathogens. Amino acid, biogenic amines and purine-specific domains were detected in all major receptor families, regulating processes as diverse as chemotaxis, gene expression, metabolism and second messenger levels, thereby indicating their universal role as signal molecules. Amino acid-specific dCache SDs were identified in humans where they serve as potential drug targets.

Conclusions: Our approach offers an universal method to identify many other functional SD families, showing great potential to narrow an important gap of knowledge in microbiology.

Abstract ID: 3697 / Poster Number: 190.17P

Bioactive molecules toward preventing oral infection: Capping of silver nanoparticles by carboxylic ligands

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Co-authors: *Jeyson Hermosilla, Ana Bustos, Gabriela Guajardo, Francisca Acevedo, Manuel Azocar*

Silver nanoparticles (AgNPs) have been used to prevent infections, rejection and control of implant deterioration. Silver has a well-tolerated tissue response, a low toxicity profile, and effective antimicrobial properties. The antibacterial activity of AgNPs depend on some factors such as: size, shape, oxygen availability, and surface coating. In connection with the latter, there is a great affinity and versatility in the interaction of silver with carboxylic (COO-) ligands. The aim of this work was to study the feasibility of using as capping agents, molecules that possess a COO− group in their structure, and their antimicrobial efficacy against periodontal bacteria. Inorganic chemistry research was conducted to synthesize AgNPs functionalized with diclofenac (dc), ketorolac (kt), cysteine (Cyst) and sodium citrate (NaCitr). Then, the antimicrobial effectiveness of the different AgNPs-based conjugations on *S. mutans* ATCC 25175, *S. aureus* ATCC 12493, *P. aeruginosa*
ATCC 27853 and *E. coli* ATCC 25922 was determined by using the broth microdilution method. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined. All newly synthesized AgNPs were spherical and water-soluble, being the AgNPs/Cyst the smallest in terms of size (5-15 nm). AgNPs/dc showed the highest antimicrobial efficacy (MIC values ranging from 18.75 to 75 mg/mL and MBC values ranging from 150 to 300 mg/mL) toward all strains evaluated. The AgNPs/NaCitr displayed the lowest antimicrobial activity with MIC values of 150-300 mg/mL and MBC values higher than 300 mg/mL. In conclusion, the results showed that capping agents played a decisive role in the antibacterial ability of AgNPs.

Abstract ID: 1465 / Poster Number: 364.17P

Bio-AgNPs as novel nanoagrochemicals: fungal-assisted synthesis, antimicrobial activity and impact on growth and biochemical parameters of maize

Presenting author: Ms. Julia Nadrowska, Nicolaus Copernicus University in Torun, Toruń, Poland

Co-authors: *Joanna Trzcińska-Wencel, Mahendra Rai, Patrycja Golińska*

Biosynthesis of nanoparticles (NPs) is an efficient and environmentally friendly approach to produce new agents for agricultural applications.

The aim of this study focused on the mycosynthesis of silver nanoparticles (AgNPs) and evaluation of their potential for agricultural use on the basis of the their antimicrobial activity against fungal plant pathogens and their effect on the growth and antioxidant system of maize after grain sterilization with selected AgNPs concentrations.

Biosynthesized AgNPs were small, spherical with crystalline structure and various functional groups on their surface. AgNPs inhibited spore germination and mycelial growth of fungal phytopathogens. Pre-sowing treatment of grains with AgNPs at a concentration of 32 µg mL-1 sterilized the surface of maize grains without negative effects on germination and stimulated shoot elongation and biomass accumulation of maize. However, changes in biochemical parameters (H2O2, malondialdehyde, glutathione, ascorbate, antioxidant enzymes activity) were observed in 14-day old plantlets after application of higher (128 and 512 µg mL-1) concentrations of AgNPs. The peroxidase (POX) and superoxide dismutase (SOD) activities were enhanced in leaves and roots, respectively, while higher ascorbate content in leaves and roots and increased glutathione content in caryopsis were noted. The decreased concentrations of H2O2 were estimated in all organs, except stems.

The results indicate that Fusarium solani is an efficient system for NPs synthesis. Mycosynthesized AgNPs, due to their unique properties, exhibit a potential for use in agriculture as a grain sterilization and antimicrobial agent, and growth stimulator to increase maize growth performance.

Abstract ID: 3555 / Poster Number: 394.17P

Bio-based mulch films as a sustainable agriculture approach founded on functionalized biodegradable materials

Presenting author: Dr. Carlos Barreiro, Universidad de León, León, Spain

Co-authors: *Sara Pérez-Gutiérrez, Ana Ibañez, Mª Fernanda Vasco, Sonia García-Alonso, Javier Rúa, Mª Rosario García-Armesto, Elías R. Olivera*

Plastic is part of countless aspects of modern life, and its recycling is a European concern due to the widespread environmental wastes, which has become one of the top scientific challenges, nowadays.

The plasticulture is an evident practice in various stages of agricultural production, from the construction of greenhouses to the crops protection through mulching techniques. However, the degradation of these plastic materials in soil leads to microplastics accumulation with harmful effects on the environment and, on the other hand, the recycling post-harvest is a low-efficiency process due to the vegetal and soil contaminants. Thus, the development of realistic self-degrading plastics is a promising solution requiring an interdisciplinary approach (material engineers, biotechnological experts, farmers, ...) to design effective, sustainable and functional materials, promoting a more environmentally friendly agriculture.

The BioPac project (no. TED2021-131864B-C21), funded by the Ministry of Science and Innovation, addresses this challenge by creating bioplastics functionalized with microorganisms that promote the biodegradation of the material, which would help to mitigate the accumulation of micro- and nano-plastics in soils, and to combat different plant diseases. Thus, two microbial strains have been selected, optimized their growth process, and integrated into a starch-based bioplastic. Viability confirmation of the microbial integration, as well as the efficacy of the process to accelerate plastic degradation are being used as a whole proof-of-concept.

Functionalized plastic materials suitable for agriculture applications is a promising research field, which present room for improvement, where Biotechnology has a relevant role as the current work is presenting.

Abstract ID: 3293 / Poster Number: 327.16F

BioBIVE: Development of Bio-Based Delivery Systems to Fight Fungal Phytopathogens in Horticultural Crops

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Horticultural crop pathogens represent a recalcitrant challenge typically addressed with synthetic pesticides, causing significant environmental harm. As an alternative, **bioactive agents** (basic substances, microbial antagonists, and seaweed extracts) offer sustainable solutions while maintaining effective pathogen control. Simultaneously, the plasticulture that supports the agricultural sector, particularly in mulch films, highly contributes to the accumulation of microplastics in soils. Addressing both issues, biodegradable platforms based on **functionalized bioplastics** emerge as eco-friendly replacements.

The objective of the EU-funded **project BioBIVE** (no. 101130442) is to develop **biodegradable delivery systems** for the controlled release of bioactive agents, which target fungal pathogens in three key European horticultural crops (tomatoes, strawberries, and carrots). These systems combine three types of bio-platforms (bioplastic mulch, biochar, and sprayable mulch) with diverse **bioactive compounds**, such as basic substances, antagonistic microorganisms, and seaweed extracts, to maximize efficacy.

Preliminary findings showed a high fungicidal activity of different basic substances against important **fungal phytopathogens** (such as *Rhizoctonia solani*, *Botrytis cinerea* or *Fusarium oxysporum*, among others), also presenting themselves as an effective eco-sustainable alternative to current chemical fungicides. Additionally, laboratory trials with antagonistic bacteria and seaweed extracts showed potential in inhibiting pathogen growth, highlighting their complementary role alongside basic substances in sustainable crop protection strategies.

In conclusion, this study focuses on the potential of biodegradable systems to replace conventional pesticides, contributing to **reduced environmental impact** while ensuring **effective crop protection**.

Abstract ID: 527

Biochar combined with mangrove root endophytic bacteria enhances tomato seedling growth under saline stress and reduces chemical fertilizer application

Presenting author: Prof. Munawwar Ali Khan, Zayed University, Dubai, United Arab Emirates

Co-authors: *Soumaya Tounsi-Hammami, Salama Ali Almehairi*

Introduction: Growing crops in arid and hyper-arid regions presents significant challenges due to limited precipitation, high evaporation rates, and salt stress caused by saline soil and water. These factors disrupt vital physiological processes in beneficial vegetable crops. Additionally, the widespread use of chemical fertilizers and soil degradation are pressing global issues that require immediate attention. Applying biochar and indigenous plant growth-promoting bacteria has been identified as potential solutions to address these challenges.

Objective: This study aimed to investigate the potential of biochar produced from agricultural waste and Indigenous halotolerant plant growth-promoting bacteria from the mangrove ecosystem. The aim was to examine their effects on tomato crop growth under saline stress and with reduced synthetic NPK fertilizers.

Method: Sterilized tomato seeds were planted under a growth room chamber at 25/22° C day/night temperature, 16/8 hours light/dark period, and an average of 250 µmol m−2 s −1 light intensity as measured with a handheld spectrometer (LICOR, USA, model LI-180). Five different treatments were applied: Control (100%NPK), Control with 50% NPK, Control with biochar + 50% NPK, Control with PGPB + 50% NPK, Control with both biochar (2%) and plant growth-promoting bacteria (Figure 1). Each treatment was replicated five times, utilizing a total of 25 pots. Several plant growth parameters were measured, including digital biomass, plant height, average greenness, projected leaf area normalized difference vegetation index (NDVI), and plant senescence reflectance index (PSRI). These measurements were conducted using an automated plant phenotyping instrument, PlantEye F600. ANOVA was employed to assess variation among the treatments, and the treatment means were compared using LSD tests in R statistical software, with a significance level set at $p < 0.05$.

Findings: Significant changes in plant growth metrics were observed across various treatments when comparing digital biomass, average greenness, plant height, projected leaf area, and the normalized difference vegetation index (NDVI) for tomato plants under

non-saline conditions, saline stress, and reduced chemical fertilizers. Under non-saline conditions, the IN-50%NPK treatment (Figure 1 A) showed the largest leaf area. It maintained a relatively high leaf area even under saline stress pf 150 mM NaCl (Figure 1 B). In contrast, a similar outcome was observed for treatments that included bacterial inoculants, biochar and reduced use of synthetic fertilizers.

Conclusion: Our study demonstrates the positive impact of biochar, both individually and in conjunction with plant growth-promoting bacterial inoculation, on alleviating salt stress and enhancing plant growth metrics while reducing synthetic fertilization by up to 50%.

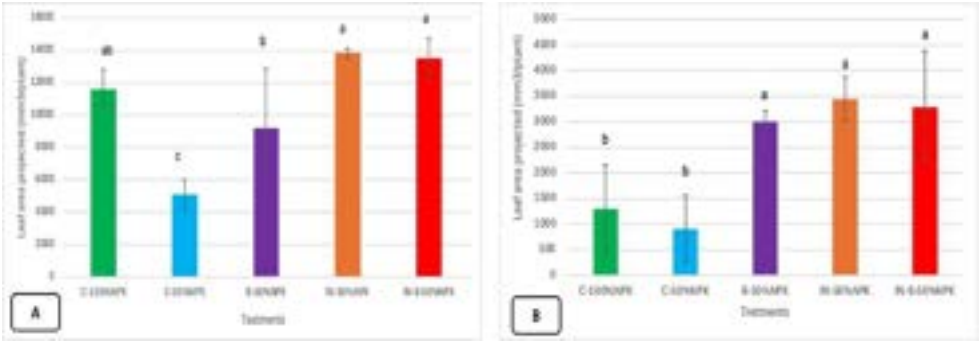


Figure 1: Leaf area projected (LAP) of tomato plants after various treatments under non-saline (A) and saline conditions (B). (Treatment with the same letters is not significantly different).

Abstract ID: 3295

Biocoatings: Confining genetically modified Escherichia coli in coatings for hydrogen production

Presenting author: Ms. Kathleen Dunbar, University of Surrey, Guildford, United Kingdom

Co-authors: *Alexia Beale, Sarah Montgomery, Beatriz Dominguez, Joseph Keddie, Suzie Hingley-Wilson*

Introduction: Hydrogen offers a source of energy that does not produce any greenhouse gases (GHG) when combusted, and hence the global demand for hydrogen fuel is growing. Biohydrogen is an attractive alternative to more traditional hydrogen production methods because it does not require fossil fuel feedstocks, however, the yield needs to be increased for biohydrogen to be commercially competitive.

Of interest here is hydrogen production from biocoatings, which are waterborne colloidal coatings containing live bacteria. Biocoatings have the potential to overcome many of the limitations presented by traditional bioreactors, including contamination and difficulties in the transport of bacteria.

Objective: The aim of the research was to genetically modify *Escherichia coli* and confine these bacteria in biocoatings for the purpose of increasing their hydrogen production.

Method: Single knockouts of genes from the mixed acid fermentation pathway were initially investigated by culturing anaerobically in Balch tubes for 48 hrs, and the headspace gas was measured using a residual gas analyser. The best performing single gene knockouts were then combined to produce double knockouts. *E. coli* were confined within a biocoating using a gelation method, wherein salt induces the coagulation of the colloidal particles. The colloidal particles were partially coalesced under 40% humidity. This method, which reduces osmotic stress, significantly increased the bacterial viability compared to a conventional manufacturing method with full drying. Hydrogen production rates from the biocoatings were then measured using hydrogen microsensors (Unisense).

Findings: Double knockouts of genes involved in the mixed acid fermentation pathway were found to increase the hydrogen yield, overcoming one of the key limitations of biohydrogen for large-scale production. The best performing modified bacteria had a hydrogen production rate that was 100 times higher than the wildtype strain. Interestingly, the production yield was higher than obtained from bacteria in liquid suspension, suggesting a positive impact of cell confinement. The production of carbon

dioxide (a GHG) by the double knockout strains was also significantly lower than the wildtype.

Conclusion: This method of hydrogen production from modified bacteria in biocoatings offers the benefits of not relying on fossil fuel sources and having a relatively high yield in combination with a low emission of carbon dioxide.

Abstract ID: 3741 / Poster Number: 346.15S

Biodegradation and Backdiffusion: Study and influence of back diffusion effect on organochlorine biodegradation mechanisms in aquifer systems

Presenting author: Ms. Danaé Barollier, BRGM, Orléans, France; Université Aix-Marseille, Marseille, France

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Method: ***qPCR*** (quantitative polymerase chain reaction) for functional genes and specific species targeting (on DNA and RNA (converted to cDNA) for quantification and gene expression analysis); ***metagenomic 16S rRNA sequencing***.

This study aims to characterize the biodegradation of organochlorine compounds, such as tetrachloroethylene (PCE) and 1,2-dichloropropane (1,2-DCP) in a contaminated aquifer beneath an industrial site in the Jura region in France. These compounds are notable for their persistence and widespread presence at contaminated sites [1,2]. While PCE and 1,2-DCP degradation pathways are known [3], their degradation mechanisms specifically under anaerobic conditions and in aquifer systems have been less studied [4]. The study utilizes a multidisciplinary approach, combining molecular biology, and isotope geochemistry.

The biodegradation kinetics of PCE, and 1,2-DCP were studied under anaerobic conditions using microbial communities from two on-site piezometers. Contaminant concentrations and their degradation metabolites were monitored. The microbiological analysis consisted of qPCR on DNA and RNA, metagenomic sequencing of the 16s rRNA gene to track the diversity, abundance and activity of microbial communities capable of reductive dechlorination. Compound-specific isotope analysis (CSIA) was used to establish the initial isotopic ratios of pollutants and monitor their evolution, thereby quantifying the extent of biodegradation. A combined, carbon and chlorine CSIA approach was applied to evaluate specific reaction pathways involved.

The changes in major ions concentrations (e.g., nitrate, nitrite, ammonium, sulfate, etc.) were measured using ion chromatography. The physicochemical conditions such as pH, conductivity redox potential and oxygen concentration were also monitored.

PCE and 1,2-DCP concentrations decreased over time, in conjunction with isotopic enrichment. The pollutant reduction rate (%) for PCE and 1,2-DCP was significant. Their degradation products were also monitored, as they were further biodegraded.

The objective was to characterize the microbial communities indigenous to the site's groundwater that are involved in PCE and 1,2-DCP degradation under aquifer-like conditions. Specifically, a focus is set on the degradation efficiency, the microorganisms involved and the genes linked to degradation. In addition, the study establishes the link between isotopic fractionation and the microbial activity responsible for pollutant degradation on this site.

Abstract ID: 531 / Poster Number: 266.17P

Biodiversity and enzymatic activities of fungal communities in Italian oleic ecosystems

Presenting author: Mr. Michele Avesani, University of Verona, Verona, Italy

Co-authors: *Giacomo Zapparoli*

Olive oil and its by-products are ecological niches for various yeast and filamentous fungi, and their occurrence can significantly influence olive oil quality. Fungal esterase-lipase enzymes can negatively affect it by increasing free fatty acids and favouring auto-oxidation. Concurrently, β-glucosidase positively contributes to it by reducing bitterness by breaking down phenolic compounds.

This study aimed to isolate and identify fungal species from olive oil and by-products and evaluate the principal enzymatic activities of fungal isolates associated with olive oil quality.

Olives, olive oil, pomace, and paste samples were collected from three production areas in Verona (Italy). Yeasts and filamentous fungi were isolated, identified and screened for esterase-lipase and β-glucosidase activities. Those found to be positive were further analyzed quantitatively.

A total of 66 yeast isolates and 10 filamentous fungi, belonging to 22 and 7 species, respectively, were identified. The screening revealed that 25 yeast and 10 fungal isolates exhibited significant enzymatic activities. In the quantitative analysis, the esterase-lipase activity was especially prominent in strains of F. magnum, G. candidum, and R. graminis. Nakazawea. molendinolei OVI2 exhibited the highest β-glucosidase activity. Among filamentous fungi, P. brevicompactum and C. perangustum strains showed notable β-glucosidase activity, while esterase-lipase activity was marked in isolates of C. allicinum, C. perangustum, and P. brevicompactum. This study provided additional insights into the fungal community inhabiting oleic ecosystems and their enzymatic activities involved in olive oil quality. Further studies are necessary to evaluate their specific impact on the quality of olive oil during the storage.

Abstract ID: 2295 / Poster Number: 355.15F

Bioenergetic adaptations of Thermus scotoductus K1 to carbon source and pH variations in extreme environments

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Co-authors: *Karen Trchounian*

Thermophilic bacteria are model organisms for studying survival strategies in extreme environments. Key bioenergetic parameters, such as ion exchange, membrane potential, and proton motive force (Δp), are crucial for understanding these adaptations. This study investigates the effects of 2 g/L of sole glucose, fructose, galactose, lactose, or starch and pH values (8.5 and 6.0) on ΔpH, membrane potential (ΔΨ), and Δp in *Thermus scotoductus K1*. At pH 8.5, intracellular pH values were highest, with glucose yielding the largest ΔpHin/out (2.3). The lowest intracellular pH was recorded with starch (7.9) and galactose (8.0). ΔΨ was strongly affected by environmental pH, ranging from −39 to −71 mV at pH 6.0 and −95 to −170 mV at pH 8.5. Glucose produced the highest ΔΨ (−170 mV at pH 8.5) and galactose the highest at pH 6.0 (−82 mV). The proton motive force (Δp) was highest with glucose (+324 mV) compared to the absence of carbon sources (+223 mV) and most other carbon sources. Lactose produced a Δp of +70 mV at pH 8.0 and an inside-positive Δp at pH 6.0, contrasting with pH 8.5. These findings reveal adaptive bioenergetic strategies in *T. scotoductus K1*, highlighting the interplay between environmental pH, carbon sources, and pH stress response mechanisms.

Abstract ID: 2074 / Poster Number: 373.15F

Bioenergetic differences in Saccharomyces cerevisiae ATCC 9804 during growth on glucose or glycerol under aerobic conditions

Presenting author: Anahit Shirvanyan, PhD, Scientific-Research Institute of Biology, Yerevan State University, 0025 Yerevan, Armenia; Department of Biochemistry, Microbiology and Biotechnology, YSU, 0025 Yerevan, Armenia

Co-authors: *Nicoletta Guaragnella, Karen Trchounian*

Crabtree-positive *S. cerevisiae* mid-exponential growth phase cells perform respiro-fermentative metabolism in glucose even under aerobic conditions, while in glycerol, they rely solely on respiration. However, the metabolic shift in these conditions and energy production mechanism remain unclear. This study investigates specific growth rate (SGR), plasma membrane proton gradient (ΔpH), and ATP yield changes of *S. cerevisiae* ATCC 9804 industrial wine strain during aerobic growth on glucose or glycerol. The results show a higher SGR on glucose (0.4 h^{-1}) with a doubling time of 1.7 h compared to glycerol (0.2 h^{-1} and 4.3 h, respectively). Glucose consumption rate was significantly higher (1.7 g L^{-1}) compared to glycerol (0.06 g L^{-1}). Intracellular pH was slightly lower on glucose (6.8) than in glycerol (7.0), which directly correlates with ΔpH changes (2.33 on glycerol and 2.13 on glucose). This can be due to the decrease in intracellular pH via acids produced during fermentation, while in glycerol, protons are used for oxygen reduction in the mitochondria, resulting in higher cytoplasmatic pH. Gibbs free energy was lower (-1367 kJ/mol) on glycerol compared to glucose (-232); however, ATP yield h^{-1} $mole^{-1}$ substrate was higher on glucose (22) compared to glycerol (~16) in exponential growth phase cells, reflecting the higher efficiency of respiro-fermentative metabolism compared to fully respiratory metabolism in aerobic conditions, which results in higher growth rates. These findings provide insights into the bioenergetic reprogramming of the industrial *S. cerevisiae* ATCC 9804 strain, which can be used to enhance production efficiency in yeast-based industries.

Abstract ID: 2157 / Poster Number: 160.15P

Biofilm formation correlates with resistance to human serum in multidrug resistant Klebsiella pneumoniae (MDR-Kp)

Presenting author: Mrs. Hadas Fulman-Levi, Ariel University, Ariel, Israel

Co-authors: *Mali Salmon-Divon, Shiri Navon-Venezia, Polina Geva*

Introduction: MDR-Kpn is a major causative pathogen of bloodstream infections with limited therapies and fatal outcomes.

Objective: The pathogenicity underlying MDR-Kp survival in the blood is still elusive. We hypothesized that biofilm production promotes MDR-Kp ability to thrive in serum.

Methods: We analysed 45 MDR-Kp clinical isolates of different genetic lineages. Bacterial fitness was assessed on rich and defined medium. Biofilm production was assessed with crystal violet and visualized using confocal microscopy. Serum resistance was determined by survival curves performed on 40% normal human sera (NHS). Expression levels of mrk genes (Type-3 pili) were determined with qRT-PCR. A multivariate linear regression model was applied to evaluate the effect of biofilm formation on serum-resistance.

Findings: Most of the isolates exhibited similar growth fitness in BM2 but exhibited varying response phenotypes to NHS. Biofilm production in BM2 and in 40% NHS varied significantly between isolates. We discovered a positive correlation between biofilm formation, mrk genes expression, and level of serum resistance, supporting the involvement of biofilm production in serum survival. Overexpression of mrk genes in MDR-Kp serum sensitive strain increased biofilm production (by-265%) and subsequently serum resistance (by-700%). Regression analysis further confirmed that biofilm formation in NHS significantly contributed to increased serum survival ($p < 0.001$; $\beta = 2.33$).

Conclusion: We describe a direct correlation between *mrk* operon-mediated biofilm production and serum survival, suggesting a possible strategy for MDR-Kp in BSIs pathogenesis. The findings may be beneficial for predicting clinical strains' serum survival and used for future design of targeted diagnosis and therapy.

Abstract ID: 2482

Biofilm Initiation Driven by Orientation Patterns in Rod-Shaped Bacterial Cells

Presenting author: Dr. Fumiaki Yokoyama, The University of Tokyo, Tokyo, Japan

Co-authors: *Kazumasa Takeuchi*

When do bacterial cells form a biofilm, and where does it begin within a population? Understanding the biofilm formation process is not only biologically intriguing but also crucial for its regulation in clinical and industrial applications. While the pre-biofilm and maturation phases—characterized by cell attachment and extracellular matrix production—are well-studied, the initial steps of structural biofilm formation remain poorly understood.

We hypothesize that mechanical stresses generated by orientation patterns of growing bacterial cells upregulate extracellular matrix gene expression, triggering biofilm initiation.

To test this hypothesis, we confined *Escherichia coli* cells expressing green fluorescent protein tagged to a colanic acid (CA) production gene, using soft agar pads and microfluidic devices (Figures A and B). Dynamics of cell growth and CA production were captured using a confocal fluorescence microscope and quantified through image analysis.

Under soft agar, growing cells collided and protruded along the z-axis, with increased CA production more than non-protruding cells (Figure A). In a monolayer confiner device, cells formed orientation domains (Figure B). Topological defects, i.e., points of cell orientation mismatch, were formed between these domains (Figure C left). Notably, CA production was concentrated around a certain type of defects characterized by accumulated mechanical stress, suggesting a correlation between orientation patterns and matrix production (Figure C middle and right). Quantitative analysis for all the pixels confirmed that CA secretion was initiated near the defects (Figure D).

Our findings suggest that bacterial cells accumulate at specific points defined by orientation, experiencing mechanical stress by growing cells that triggers extracellular matrix production for biofilm initiation. These insights could contribute to developing strategies for biofilm regulation by aligning cells on a patterned substrate or structured confinement.

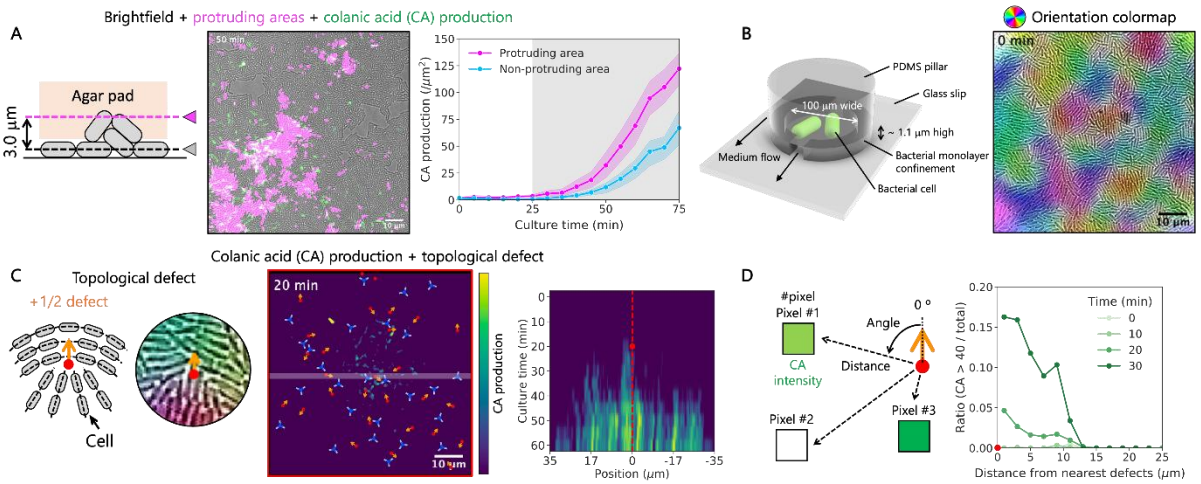


Figure: Production of colanic acid (CA) around topological defects in a rod-shaped *Escherichia coli* population during biofilm initiation. (A) CA production from protruding cells confined under an agar pad. (B) Schematic representation of a microfluidic monolayer confiner and a colormap showing the orientation of rod-shaped cells within the confinement. (C) Sketch and microscopic image of a defect in rod-shaped cells (left) and their association with CA production (middle). The kymograph (right) was taken along the white line in the middle image, with the red dot marking a defect. (D) Quantification of CA production as a function of distance from the nearest defects.

Abstract ID: 1627 / Poster Number: 244.17P

Biofilm Modulation and Transcriptomic analysis under stress conditions in Halomicronema sp

Presenting author: Marina Caldara, PhD, University of Parma, Parma, Italy

Co-authors: *Henk Bolhuis, Marta Marmiroli, Carola Carlini, Nelson Marmiroli*

Introduction: In nature, bacteria frequently form diverse communities encased in a complex structure known as biofilms, here the extracellular matrix, produced by microorganisms, acts as the primary defense between the cells and their surroundings. It consists mostly of water, extracellular polymeric substances (EPS), lipids, proteins, and DNA. EPS, mainly polysaccharides, serve multiple functions, including safeguarding against dehydration, UV radiation, viruses, and the capture of nutrients or metal ions. Cyanobacteria develop biofilms and possess distinct traits like oxygenic photosynthesis, nitrogen fixation, remarkable adaptability to various abiotic stressors, and the capacity to release a variety of metabolites and hormones.

Objective: This research focused on the characterization of the cyanobacterium *Halomicronema sp.* strain, isolated from a brackish habitat.

Methods: The study involved microscopic imaging, assessment of phenolic content and antioxidant activity, identification of compounds that interfere with biofilm formation, and transcriptomic analysis through RNA sequencing and real-time PCR. Gene expression analysis concentrated on genes involved in EPS production and biofilm-related transcription factors.

Findings: The findings led to the identification of *wza1* and *wzt* as EPS biomarkers, as well as *luxR-05665* along with genes from the TetR/AcrR and LysR families, as potential biomarkers valuable for studying and monitoring biofilm formation under various environmental conditions.

Conclusions: *Halomicronema sp.* can grow even under harsh abiotic stresses, such as high salinity, and exhibits good antioxidant properties. An interesting application will be the possibility to test if this bacterium has “Plant Growth Promoting” capacities which can support crop growth in arable lands with high salinity.

Abstract ID: 2116

Biofilm origin and stressor's legacy modulate the effects of new stressors on river biofilms

Presenting author: Mr. Javier Ortiz Rivero, Catalan Institute for Water Research, Girona, Spain

Co-authors: *Paula Siñeriz, Anna Freixa, Sergi Sabater*

Introduction: Mediterranean rivers are subject to co-occurring stressors, leading to alterations in water flow, temperature, and contaminant inputs. They affect microbial communities and their ecological functions, though effects may differ according to the structure (origin) of biofilms. Stressors legacy may modulate the effects of other new stressors, having altered the composition of microbial communities and their associated functions. This, in turn, determines how microorganisms respond to new stressors.

Objective: To study how the different structure and origin of river biofilms responds to the legacy effects of past stressors when these microbial communities are exposed to new stressors.

Methods: An experiment was conducted using artificial streams colonized with biofilm communities from several origins and structure (five different rivers). Biofilms were exposed to pollutants (herbicides, pesticides) for two weeks, followed by hydric stress for another two weeks. Biofilms were then allowed to recover, once stressors were suppressed, for three other weeks. Potential changes in the microbial community were evaluated every change of stressor (4 times during the experiment) by high-throughput sequencing of 16S rRNA and 18S rRNA gene amplicons, and the response of biofilm functions (enzymatic activities, primary production, among others) were also evaluated.

Findings: Pollutants impacted different functions in distinct ways; primary production increased in the presence of pollutants, but enzymatic extracellular activities glucosidase and leucine activity decreased. Hydric stress negatively affects microbial community structure and functions, this having a general impact on the different biofilms. During the recovery period, new communities replaced the original microbial communities, resulting in a delay in the functional capacity of the biofilms. Responses diverged between the origin of biofilm communities, those more complex responding faster.

Conclusions: The findings emphasize the need to assess the combined effects of stressors on microbial biofilm communities in order to better anticipate and mitigate

their impact on river ecosystems, particularly in the context of global change, and to evaluate their resilience to disturbances.

Abstract ID: 1802

Biofuels and bioengineering of cyanobacterial strains: the impact of uptake hydrogenase on hydrogen production

Presenting author: Dr. Galyna Kufryk, Grand Canyon University, Phoenix, United States

Introduction and Objectives: Cyanobacteria are a diverse group of photoautotrophic prokaryotes that are found in a variety of environments and can be grown in the laboratory. With genomic information available for more than 130 cyanobacterial strains, many varieties of cyanobacteria can be genetically engineered to produce biofuels, including hydrogen. Cyanobacteria are ideal cell factories for hydrogen production because they have low nutrient requirements and are capable of using light to generate biomass from water and carbon dioxide.

Cyanobacteria produce hydrogen through two key enzymes, nitrogenase and bidirectional hydrogenase, and oxidize molecular hydrogen by uptake hydrogenase.

The main role of uptake hydrogenase is to protect nitrogenase from oxidative damage, and prevent feedback inhibition of both nitrogenase and bidirectional hydrogenase, which reduces the net gain of molecular hydrogen. Inhibition of the hydrogen-oxidizing activity is important in order to maximize increasing hydrogen yield from cyanobacterial cultures.

Uptake hydrogenase has two subunits; the small subunit directs electron transport to the large subunit, which has an active site that binds molecular hydrogen. These subunits are encoded by *hupS* and *hupL* genes, respectively, that can be genetically modified to reduce activity of the uptake hydrogenase and increase hydrogen production. The objective of this study was to investigate the impact of uptake hydrogenase deletion on growth of cyanobacterial cultures and hydrogen production in cyanobacteria.

Methods: Cloning of *hupSL* operon was completed according to the standard cloning protocols. *HupS* and *hupL* genes were inactivated in *Anabaena* sp. PCC 7120 and *Anabaena variabilis* ATCC 29413 by insertional mutagenesis, and the knockout mutants were segregated and verified by sequencing. Chlorophyll content of cyanobacterial cultures was calculated based on the optical density of methanol extracts. Hydrogen production in the liquid cultures of mutant and wild type strains was measured by gas chromatography. Nitrogenase activity was assessed by acetylene reduction method and gas chromatography. Light microscopy was used for the morphological characterization of cyanobacterial strains.

Findings and Conclusion: Genetic deletion of uptake hydrogenase in cyanobacteria affected the net hydrogen yield in various ways. In *Anabaena* sp. PCC 7120 it increased hydrogen production by 4-7 fold while in *Anabaena variabilis* ATCC 29413 it reached a 5-fold improvement, compared to the wild type strain. Uptake hydrogenase deletion strains demonstrated stable activity of nitrogenase enzyme over the course of cultivation. The growth rate of the Δ*hupSL* mutant and the wild type of the same cyanobacterial species were comparable under the same cultivation conditions. Morphological characteristics of cyanobacterial filaments and the distribution of vegetative cells and heterocysts were followed on daily basis for the two consecutive weeks of cultivation, and the results showed similarities between the wild type and Δ*hupSL* mutant strains of the same species of cyanobacteria.

Based on the current findings, uptake hydrogenase deletion strains can be the starting point for further genetic modifications for the purpose of enhancement of their hydrogen-producing capacity. Hydrogen production makes cyanobacteria a promising source of renewable biofuel. Accumulation of biomass by cyanobacteria is linked to carbon dioxide sequestration. It can help reduce the carbon footprint by producing polymers of carbon. The ability to lower the extent of carbon dioxide pollution as well as to produce energy dense hydrogen makes cyanobacteria significant in biofuel research.

Abstract ID: 4131 / Poster Number: 302.15P

Biogeography shapes the Microbiome of Argan fruits

Presenting author: Dr. Fatima Zahra Aliyat, African Genome Center (AGC) Mohammed VI Polytechnic University (UM6P), Ben Guerir, Morocco

Co-authors: *Jean Legeay, Jamal IBIJBIJEN, Mohamed HIJRI, Bulbul AHMED*

Argan (*Argania spinosa*) is an endemic woodland species in Morocco. Argan fruit has multiple uses for food, cosmetic and medical purposes. The fruit-associated microbiome is essential for maintaining plant health, influencing fruit quality, and mediating ecological interactions. However, the mechanisms underlying the effects of argan fruit microbiome remain unquantified. In this study, we investigated the diversity of Argan fruits with biogeographical influence. To understand how biogeography influences argan fruit microbiome, we characterized bacterial and fungal communities from 36 argan trees, across 4 sites (Agadir, Essaouira, Rabat, and Berkane) with altitudes ranging from 69 – 800 m sea level. We performed amplicon sequencing of 16S rRNA gene for bacteria and ITS for fungi. Our finding revealed that microbial community structure was affected by sites. Bacterial communities showed distinct taxonomic profiles throughout regions, with dominance of 17% of *Tatumella* sp. and fungal communities showed with the dominance of *Cladosporium* by 11%. We found significant variations ($p < 0.05$) in microbial richness and evenness across altitude gradients (altitude>100, 100-500, 500<altitude). However, seed weight showed a significant difference across sites, the weight range between 2.0 to 14.5 g/fruit and significant difference in weight was observed across locations ($p = 0.0108$). Our results suggest that, in addition to fruit weight, biogeographical distributions (level and distance from the sea level) differentially impact the argan fruit microbiome, which might affect the fruit health of argan grown in different locations.

Abstract ID: 1046 / Poster Number: 150.16P

Biological Activities of Garcinia kola Leaf and Seed Extracts on Bacteria from Human Feces

Presenting author: Ms. Gladys Anyanwu, Federal Polytechnic Nekede, Owerri, Nigeria

Co-authors: *Joseph Uwaezuoke, Kenneth Yongabi, Chinyere Ohalete*

Introduction: Medicinal plants are crucial for the synthesis of bioactive molecules to treat drug-resistant infections, providing an alternative to chemosynthetic medications (Kraupner et al., 2018; Bengtsson-Palme et al., 2018). They are found in ecosystems as curative ingredients for various diseases. However, cultivation faces challenges like overharvesting, ecological distortion, and habitat destruction. The use of plant bioactive compounds can be infeasible due to small product accumulation, long maturation periods, and difficulty in recovering metabolites (Palanichamy et al., 2018).

Objectives of the study: Isolate and identify bacteria from human faeces, determine the antibacterial activities of *Garcinia kola* leaf and seed extract against bacteria from human faeces, determine the minimum inhibitory and minimum bactericidal concentrations of *Garcinia kola* leaf and seed extracts.

Methods: The study involved extracting ground seeds and leaf powder, soaking them in ethanol, filtering, and storing. Stool samples were homogenized, inoculated, and tested for antibacterial activities on nutrient and eosin methylene blue agar plates.

The antibacterial activities of leaf and seed extracts were tested using a standard agar well diffusion method.

Results: *Salmonella, Streptococcus, Staphylococcus* and *Escherichia* species were isolated. The leaf extract demonstrated superior antibacterial activity against Staphylococcus species, with zones of inhibition ranging from 18mm to 28mm.

Conclusion: The study found that Garcinia kola leaf and seed extracts have antibacterial properties against human bacterial isolates, with leaf extract showing better antibacterial activity at lower concentrations, suggesting their potential in complementary and alternative medicine.

Abstract ID: 2614 / Poster Number: 400.16P

Biological Characterization of Pooled Postbiotics from Akkermansia muciniphila and other intestinal wound healing candidate strains

Presenting author: Ms. Diana Almeida, Universidade Católica Portuguesa (UCP) – Escola Superior de Biotecnologia (ESB), Porto, Portugal

Co-authors: *Melissa Garcia, Sónia Mendo, José Andrade, Ana Maria Gomes*

Intestinal damage and dysbiosis derived from enteric infections can contribute to the onset of gastrointestinal and systemic conditions when mucosal repair mechanisms fail to maintain barrier integrity. These mechanisms involve a complex immunological network integrated with commensal microbiota, including Akkermansia muciniphila and certain aerotolerant strains. The enhancement of this microbial contribution may represent a viable strategy for promoting effective mucosal healing interventions.

Our study aimed to investigate interaction effects between A. muciniphila and lactobacilli/bifidobacteria strains, in the form of cell-free supernatants or postbiotics, focusing on their antioxidant and antimicrobial activities.

Akkermansia muciniphila and selected lactobacilli and bifidobacteria strains postbiotics were pooled in equal proportions (1:1). The following analyses were performed on individual and pooled postbiotics: organic acid and sugar content (HPLC), antioxidant activity (TPC, DPPH, and ABTS assays), antimicrobial (broth microdilution) and antibiofilm activity against the foodborne pathogens Listeria monocytogenes, Yersinia enterocolitica, Salmonella Typhimurium, and Escherichia coli.

Ten lactobacilli and bifidobacteria strains, selected for their intestinal pro-regenerating and antimicrobial properties, were used for pooling. This study revealed diverse interaction effects between pooled A. muciniphila postbiotics and probiotic strains in antioxidant assays. Pooling generally resulted in neutral or additive effects ($p > 0,05$) while ABTS indicated possible antagonistic interactions ($p < 0,05$) when compared to the expected results. Pooled postbiotics did not significantly improve antimicrobial activity against foodborne pathogens.

This work explores how interactions between microbial-derived metabolites can modulate and enhance microbial characteristics. Further studies are required (needed)

to validate these findings in more complex systems, especially those simulating wound healing.

Abstract ID: 4018 / Poster Number: 368.17P

Biological Inputs in Passion Fruit Seedling Development and Microbiological Evaluation of the Substrate

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Yellow passion fruit (*Passiflora edulis*) is economically significant in Brazil's tropical fruit industry. High-quality seedlings are crucial for successful orchard establishment and to prevent production losses. This study evaluated the effects of biological inputs and application methods on seedling growth and substrate microbiology. Two commercial cultivars, Giant Yellow (GY) and FB200, were tested using a completely randomized factorial design with six treatments and three application methods: seed coating, substrate incorporation, and foliar inoculation. Treatments included: control, biological inputs *Trichoderma asperellum* (1×10^{10} CFU/g) Lallemand Inc., *Bacillus subtilis* (3×10^{9} CFU/g) Lallemand Inc, combined *T. asperellum* + *B. subtilis* at 0.5% and 1%, and an organomineral foliar fertilizer. For the GY variety, the best results were observed with *B. subtilis*, the combined formulations (0.5%–1%), and the organomineral fertilizer, particularly with substrate incorporation. These treatments enhanced leaf number, plant height, and stem length. In FB200, the best performance was recorded with combined formulations (0.5%–1%). Substrate analysis showed no statistical differences among treatments for FB200, but significant differences were found in GY's rhizosphere regarding colony-forming units. β-glucosidase activity varied significantly between treatments, with the highest activity in FB200's rhizosphere at 1% and in GY under specific application methods. Acid phosphatase activity increased with bioinputs in all treatments. Overall, biological inputs improved seedling morphology, demonstrating their potential to enhance passion fruit cultivation.

Abstract ID: 1210 / Poster Number: 357.17P

Biolubricants production by fermentation of dairy effluent "scotta": optimization of conversion parameters in Lipomyces starkeyi and Cutaneotrichosporon oleaginosus

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The use of fossil fuels as a predominant energy source has been a persistent practice, playing a substantial role in the current climate crisis. Nowadays, there is a pressing need to shift from fossil fuels to renewable energy alternatives, such as biofuels and biolubricants, which offer a relatively reduced environmental impact. Single-cell oils (SCOs) obtained from microorganisms have attracted significant attention in biodiesel and biofuel production, due to the capacity of microorganisms to grow on waste and effluent substrates.

In the present study, the production of SCOs by *Lipomyces starkeyi* and *Cutaneotrichosporon oleaginosus* was assessed. As economical sustainability of the process is a key issue, scotta, a dairy effluent, was used as substrate. Fermentation processes were conducted under anaerobic conditions in batch, at two distinct temperatures (25 °C and 30 °C) to investigate the impact of temperature on yield and rheological characteristics of oil. The highest yields of microbial oil were obtained at 25 °C from fermentations of both *L. starkeyi* (2.44 g L^{-1}) and *C. oleaginosus* (1.88 g L^{-1}).

Furthermore, at higher temperatures, there was a decrease in unsaturated FAs, whilst the content of shorter-chain saturated FAs increased, thus ensuring the oil had adequate viscosity. Peroxide analysis yielded very low values ranging from 0 to 0.23 meqO2 eq $^{-1}$ for all the samples, with the lowest value recorded for *L. starkeyi* at 30 °C. These results underscore the high oxidative stability of yeast oil, thereby affirming its competitive performance in comparison to vegetable oils.

Abstract ID: 1482 / Poster Number: 291.17P

Biomass and bioethanol production from dairy waste by Saccharomyces cerevisiae ATCC 13007

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The growing population has increased fossil fuel consumption, leading to resource depletion, higher greenhouse gas emissions, and accelerated global warming. As a result, alternative ways for sustainable biofuel production are crucial. This study aims to investigate the biomass and bioethanol production rate from dairy industry side streams (whey) using the glucoamylase-producing *S. cerevisiae* ATCC 13007 beer strain. To obtain glucose and galactose from lactose, whey was pretreated by β-galactosidase under the 100 °C. *S. cerevisiae* ATCC 13007 was cultivated under oxygen-limited conditions at pH 5.0. Volatile solids (VS) was estimated using muffle furnace and protein content was determined by the Kjeldahl method, bioethanol production was quantified using a bichromate-sulfuric acid, and carbohydrate content was analyzed by the sulfuric acid-phenol method. 2.6 g L-1 g-1 dry mass protein and 8.9 g L-1 g-1 dry mass carbohydrates were determined in the pretreated whey. The yeast specific growth rate was 0.12 h^{-1} in both non-diluted and 2×diluted samples. The initial VS concentration in whey was 4.5 g L-1, which increased 3-fold at 24h of growth in the non-diluted sample, while in the 2×diluted sample non-significant changes were observed. Full consumption of whey proteins was observed in both non-diluted and 2×diluted samples at 96h of growth. Compared to non-diluted sample, the ethanol production yield (97.8 mM) was stimulated 2-fold at 24h of growth in the 2×diluted sample. Results can be used for the enhancement of renewable bioethanol production by *S. cerevisiae* ATCC 13007 using whey as a promising and sustainable, cheap feedstock.

Abstract ID: 4148

Biomining E-Waste: Exploring the Potential of Fe(II)-Oxidizers for Sustainable Metal Extraction

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Modern society is increasingly dependent on electronic technology. Devices such as laptops, smartphones, and tablets have become essential in most developed countries, with demand rapidly growing in developing regions. As the lifespan of electronic devices shortens and technological advancement accelerates, electronic waste (e-waste) generation is rising at an unprecedented rate.

Improper disposal of e-waste poses serious environmental and health risks due to the presence of toxic substances such as heavy metals (e.g., Pb, Hg, As, Cd, Cr), polychlorinated and polybrominated biphenyls, and epoxy resins[1]. At the same time, e-waste is a valuable secondary source of strategic and critical metals, including Au, Ag, Pd, Ti, Ga, Ta, Co, Cu, Fe, Al, Zn, Sn, Pb, and Ni[2]. Components such as printed circuit boards (PCBs) are also enriched in rare earth elements (REEs) – a group of 17 chemically similar metallic elements critical for modern technologies[3].

Current recycling approaches for critical raw materials (CRMs) from e-waste—primarily pyrometallurgy, hydrometallurgy, and pyrolysis – suffer from major limitations, including high energy consumption and environmental hazards. In contrast, microbially mediated methods such as bioleaching and biohydrometallurgy have gained significant attention as more sustainable alternatives. These methods rely on biological oxidation and complexation to mobilize metal ions from solid matrices and are generally safer, more environmentally friendly, and cost-effective compared to chemical methods.

Acidophilic and mesophilic chemolithoautotrophs – particularly Acidithiobacillus ferrooxidans, which oxidizes both Fe^{2+} and sulfur compounds—are commonly used in bioleaching due to their ability to operate under highly acidic conditions. While A. ferrooxidans has been extensively studied and applied in biomining, its potential for recovering critical elements from e-waste remains underexplored. Furthermore, expanding the portfolio of Fe(II)-oxidizing bacteria for biomining applications is necessary.

This study aims to: (1) evaluate the bioleaching potential of A. ferrooxidans in extracting critical elements from PCBs, and (2) compare its performance to newly enriched consortia of Fe(II)-oxidizing microbes.

Our analysis revealed that PCBs are a rich source of metals, particularly Cu > Au > Ni > Fe (300,000 to 1,700 ppm), along with Sr, Zn, Cr, Ag, Sb, W, Pd, Y, Mn, Pb, As, Dy, and Li (400–25 ppm). After 11 days of incubation with A. ferrooxidans, efficient mobilization of core PCB elements (Fe, Cu) was observed, as well as significant recovery of light REEs (Pr, Cs), heavy REEs (Er), and other critical (Ir, Ba, Pd, W, Zn, Mn, Co, Cr) and precious metals (Au, Ag). Surprisingly, the spent medium (without microbial biomass) demonstrated higher leaching efficiency than the medium containing active A. ferrooxidans cells.

Newly enriched cultures of moderately acidophilic Fe(II)-oxidizers showed moderate efficiency in mobilizing Fe, Cu, Zn, Sr, Cr, and Li. Further enrichment is needed to increase biomass yield and enhance leaching efficiency. Nonetheless, both systems demonstrated promising potential for eco-friendly metal recovery from e-waste.

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Abstract ID: 674 / Poster Number: 270.17P

Bioremediation of Arsenic in Rice Harnessing Native Rhizobacteria for Sustainable Agriculture

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Arsenic contamination in rice, caused by the use of arsenic-laden groundwater for irrigation, is a critical issue affecting millions in Asia, particularly in Bangladesh and India. While chemical methods for detoxifying groundwater are available, they are often expensive and inaccessible to rural communities. To promote sustainable agriculture, alternative approaches that are cost-effective and eco-friendly must be explored. This study investigates the bioremediation potential of native arsenic-tolerant rhizobacteria, focusing on their dual ability to promote plant growth and reduce arsenic bioaccumulation in rice.

Rhizobacterial isolates were obtained from the rhizosphere of paddy fields Northeast India, and their tolerance to arsenite and arsenate was evaluated using minimum inhibitory concentration assays. In vitro tests for plant growth-promoting traits were conducted, followed by greenhouse experiments using arsenic-spiked soil to assess the isolates' efficacy in enhancing plant growth and mitigating arsenic accumulation. Post-harvest, total arsenic content in soil, roots, and shoots was quantified using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Statistical analyses were performed to compare arsenic bioaccumulation in control versus treated plants.

The results demonstrated the potential of these native rhizobacteria as a sustainable bioremediation strategy, reducing arsenic uptake in rice and promoting healthier plant growth. These findings provide a foundation for developing low-cost, eco-friendly solutions to address arsenic contamination in rice cultivation, offering a sustainable pathway for improving food safety and public health in affected regions.

Abstract ID: 211 / Poster Number: 341.16P

Bioremediation Potential of Lipid-degrading Bacteria Isolated from Food Canteen Wastewater

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Co-authors: *Adeoye Oluwole*

Introduction: Lipid-rich wastewater from food canteens poses environmental challenges due to high fatty acid content and elevated Biological Oxygen Demand (BOD), impeding water quality and degradation processes.

Objective: This study aimed to isolate and characterize bacteria from food canteen wastewater and evaluate their bioremediation potential for degrading lipids in oil-rich wastewater.

Methods: The wastewater samples were analyzed for microbial load using 10-fold serial dilution and Pour Plate methods. The isolates were screened for lipolytic activity using Tributyrin-amended medium. Lipolytic bacteria were identified using 16SrRNA sequencing. These strains were applied to wastewater bioremediation over a period of 12 weeks, with periodic monitoring of microbial load, BOD and fatty acid content using Total Viable Count, a 5-day BOD test as described by standard methods (APHA) and Gas chromatography respectively.

Findings: Total heterotrophic bacterial counts ranged from 5.8 ± 0.05 to 6.4 ± 0.08 Log10 CFU/mL. Two isolates with the highest lipolytic activity, Pseudomonas aeruginosa WLS4 and Proteus mirabilis CBNRA, reduced fatty acid concentrations from 2.45 ± 0.10 g/L to 0.65 ± 0.08 g/L. BOD decreased from 410 ± 15 mg/L to 120 ± 10 mg/L (70.7%) over 12 weeks, improving water quality. The microbial load in treated samples increased steadily from the 2nd week to a peak value of 8.1 ± 0.12 Log10 CFU/mL on the 8th week and subsequently, decreased from the 10th to the 12th week.

Conclusion: This study demonstrates the potential of Pseudomonas aeruginosa WLS4 and Proteus mirabilis CBNRA for bioremediation of lipid-rich food canteen wastewater, significantly reducing fatty acids and BOD.

Abstract ID: 3026 / Poster Number: 162.17P

Biosafety assessment of food industry by-products and derived soil improvers for a safe use in agriculture

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Recycling food processing residues streams using environmentally friendly technologies able to inactivate harmful microbes represents a practical approach towards circular economy, particularly in the production of soil improvers for sustainable agriculture.

The aim is to evaluate the biosafety of raw materials and soil improvers obtained by sustainable and high-quality standardized valorisation processes able to inactivate relevant biological hazards.

Culture-based and molecular analyses were considered to evaluate both microbial pathogens and potential antibiotic resistance genes in both raw materials and end products. Different DNA extraction methods were used on different soil improver matrices (e.g. compost, biochar, digestate, biostimulant, and meat and bone meal) to standardize the experimental procedure. Compost and digestate samples were also analysed for the enumeration of *Clostridium perfringens*, *Escherichia coli*, *Enterobacteriaceae*, *Enterococci* and extended spectrum beta-lactamase producing (ESBL) *Escherichia coli* and for the detection of *Salmonella*.

The preliminary screening of different DNA extraction methods permitted to identify the most effective method for each type of sample to obtain high-quality DNA for subsequent metagenomic analyses and the development of a dedicated protocol for DNA extraction tailored to the different raw material and soil improver matrices. Microbiological parameters were examined following Standard Operating Procedures previously developed for the biosafety assessment of residues and soil improvers. Samples were analyzed in quintuplicate for each replicate. Overall, all compost and digestate samples met the safety criteria.

Microbiological monitoring is crucial to prevent the escalation of antibiotic resistance. Culture-based and molecular analysis will permit to assess the biosafety of residues and soil improvers.

Abstract ID: 401 / Poster Number: 412.15P

BioSmart Fertilizers: Engineered Microbial Solutions for Sustainable Agriculture and Climate Resilience

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The agricultural sector faces increasing challenges to ensure food security for a rapidly growing global population, requiring extensive use of synthetic fertilizers. This causes significant ecological perturbation and exacerbates climate change. In this environmental crisis, extreme weather events become more frequent, threatening crop survival and deepening social inequalities. To address these issues, we propose the development of "BioSmart" fertilizers, using hyper-modified microorganisms functioning as a synthetic microbial community.

These microorganisms will be designed to sense and respond to diverse environmental cues, including nutrient deficiencies, heavy metal contamination, or pathogen presence, and execute targeted actions to resolve these challenges. Using advanced synthetic biology tools, the project aims to engineer robust microbial systems capable of processing complex environmental inputs and implementing precise responses, such as pollutant degradation, nutrient availability, and antimicrobial production.

This project operates at the intersection of synthetic biology, plant science, soil ecology, and environmental science, emphasizing the need for a multidisciplinary framework. The current phase of the project focuses on assessing technological feasibility and establishing a collaborative research consortium with complementary expertise.

This initiative has the potential to transform agriculture by enabling more sustainable practices that reduce environmental impacts, enhance resilience to climate challenges, and secure global food supplies. The outcomes will drive progress toward an equitable and sustainable future for agriculture and society at large.

Abstract ID: 596 / Poster Number: 187.15P

Biostimulation improves biodegradation compared to dispersant application in Arctic Ocean oil spill scenarios

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Accidental marine hydrocarbon (HC) spills cause substantial environmental damage in marine ecosystems (1,2). Future spills could lead to complex spill response conditions, due to oil exploration activities moving into deeper waters and less impacted regions like the Arctic (3-5). Chemical dispersants are solvent-surfactant mixtures applied to disperse oil into the water column. The ecological impact of chemical dispersant application, especially on native microbial oil-degrading populations, is still under debate (6,7). Biostimulation represents an alternative remediation approach by enhancing naturally occurring oil biodegradation processes, typically by supplying limiting nutrients (8). The main objectives of this study were to characterize the response of microbial communities from the central Arctic Ocean to a simulated oil spill scenario and to compare the effects of nutrient or dispersant addition on the microbial community composition and their oil biodegradation potential.

Arctic Ocean seawater microcosms were amended with crude oil components as water-accommodated fractions (WAF) ± nutrients (N; i.e., nitrate, ammonium, phosphate), oil-dispersant mixtures as chemically enhanced WAFs (CEWAF), or dispersant alone, and incubated for 32 days at 1°C in triplicates. Nutrients, DOC, and HC concentrations were monitored during the course of the experiment. Microbial community composition and cell numbers were investigated using 16S rRNA gene amplicon sequencing, qPCR, and cell counts. Microbial activity was measured using ^{3}H-leucine incorporation assays.

Total petroleum hydrocarbon equivalents revealed significantly higher (2.4-fold; $p = 0.01$) persisting levels of HCs in dispersant-containing treatments compared to both WAF treatments after 32 days. The n-alkanes ($C_{12–18}$) decreased significantly ($p = 0.04$) in all treatments, with the CEWAF treatment reaching the lowest remaining levels (11% of T_0 concentrations) after 32 days. DOC concentrations in both WAF treatments decreased significantly, reaching background levels within 12 days in the WAF+N treatment ($p = 2\times10^{-6}$) or 32 days in the WAF treatment ($p = 1\times10^{-7}$). In the dispersant-containing treatments, DOC content remained significantly higher ($p = 2\times10^{-6}$) after 32 days.

After 32 days, the microbial activity in dispersant-containing treatments had decreased to levels similar to control treatments, while the activity in both WAF treatments was significantly ($p = 7\times10^{-5}$) higher, with the nutrient-amended WAF treatment showing the highest sustained activity. Cell counts showed that the observed increase in bacterial productivity was followed by an increase in cell numbers after 12 days (4.5- to 8.7-fold), with nutrient-amended treatments reaching the highest average cell numbers after 32 days. Dispersant-containing treatments exhibited an enriched taxon after 5 days (*Sinobacterium*; up to 16%) and were mostly dominated by *Colwellia* for the remainder of the experiment (up to 82%). *Neptunomonas* was enriched in both WAF treatments (up to 20%), with other taxa only enriched in either WAF (*Loktanella*; 4%) or WAF+N (*Profundimonas*, *Psychroserpens*, *Pacificibacter*; 10–22%) treatments.

The response of the Arctic seawater community to a simulated oil spill scenario revealed a large local potential for oil biodegradation. Biostimulation and chemical dispersant application resulted in different respective microbial communities and biodegradation dynamics with the dispersant addition leading to more drastic changes in microbial community dynamics than nutrient addition when compared to the WAF-only treatment.

Abstract ID: 3392

Biosynthesis and function of capsular heptose in Campylobacter jejuni

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Co-authors: *Matthew Myles*

Background: *Campylobacter jejuni* (CJ) is a commensal in many animals but causes severe diarrhea in humans who ingest contaminated water or meat. Its surface capsule confers protective functions in different environmental conditions in a strain- and species-specific manner. A unique feature of the CJ capsule is the presence of a modified heptose of unknown function which differs across strains. We investigated its biosynthesis and function in 2 CJ strains: NCTC11168 that comprises 3,6-OMe-L-*gluco*-heptose, and 81-176, that comprises 6-deoxy-D-*altro*-heptose. We surmised that if the heptose supports capsule function and virulence, the enzymes involved in its production can be exploited to derive novel treatments for campylobacteriosis. These would be valuable considering the rising antibiotic resistance of CJ.

Objectives: We aimed to determine the biosynthetic pathways of 2 differently modified heptoses and elucidate the mechanism of action of the enzymes involved. We also aimed to determine the biological role of the heptose in strain NCTC 11168, where the location of heptose as a branching sugar allows to generate knockout mutants of biosynthetic enzymes that still produce capsule but lack the modified heptose.

Methods: We overexpressed enzymes from the capsular cluster to elucidate their role in heptose modification using capillary electrophoresis to follow catalysis and identified key residues by site directed mutagenesis. We generated capsule-less and heptose-less mutants in strain NCTC 11168 which we used to elucidate the function of the capsule and its heptose using *in vitro* stress models, animal models and tissue culture models.

Results: We elucidated the function of 7 enzymes and showed their sequential activity in similar pathways accross both CJ strains though the enzymes specificities led to different heptoses. We identified key catalytic residues and several residues that govern substrate or epimerization specificity, which will support the design of inhibitors. Further, we showed that inactivation of heptose modification genes decreased susceptibility to bile salts, increased sensitivity to serum killing, and abrogated invasion of human intestinal cells. We also showed that the capsule and heptose support persistence in chicken and drosophila intestines, and enhance virulence in *Galleria*. The capsule had

limited antiphagocytic activity but decreased adherence to chicken macrophages and decreased intracellular survival in human and chicken macrophages, while the heptose supported adherence to human macrophages and survival within, and increased uptake by chicken macrophages. Also, the heptose mitigated the capsule-triggered nitric oxide production in chicken macrophages and protected against nitrosative assault. Finally, CJ NCTC11168 elicited strong cytokine production in both macrophage species but quenched ROS production independently from capsule and heptose, and while the capsule and heptose did not protect against oxidative assault, they favored growth in biofilms under oxidative stress.

Conclusion: Our data suggest that the capsule and its heptose are important for interactions with various hosts and their innate immune defenses though they often have antagonistic actions. As the wild-type capsule with its heptose is optimal to resist innate defenses in strain NCTC11168, inhibitors of our heptose modification enzymes would provide a means to reduce chicken colonization, thus decreasing meat contamination and downstream human campylobacteriosis.

Abstract ID: 280 / Poster Number: 151.15P

Bio-synthetic gene clusters found in microbial communities inhabiting a high-altitude thermal system

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Thermal systems are characterized by the flow of water that is hotter than ambient air carrying a wide array of solutes and gasses. Lirima is a high-altitude thermal system located in the Chilean altiplano that is a terrestrial hydrothermal system consisting of an area of 500 mts2 containing ponds with water temperature ranging from 42-80 ºC. The extreme conditions generate a reservoir for the study of novel taxonomic groups and metabolic potential. This study aimed to determine the genomic potential of this community to produce secondary metabolites that enables inhabiting microorganisms to thrive under a great number of stressors including, high solar radiation and temperature, and high metal(oids) concentrations. We found that taxonomy composition of the communities inhabiting these sediments and microbial mats are dominated by photoheterotrophic bacteria. Moreover, we determined that these microorganisms have great potential for metabolites with different chemical nature including lantipeptides, lasso peptides, ranthipeptides, RiPPs, NRPS, betalactones, PKS, among other secondary metabolites like terpenes, ladderanes and RRE containing molecules that could be the scaffold for building a wide array of bioactive compounds including unknown metabolites. In this study we report the presence of several biosynthetic gene clusters with the potential for antimicrobial activity of different chemical nature and a wide array of pharmacological applications. This study contributed to the characterization of the unique yet fragile ecosystem of Lirima that is currently under anthropogenic stress and is promoted as an ideal natural laboratory to study adaptations to stress and the evolution of life.

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Abstract ID: 2779 / Poster Number: 382.17P

Biotechnological valorization of brewer's spent grains through microbial fermentation and High-Pressure Homogenization (HPH) treatments to obtain functional ingredients for food formulations

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The FAO estimates that about one third of the food produced for human consumption is lost every year, with a significant proportion of it being wasted or underutilized, including cereals, fruits and vegetables. In this context, the PROACTIVE project (PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2020, Prot. 2020CNRB84) aims to valorize several food by-products to obtain ingredients rich in bioactive peptides for the fortification of healthy foods. Among the biotechnological approaches, microbial fermentation with tailored strains or treatments such as High-Pressure Homogenization (HPH) could potentially improve the increase of bioactive peptides in food by-products such as brewer's spent grains (BSG). Therefore, tailored fermentations of BSG with selected yeast and lactic acid bacteria strains were performed after substrate optimization and HPH treatments (500-1000 Bar). The fermented samples were analyzed for their peptide content, and the best conditions, both the liquid supernatants and the peptide extracts were better characterized. These samples were analyzed for microbial growth and absence of cytotoxicity in the intestinal cell lines Caco-2 and HT-29 using the MTT assay. Furthermore, the protective effect of the samples on Caco-2 and HT-29 cells against SDS-induced inflammatory stress as well as their anti-inflammatory effect on macrophages (RAW264.7 cells) was investigated by measuring nitric oxide (NO) production. Overall, fermentation with yeasts and bacteria resulted in products with different peptide contents and functional properties, depending on the strain used and the HPH treatment applied. In future studies, the most promising fermented products will be used for the development of value-added foods.

Abstract ID: 846 / Poster Number: 275.17P

Biotechnological valorization of mining: Enhancing the production of industrially relevant bioactive compounds

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Co-authors: *João Costa, Teresa Rocha-Santos, Tânia Caetano, Ruth Pereira, Sónia Mendo, Joana Lourenço*

Among the various classes of waste, naturally occurring radioactive materials (NORM) are generated by several industries. However, the lack of strategies for their re-use and valorization presents significant hazards to the environment due to their chemical and radiological properties. Fungi appear as promising sources of industrially relevant biomolecules. In this study, we evaluated growth and metabolic responses of three species (*Pleurotus ostreatus, Pholiota nameko* and *Trametes versicolor*), of white-rot fungi exposed to low concentrations of a uranium mine effluent containing NORM and metals. The focus of our research was on the production biotechnology-relevant compounds by these species. The growth rate was assessed in three different culture media, with and without the uranium mine effluent (1% V/V). The metabolic profile was analysed with FTIR-ATR spectroscopy.

An increase on the growth rate (not statistically significant) was observed in the samples containing uranium mine effluent. Notably, *T. versicolor* produced droplets during growth and demonstrated significant metabolic changes when exposed to the effluent. These changes were associated with the production of laccase, melanin, and oxalic acid. The results highlight the importance of exploring the production of industrially relevant bioactive compounds while supporting the development of circular economy strategies for the re-use and valorization of NORM-containing waste.

Abstract ID: 2263 / Poster Number: 310.17P

Biotechnology at the Edge: Does Lithium Drive Exopolysaccharide Production?

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The Salar de Uyuni (SdU), located in the central depression of the Bolivian Altiplano, represents the largest hypersaline ecosystem on the planet. This system has been defined as a polyextreme environment mainly due to its high salinity and elevated concentrations of chaotropic salts like lithium, known for its toxicity and industrial significance. Previous studies have isolated a collection of microorganisms from this hypersaline environment, sourced from salt and sediment samples of the SdU.

The aim of this study is to investigate the biotechnological potential of exopolysaccharide (EPS) production as a protective mechanism against high salinity conditions in chaotropic-kosmotropic salts. For this purpose, *Halomonas* bacteria were grown in the absence of any salts, with NaCl (kosmotropic salt), and LiCl (chaotropic salt), using glucose as the sole source of carbon and energy. The results obtained showed differences in bacterial growth and an increase in EPS production in the presence of LiCl. The subsequent characterization of the EPS from each condition revealed differences in the compositional concentration of their monomers and their biotechnological outcomes. Simultaneously, a genomic analysis was performed to identify potential EPS biosynthesis pathways, proposing several metabolic routes that may be involved in their production.

This study suggests a sustainable and industrially competitive alternative for replacing antioxidants, antitumor agents, or traditional fossil-based plastics. Moreover, the study proposes the use of these EPS as an economically viable tool to enhance bioleaching processes, aligned with the circular economy, to address the environmental challenges in mining industry.

Abstract ID: 1928 / Poster Number: 101.17P

Bivalve mussels are good bioindicators of the spread of antimicrobial resistance in freshwater reservoirs

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Antimicrobial resistance (AMR) is a global emerging problem and developing new methods to monitor its spread is crucial. Accordingly, bivalve mussels were tested as bioindicators of the dissemination of AMR in freshwater reservoirs, due to their capacity for filter water and concentrate bacteria. Zebra and quagga mussels were collected from Swiss lakes of the Seeland region, impacted by anthropogenic activities. Mussels and water were collected monthly (n=5) in the same sampling points. Absolute numbers of third-generation cephalosporin-resistant Enterobacterales were always higher in mussels (up to 10^4 CFU/g) than in water (up to 10^1 CFU/100 ml). After enrichment, 152 presumptive cephalosporin- and carbapenem-resistant strains were isolated from mussels (n=90) and water (n=62) and a selection of 85 isolates were sequenced. As a result, many *E. coli* isolates (32 out of 35) harboured a $bla_{CTX\text{-}M}$ gene, which as an extended-spectrum beta-lactamase (ESBL) and a great part of them (24 out of 35) were isolated in mussels. Only *E. coli* from mussels harboured the bla_{OXA} gene (n=5), being $bla_{OXA\text{-}48}$ in 3 cases, that confers resistance to carbapenems. The other Enterobacterales harboured mainly AmpC beta-lactamases, and 3 *Enterobacter* isolates carried the *mcr-10* gene, conferring colistin resistance. Three *Acinetobacter* isolates carried the $bla_{OXA\text{-}24}$ gene, with strong carbapenemase activity. To conclude, our results highlight the role of environmental bacteria on the maintenance of clinically-relevant antibiotic resistance genes, which is of great importance from the One Health perspective. Moreover, it was demonstrated that sampling freshwater bivalves may be an efficient way to monitor AMR in environment.

Abstract ID: 3809

Boosting Antimicrobial Photodynamic Inactivation of Pseudomonas aeruginosa Biofilms in Chronic Wounds with a Colistin-Berberine-Gentamicin Combination

Presenting author: Assoc. Prof. Manuel Simões, University of Porto, Porto, Portugal

Co-authors: *Ariana Gonçalves, Miguel Leitão, Anabela Borges*

Background: *Pseudomonas aeruginosa* biofilm-associated infections in chronic wounds pose significant therapeutic challenges, especially due to the reduced effectiveness of traditional treatments. Although antimicrobial photodynamic inactivation (aPDI) is a promising therapeutic strategy, its action against Gram-negative bacteria remains suboptimal. To enhance aPDI efficacy, this study investigates the use of subinhibitory concentrations of colistin (Col) in combination with berberine and gentamicin (Ber-Gen) against *P. aeruginosa* biofilms.

Methods: A clinical strain of *P. aeruginosa* isolated from a diabetic foot ulcer was used. Membrane permeability changes, induced by Col, were quantified through flow cytometry, while the optimal synergistic concentration of the triple combination (Ber-Gen-Col) was determined by checkerboard assay. The impact of blue light (420 nm, 30 mW/cm^2, 10 min) on the treatment's effectiveness was tested after one or three aPDI cycles (administered every 24 h for up to 3 days). Biofilm eradication was assessed by measuring biomass, metabolic activity, and culturability (by crystal violet, resazurin, and colony-forming units (CFU/cm^2), respectively).

Results: At subinhibitory concentrations (2 µg/mL), Col increased membrane permeability by 15 %, enhancing the photodynamic effect of Ber-Gen-Col. The combination resulted in a nearly complete inactivation of biofilm cells, achieving a 7-log CFU/cm^2 reduction and over 90 % reductions in biomass and metabolic activity after single or multiple irradiation cycles. However, sustained bacterial growth suppression was only observed after repeated treatments, with biofilm regrowth occurring within 24 h after a single cycle.

Conclusion: These findings highlight the potential of integrating subinhibitory Col concentrations with repeated aPDI cycles as a novel strategy to effectively combat *P. aeruginosa* biofilms in chronic wound infections.

Abstract ID: 3684 / Poster Number: 382.16F

Boosting Non-conventional Crops Growth with a Community Biofertilizer

Presenting author: Paola Andrea Reyes-Caldas, PhD, Universidad de los Andes, Bogotá, Colombia

Co-authors: *Yeimy Alejandra Montenegro Bacca, Laura Camila Samboni Claros, Martha Cardenas, Maria Fernanda Mideros, Javier Tabima, Adriana Bernal*

It is expected that climate change will increase the vulnerability of crop production systems worldwide. At the same time, glasshouse gases derived from agrichemical inputs are one of the main drivers of climate change. The use of PGPB in agriculture promises to help alleviate the global warming crisis, being an inexpensive and sustainable practice to help farmers to achieve profitable clean production. However, there is a lacking body of proof to support the efficacy of these soil amendments in the field, especially for non-conventional crops. Here, we study the effect of an organic fertilizer and a biofertilizer in the soil health (microbial diversity), germination and growth of Sacha Inchi (*Plukenetia volubilis*), a native superfood from the Amazon. We used an in-house preparation from the local community as organic fertilizer and an artificial consortium composed of three PGPB previously isolated from *P.volubilis* tissue as a biofertilizer, alone or in combination. A chemical fertilizer and a mock treatment were used as controls. The effect of the treatments on seed germination were evaluated in vitro while the effect on growth and production was evaluated in field experiments in the Department of Caquetá, Colombia. Results indicate *P.volubilis* germination and growth benefit in the presence of organic fertilizers and biofertilizers, and its addition shapes the microbial community associated with *P.volubilis* rhizosphere in the field. Results from this study highlight the importance incorporating plant growth promoters to boost production of non-conventional crops and improving the growers' livelihood.

Abstract ID: 3987 / Poster Number: 364.16P

Brewers' spent grain- based substrates as organic fertilizers

Presenting author: Angela Bianco, PhD, University of Sassari, Department of Agricultural Sciences, Sassari, Italy

Co-authors: *Sara Melito, Matteo Garau, Vittoria Giannini, Giacomo Zara, Davide Assandri, Safa Oufensou, Roberta Coronas, Niccolò Pampuro, Marilena Budroni*

Brewers' Spent Grain (BSG) is the primary byproduct of breweries, with an estimated worldwide production of about 38 million tons annually. The rich nutritional profile makes BSG a valuable resource for various biotechnological applications. In the era of climate change, it is crucial to identify eco-sustainable processes, such as composting, vermicomposting and pyrolysis to manage and recycle BSG into a valuable resource. This study, therefore, aimed to evaluate the effectiveness of BSG-based substrates – namely compost (CP), compost enriched with a microbial consortium (CPE), vermicompost (V), and biochar (B) – on the growth parameters, yield, and physiological responses of Lactuca sativa L. var. capitata "Regina dei ghiacci" at (i) nursery stage, and BSG-based substrates were mixed at 2 and 4% with peat and compared against two control treatments: 100% peat (C1) and peat with ternary chemical fertilizer (C2). At the nursery stage, the application of substrates V, CP, and CPE, all added with 4% peat, resulted in plant productivity comparable to that obtained using the chemical fertilizer (C2). Additionally, plants treated with BSG-based compost exhibited higher root development (C 4% and CPE 4%), as well as higher chlorophyll (α and β) (CPE 4%), and carotenoid contents (CPE 4%). These results provide evidence that processed BSG can be applied as a biofertilizer in agriculture, addressing the challenge of brewery's waste recycling and suggesting the possibility of including BSG-based biofertilizers among those admitted to organic horticulture (Bianco et al., 2024).

Abstract ID: 1234 / Poster Number: 087.17P

Bridging breastfeeding and milk resistome to the establishment and transmission of antibiotic resistance genes in the infant gut during the first month of life

Presenting author: Mr. Zhe Pan, APC Microbiome Ireland, Cork, Ireland; School of Microbiology, University College Cork, Cork, Ireland

Co-authors: *Conor Feehily, Fionnuala M. McAuliffe, Paul D. Cotter, John MacSharry, Douwe Van Sinderen*

The infant resistome, the collection of antimicrobial resistance genes (ARGs), is critical for adaptions to environmental pressures and the establishment of the gut microbiota. Metagenomic sequencing was performed to study infant resistome establishment and transmission of ARGs from mothers/breastmilk to infants using a total of 431 samples collected from infant stools within one-week (n=119), at one-month (n=119), from maternal stool post-partum at one-month (n=120), and from breastmilk (n=73). Our results revealed that multidrug resistance contributed most to ARG abundance during the first month. Furthermore, breastfeeding selectively increases the number of bacteria carrying more than one ARG, while higher abundance of *Escherichia coli* decreases the functional redundancy of ARG-harboring bacteria. *Bifidobacterium longum* exhibited the most antagonistic relationships with 21 ARGs at 1 month in breastfed infants, instead, formula feeding led to antagonistic relationships between *Bifidobacterium bifidum* and four ARGs. The milk resistome was dominated by the macrolide-lincosamide-streptogramin class, however, potential transmission of ARGs was observed from the quinolone class, by which breastfeeding together with maternal antibiotic usage increases the transmission possibility of *srdM* from milk to infant with *Staphylococcus aureus* being the carrier of *srdM* in milk and infants. Taken together, our study reveals that breastfeeding together with age and antibiotic usage shapes gut resistome establishment and transmission and identified the beneficial role of *Bifidobacterium* species and other species in modulating the resistome and functional redundancy of ARG-harbouring bacteria, suggesting the importance of breastfeeding in affecting resistome in early life.

Abstract ID: 3561

Bridging the gap between culture-dependent methods and metagenomic predictions: integrating culturomics and bioinformatics for comprehensive water kefir characterisation

Presenting author: Dr. Guerrino Macori, University College Dublin, Dublin, Ireland

Co-authors: *Mingwei Hong*

Introduction: Advances in sequencing technologies and bioinformatics are revolutionising microbiology by enabling the detailed characterisation of complex microbial communities. However, discrepancies between culture-dependent methods and metagenomic predictions pose significant challenges, particularly in food safety and microbiome research. The presence of DNA from dead cells is hypothesised to contribute to these gaps, affecting the accuracy of metagenomic analyses.

Objectives: This study aimed to bridge the gap between culture-dependent and metagenomic methods by integrating culturomics and bioinformatics, using water kefir as a microbiome model. A secondary objective was to investigate the role of dead cells in these discrepancies by employing propidium monoazide (PMA) treatment and developing a robust analysis and prediction pipeline.

Methods: Water kefir samples were analysed after aerobic and anaerobic fermentations. Culture-dependent techniques involved classical microbiology methods, novel media, and in-situ culturing using iChip to isolate key probiotic species (*Gluconobacter*, *Liquorilactobacillus*, *Lactiplantibacillus*, *Lentilactobacillus*, and *Lacticaseibacillus*). These were complemented by metagenome-assembled genomes (MAGs) derived from shotgun sequencing and full-length 16S rRNA gene sequencing using Nanopore technology. PMA treatment selectively inhibited amplification of DNA from dead cells, with heat-killed controls and live-dead mixtures serving as experimental references. DNA was extracted with dedicated extraction kit and analysed through bioinformatics pipelines.

Findings: PMA treatment revealed that non-viable cells partially contributed to discrepancies between culture-dependent and metagenomic predictions. Novel MAGs were recovered, offering insights into microbial diversity and functionality. These MAGs aligned with isolates recovered through culturing and iChip, reducing the gap between culture-dependent and culture-independent methods. Nevertheless, a portion of

microbial dark matter remains, representing unculturable or improperly predicted microorganisms. The combined use of PMA, novel culturing techniques, and robust bioinformatics pipelines enhanced strain-level characterisation of the water kefir microbiome.

Conclusion: This study demonstrates that integrating culturomics, PMA treatment, and bioinformatics improves the reliability of metagenomic predictions while addressing discrepancies with culture-dependent methods. These findings advance the understanding of microbial ecosystems in fermented foods and highlight the potential of next-generation tools in microbiome research and precision food safety.

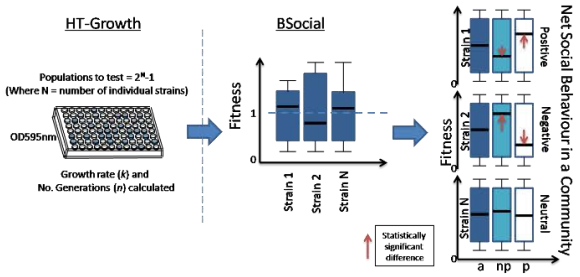
Abstract ID: 2977 / Poster Number: 462.15P

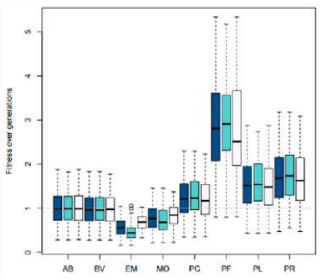
BSocial – deciphering the social behaviour of PGPBs

Presenting author: Dr. Jessica Purswani, Universidad de Granada, Granada, Spain

Co-authors: *Maria Garcia-Toledo, Belen Juarez-jimenez, Belen Rodelas, Clementina Pozo*

The application of microbial consortia in biotechnological areas such as bioremediation or biofertilisation has been shown to be much more efficient than the use of populations consisting of a single microorganisms due to the ability to increase stability and functional efficiency. The main difficulty in the study of microbial consortia lies in the large number of populations to be tested. The use of models/protocols to predict functional efficiency on a mass scale, looking at the complex interactions of more than 2 species, are the key to incorporating greater microbial diversity into the biotech industry. An example of this is the BSocial tool (http://m4m.ugr.es/BSocial.html), which assigns a social behaviour to each strain based on its contribution to the overall growth (number of generations and growth rate) of the consortium through statistical analysis, thus defining the composition of the social consortia. This tool has been used to design microbial consortia for the bioremediation of environments contaminated with petrol oxygenates and pharmaceuticals and now being used to create biofertilizers. The social behaviour of 8 PGPBs (*Azospirillum, Bradyrhizobium, Ensifer, Pseudomonas spp., Mesorhizobium*), as well as 3 functions (PGPT) of the complete combinatorial (255 populations) were analysed by colorimetry. To test initial germination toxicity, tests were carried out on Dutch cucumber (*Cucumis sativus*), cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) and pepper (*Capsicum annuum*) seeds under optimal conditions and under combined saline and drought stress. From the 3 social consortia (positive and neutral social behaviour strains) resulting from the BSocial tool, 2 had high PGPT functional potential. One demonstrated high values for all 3 functions evaluated (siderophore production, phosphate solubilisation and indole acetic acid production) whilst the other excelled in indole acetic acid production. The two functional social consortia promoted seed germination (cucumber, cherry tomato and pepper), especially under stress conditions. An increase in species richness within the consortia lead to increased functionality and stability, except for siderophore production stability. Therefore, biofertilizer consortia predictions using the BSocial tool increases the probability of selecting highly functional, non-toxic and stable PGPBs for biofertilizer use.





Strain	Social behaviour	
	No. generations	Growth rate
AB	Neutral	Negative
BV	Neutral	Negative
EM	Neutral	Neutral
MO	Positive	Neutral
PC	Negative	Positive
PF	Negative	Negative
PL	Negative	Neutral
PR	Negative	Negative

Figure 1: BSocial: from combinatorial growth data to social behaviour assignment. BSocial groups the combinatorial data into two groups for each strain: consortia in which it is present (p) and not present (np) and assigns social behaviour based on the differences between the medians of the two groups. If the median p > np, positive behaviour is assigned; if p < np, negative behaviour is assigned; and if there are no significant differences, neutral behaviour.

Abstract ID: 2724

Calcite addition to surface sediments as a CO2 removal strategy in seasonal hypoxic coastal waters promotes cable bacteria and alters the biogeochemical cycle

Presenting author: Assoc. Prof. Mirjam Perner, GEOMAR, Kiel, Germany

Co-authors: *Rebecca Bährle, Till Bornemann, Michael Fuhr, Stefanie Böhnke, André Soares, Andrew Dale, Alexander Probst, Sonja Geilert*

Enhanced benthic weathering (EBW) is considered a promising carbon dioxide removal (CDR) strategy. In EBW, alkaline minerals are added to the seafloor, thereby accelerating weathering and releasing alkalinity to the water body and fostering CDR. In the Baltic Sea, sediments exposed to seasonal hypoxic bottom waters are expected to be ideal settings for EBW. As of now, feedbacks of microbial metabolisms to EBW remain unresolved. To investigate changes of the biogeochemical cycle related to this CDR strategy, alkaline minerals, namely dunite and calcite, were added to surface sediments that are exposed to seasonal hypoxia. These sediment cores were incubated under oxygen limited conditions and the shifts in geochemistry and microbial transcriptomes were monitored over time. Calcite amended incubations indicated the highest CDR potential. Here the combination of calcite dissolution related bicarbonate bioavailability and limited oxygen availability in bottom waters were likely the main drivers of *Cand*. Electrothrix (cable bacteria) enrichment. *Cand*. Electrothrix's acidifying activity promoted carbonate- and iron monosulfide dissolution, evidenced by the accumulation of bicarbonate, manganese, iron and hydrogen sulfide in sediment porewaters. In line, as indicated by transcriptomics, overall microbial sulfur-, iron- and manganese oxidation were stimulated. Moreso, transcript abundances posited shifts towards denitrification, indicating considerable changes in biogeochemical cycling. Carbonic anhydrases (converting bicarbonate into CO_2) were upregulated suggesting a bicarbonate source in the sediments – likely related to calcite dissolution. This appeared to trigger activity of autotrophic CO_2-fixation as indicated by transcriptomic analyses. *Cand*. Electrothrix appeared to promote EBW. In turn, artificial calcite addition also increased the buffering capacity of the sediments, limiting mineral dissolution. Hence, less available ferrous iron may restrict iron-oxide formation and thus may promote toxic sulfide being released from the sediments during seasonal hypoxia/anoxia.

Conclusively, according to shifts in transcript abundances, feedbacks may include stimulated EBW but also loss of dinitrogen from sediments as well as reduced retention

of hydrogen sulfide during anoxia if sediments are already naturally enriched in carbonates. Metabolic rate measurements are urgently required to scrutinize the holistic effects of EBW on such coastal systems.

Abstract ID: 609 / Poster Number: 324.15P

Can Campylobacter jejuni form biofilm on uncoated plastic polymers?

Presenting author: Dr. Irene Ortega Sanz, Ghent University, Gent, Belgium

Co-authors: *Tessa Callaert, Andreja Rajkovic*

Biofilm formation is one the major strategies developed by bacteria to survive under extreme conditions. In the food industry, foodborne pathogens are able to adhere to and colonize diverse surfaces, such as plastic, stainless steel and glass, leading to biofilm formation, which endangers the effectivity of the cleaning and disinfection procedures. Among them, *Campylobacter jejuni* poses a significant food safety risk for causing most of the foodborne bacterial infectious gastroenteritis cases reported worldwide. However, their survival strategies are poorly understood. Previous studies have focused on evaluating the effect of (plasma-)treated plastic surfaces with improved hydrophilicity on the ability of *C. jejuni* to form biofilm (Ortega-Sanz et al., 2024). However, little is known about the impact of pristine (micro)plastics with strong hydrophobic properties in the survival of the pathogen. In this study, we analysed the ability of 5 *C. jejuni* strains to form biofilm on flat-bottomed, non-treated 96-well microtiter plates made of polypropylene (PP) and polystyrene (PS) for 96 h at 22 °C and 37 °C under aerobiosis following the crystal violet staining method. Based on the formula of Stepanović et al. (2007), that classifies the isolates as non-, weak, moderate or strong biofilm formers, only 3 isolates (including the two isolates from human origin) were able to form biofilm on PP at both temperatures, although weakly, showing optical density values at 590 nm ranging between 0.098 and 0.103 (Figure 1). However, their ability to form biofilm showed no significant difference compared to the negative control (0.092 ± 0.002) ($p > 0.05$). The findings indicate that *C. jejuni* is not really capable of forming biofilm on the hydrophobic plastic materials tested, highlighting the relevance of the surface coating on the attachment and colonization of the pathogen.

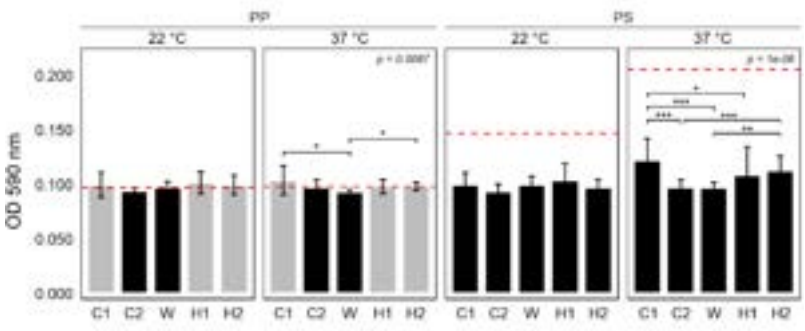


Figure 1: Biofilm formation on polypropylene (PP) and polystyrene (PS) at 22 °C and 37 °C under aerobiosis by the 5 C. jejuni isolates (from chicken –C–, water –W–, and human –H–) tested in this study. The red lines represent the cut-off OD value, defined as three standard deviations above the mean OD of the negative control. Biofilm-forming capacity varies significantly among isolates at 37 °C (Kruskal-Wallis; $p < 0.05$). The results represent mean values and standard errors of three independent experiments. Each experiment was performed in sextuplicate. Black: non-biofilm former; grey: weak biofilm former. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Acknowledgements

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Abstract ID: 1899 / Poster Number: 270.16P

Can fish be used as sentinels for AMR surveillance in riverine environments?

Presenting author: Ms. Faina Tskhay, Technische Universität Dresden, Dresden, Germany

Co-authors: *Cristoph Köbsch, Alan Elena, Johan Bengtsson-Palme, Thomas Berendonk, Uli Klümper*

Modern wastewater treatment plants (WWTPs) are inefficient at removing antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Consequently, WWTPs are critical anthropogenic sources of ARB and ARGs discharged into receiving water bodies, significantly elevating their levels in river water and sediments. However, if the enrichment of ARB and ARGs observed in environment is also mirrored in riverine wildlife has not been extensively explored.

To investigate whether river fish can serve as sentinels for antimicrobial resistance (AMR) pollution, we compared the microbiome and resistome of two common German riverine fish species—brown trout (*Salmo trutta*) and European bullhead (*Cottus gobio*)—collected upstream and downstream of a WWTP in Germany. These species were selected for their differing lifestyles: brown trout is an actively swimming fish inhabiting the water column, while European bullhead is a sedentary species with limited mobility close to the sediment.

To analyze the fish microbiome composition, we extracted DNA from fish gills, skin, and feces and performed 16S rRNA gene-based sequencing. Resistome analysis of fish samples was conducted using high-throughput qPCR targeting 27 specific ARGs. Furthermore, to trace potential changes in fish microbiome or resistome to the sources, we analyzed the microbiome and resistome of river water and sediment samples collected upstream and downstream of the WWTP as well as the discharged wastewater.

The microbiome compositions of both fish species reflected the observed variations in river water microbiome up- and downstream of the WWTP, with more pronounced changes in the trout microbiome compared to the bullhead microbiome. The correspondence between fish and river microbiomes was particularly supported by the increased relative abundance of the phyla Verrucomicrobia and Proteobacteria in both water and fish samples collected downstream of the WWTP. Although the difference in microbiome composition was significant, the variations in ARG profiles between river water up- and downstream of the WWTP were less obvious. However, wastewater

effluent caused a significant increase in the levels of individual ARGs in the river downstream of the WWTP. Notably, a more than 10-fold increase in the relative abundance of four tested ARGs (*bla*$_{OXA}$, *ermB*, *ermF*, and *tetW*) was observed. Both trout and bullhead exhibited low dissimilarity in their resistomes upstream and downstream of the WWTP. At the individual gene level, neither trout nor bullhead reflected the increase in specific ARGs detected in the river water. As a result, neither fish species appears to be an effective sentinel for assessing AMR pollution in the environment.

Our findings suggest that poikilothermic organisms, such as fish, are less effective at reflecting changes in the river resistome associated with wastewater effluent. Therefore, efficient surveillance of AMR pollution requires further research to identify alternative, reliable indicator organisms for detecting the antimicrobial contamination levels in aquatic environments.

Abstract ID: 2549 / Poster Number: 169.16P

Cancer therapy using Anti-PD-L1 cell-surface display from Salmonella

Presenting author: Mr. Seunghyeok Bang, Kyungpook National University School of Medicine, Daegu, South Korea

Co-authors: *Jeongah Kim, Samia Akter, Seung Ji Kang, Kyung-Hwa Park, Minsang Shin*

Cancer remains a leading cause of mortality worldwide, with an increasing incidence and death rate. Immune checkpoint inhibitors, particularly targeting the PD-1/PD-L1 axis, have emerged as promising cancer immunotherapies. However, challenges such as reduced peptide efficacy due to in vivo degradation and the risks associated with residual bacteria in bacterial-based delivery systems limit their clinical application.

This study aims to develop a novel cancer therapeutic using *Salmonella*-derived outer membrane vesicles (OMVs) engineered to carry anti-PD-L1 peptides. OMVs are biocompatible nanostructures naturally released by Gram-negative bacteria, offering a safe and efficient delivery platform. By modifying the extracellular loop of the OmpA protein on OMVs to conjugate PD-L1-binding peptides, this approach enhances T-cell activation and tumor suppression.

Key objectives include optimizing endotoxin-free OMVs to ensure safety, confirming PD-L1 binding specificity in vitro, and evaluating anti-cancer efficacy in vivo using mouse models. The engineered OMVs showed robust binding to PD-L1 on cancer cells, effectively reactivating T-cell activity and suppressing tumor growth. Furthermore, endotoxin-free OMVs demonstrated no toxicity, overcoming a critical limitation of bacterial-based therapies.

This research addresses the instability of peptide-based therapies and the safety concerns of residual bacteria, offering a novel platform for targeted cancer treatment. By leveraging the immunomodulatory potential of OMVs, this approach provides a promising new paradigm for immune checkpoint inhibitor-based cancer immunotherapy.

Abstract ID: 1397 / Poster Number: 372.15F

Canonical methanotrophs for high- and low-affinity methane removal

Presenting author: Dr. Adrian Ho, Nestlé Research, Lausanne, Switzerland

Co-authors: *Michelangelo Vuono, Judy Su, Carla Risso, Josh Silverman, Elodie Soussan,*

Methane contributes to the greenhouse effect, but the adverse impact can be partially abated by microbial methane-oxidizers (methanotrophs). Canonical aerobic methanotrophs are thought to be "low-affinity" methane-oxidizers, whereby the genes regulating methane oxidation are induced at >600ppmv methane. However, in anthropogenic-influenced environments (e.g., manure pits and cow barns in dairy production systems), methane emission levels are typically less than 600ppmv. This necessitates the enrichment and selection of methanotrophs with the capacity to oxidize methane at a wide range of methane concentrations for the greenhouse gas removal. Here, we characterized the methane oxidizing capacity of a commercial (Windfall Bio) and naturally-enriched methanotroph consortia, as well as pure cultures (*Methylotuvimicrobium buryatense*, *Methylosinus* sp.LW4, *Methylomonas* sp.LW13, *Methylocapsa aurea*) at <100ppmv to 1.5%v/v methane in laboratory-scale incubations. Additionally, biochar was supplemented to selected methanotroph consortia to determine whether the soil additive may increase methane oxidation rates. All methanotrophs oxidized methane at low (<100ppmv) to high (1.5%v/v) concentrations, albeit the consortia exhibited higher mean methane oxidation rates than the pure cultures under 1.5%v/v methane. The methane oxidation rate was significantly ($p>0.05$) stimulated by the addition of biochar in the methanotroph consortia. Changes in the cell density/counts *via* OD at 600nm and flow cytometry indicated growth occurring only at higher methane concentrations. Work is underway to characterize the bacterial community composition in the commercial and naturally-enriched consortia. Thus far, results show high-affinity methane oxidation by canonical "low-affinity" methanotrophs, and the capacity for methane removal at a wide range of methane concentrations for biotechnological applications.

Abstract ID: 139

Capsules, Pumps, and Phages: A New Strategy Against Antibiotic-Resistant Acinetobacter baumannii

Presenting author: Anita Tarasenko, PhD, Flinders University, Adelaide, Australia

Co-authors: *Robert Edwards, Bart Eijkelkamp*

Introduction: The global rise of multidrug-resistant (MDR) Acinetobacter baumannii presents a critical public health challenge. Fundamental resistance mechanisms, including efflux pumps, capsule-mediated protection, and biofilm formation, contribute to its ability to evade antibiotics and immune responses.

Objective: This study evaluates the therapeutic potential of bacteriophages targeting efflux pump mutants in A. baumannii while investigating the relationship between efflux pumps, capsule synthesis, and phage susceptibility.

Method: Five bacteriophages were isolated from sewage samples and characterised using host range analysis, genome sequencing, and Transmission Electron Microscopy (TEM). Phages were tested against three wild-type strains, twelve efflux pump mutants, a capsule mutant (AB5075 ΔWZC), and two FadL-deficient mutants. Capsule thickness was assessed using capsule density assays and TEM imaging. Phage genomes were screened for virulence and resistance genes to ensure safety.

Findings: All five phages demonstrated lytic activity. TEM analysis identified four as Siphoviridae and one as Myoviridae. Mutants lacking AdeB showed increased capsule thickness, correlating with phage resistance and indicating a role for the AdeB efflux system in capsule regulation. Capsule-deficient mutants (ΔWZC) were highly susceptible to phage infection, while wild-type strains and specific efflux pump mutants remained phage-sensitive. Phages utilised efflux pumps and related outer membrane structures as receptors. Genome analysis confirmed the absence of virulence and resistance genes, supporting therapeutic safety.

Conclusion: Phage therapy targeting efflux pumps offers a promising strategy to restore antibiotic efficacy and combat MDR A. baumannii infections. The link between efflux pump activity and capsule regulation provides opportunities for tailored phage therapies to address resistance effectively.

Abstract ID: 3797 / Poster Number: 136.16S

Capturing the Intricate Microbiota-Immune Interactions with High-resolution Immuno-Metagenomics at Early Onset of Multiple Sclerosis

Presenting author: Dr. Lejla Imamovic, Sorbonne University, Paris, France

Co-authors: *Simeon Nthuku, Guy Gorochov*

Introduction: Multiple Sclerosis is an inflammatory autoimmune disease of the Central Nervous System (CNS). Perturbed gut microbiota homeostasis has been previously associated with MS (Zou et al, 2022). It is hypothesized that this shift is linked to the mutual interaction of microbes with the Immunoglobulin (Ig) A antibody whose concentrations appear to be increased in the CNS during active MS (Probstel et al., 2021). Yet, the landscape of IgA-interacting microbiome from an early-onset Clinically Isolated Syndrome (CIS) to late-onset of MS disease has not been clearly defined. Our hypothesis was that the set off factors encompassing the MS-prone CIS can be traced to the gut with regard to the interaction between the gut microbiome and immune system.

Objectives: Our objective was to compressively characterize immunologically active microbiota in CIS and identify microbial signatures related to MS pathogenesis.

Method: To characterize the bacterial communities that interacts with IgA, we evaluated the gut microbiota of 18 healthy subjects and 14 CIS patients. Gut microbiota and IgA were fluorescently labelled and 10 millions of bacteria is separated into IgA-positive and IgA-negative fractions using Fluorescence-Activated Cell Sorting. Following DNA extractions, sorted fractions were deep shotgun sequenced. This allowed us to, for the first time, characterize the know and taxonomically unsigned IgA-bound microbiota in MS at the strain level. Finally, immunologically active microbiota is depicted from large cohorts in publicly available dataset on CIS and MS.

Findings: Our methodological pipeline allows us to, for the first time, depict the strain level differences between the immunological active and inert microbiota. Notably, we observed that on average 15% of bacteria interacting with IgA are unclassified at genus level. These so called “microbial dark matter” members represented up to 39% of immunologically active microbiota (average 13%). We observed that gut bacteria interacting with IgA differ in diversity, composition and interaction between healthy and CIS subjects (Fig.1). Network analysis revealed increased clustering of IgA-bound bacteria in CIS compared to healthy subjects. We depicted abundant IgA-bound bacteria

that discriminate healthy and CIS groups, including three unknown bacterial species currently annotated only at the family level. We investigated the IgA binding indices of the core microbiome and observed a significant difference in IgA-binding of Coprococcus catus and Bifidobacterium adolescentis, indicating their less likelihood of being bound by IgA in CIS.

Conclusion: We preformed, for the first time, in-depth profiling of immunologically active gut microbiota and we identified an immunological shift between clinical phenotypes that discriminate between CIS and healthy controls. Our analysis suggests that the gut microbiome of CIS patients represents a different ecological niche even before converting to chronic disease.

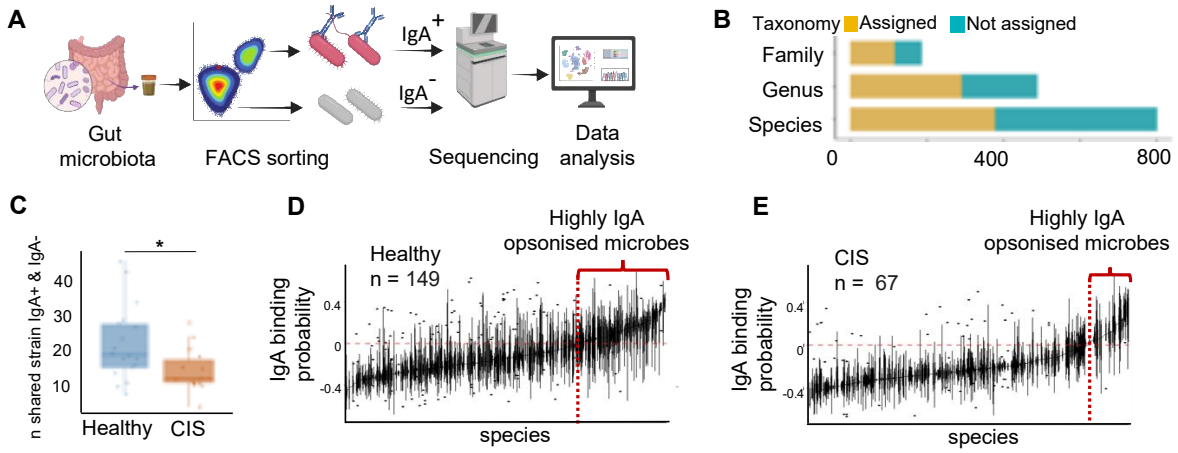


Figure 1: Capturing the Intricate Microbiota-Immune Interactions with High-resolution Immuno-Metagenomics. A. Schematic representation of the approach. **B.** Detection of immunologically relevant microbes as known, less characterised, uncultured gut bacteria. **C**. Strain-level difference observed between IgA+ and IgA- bacteria in heathy and CIS bound bacteris. **D, E**. IgA–bound microbiota is less diverse and less targeted by IgA in CIS. The overall level of binding of IgA is deceased: D- healthy controls E - CIS. Data presented in D and E are for highly prevalent strains (0.5 threshold prevalence).

Abstract ID: 1060 / Poster Number: 082.17P

Carbohydrate and antibiotics: a novel Trojan horse strategy against antimicrobial resistance

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Introduction: Antimicrobial resistance is a major health concern. In 2019, 4.95 million deaths could be associated to AMR. In 2024, WHO released its bacterial priority list, for instance, carbapenem-resistant K. pneumoniae moved from the 4th place to the 1st place. It is therefore important to find alternative ways to treat such microorganisms.

Objective: The aim of this study is to identify the transport mechanisms and degradation processes for carbohydrates and to test glycoconjugates, composed of a carbohydrate and a fluorophore or antibiotic. This way, it will be possible to understand how they enter the cells and determine their activity. Our strategy utilizes maltodextrins or cellodextrins metabolic pathways to allow entrance of our glycoconjugates.

Method: Flow cytometry is used to measure if cells incorporated these glycoconjugates. HPLC is used to measure glycoconjugates degradation, to monitor glycoconjugate degradation and antibiotic release. MIC assay is used to measure in vitro activity of the antibiotics grafted to carbohydrates.

Findings: It is expected to identify what transport channels carbohydrates use and if the glycoconjugates are able to use these transport routes. We want to determine if carbohydrate metabolism enzymes are capable of degrading glycoconjugates to release the grafted antibiotic.

Conclusion: This study brings relevant informations on the carbohydrate metabolism and our ability to hijack the metabolim to treat those multi-drug resistant organisms, using Klebsiella pneumoniae as a Gram negative model, in addition, Enterococcus faecalis will be used as a Gram positive model.

Abstract ID: 2448 / Poster Number: 439.15P

Carbon monoxide: poison or fuel for Mycobacterium tuberculosis?

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Tuberculosis is currently the deadliest infectious disease in the world, accounting for approximately 1.3 million annual deaths[1]. During infection, *Mycobacterium tuberculosis* (Mtb) is exposed to carbon monoxide, produced by macrophage heme oxygenase-1, as a defence mechanism to control the bacteria[2]. A recent study demonstrated that the saprophytic soil dweller *Mycobacterium smegmatis* can respire atmospheric CO and survive nutrient starved conditions[3]. It uses a carbon monoxide dehydrogenase (CODH) enzyme that oxidises CO into CO_2, and feeds the electrons derived from this process into the respiratory chain, thus generating energy for the cell[3]. Interestingly, Mtb possesses a homologous CODH enzyme, suggesting its capacity to employ CODH for persistence, by oxidising CO produced by macrophages. Here, we aimed to determine if Mtb is tolerant and capable of utilising CO for prolonged persistence. We confirmed that *M. tuberculosis* mc^26206 was tolerant to high CO and was able to grow after adapting to this gas. Preliminary proteomics analysis of CO exposed Mtb showed several proteins were more abundant, including siderophores and several dormancy regulated genes. We are currently in the process of constructing deletions, via homologous recombination, of genes that are thought to be important for CO tolerance. The ability of Mtb to consume CO at environmentally relevant concentrations was investigated using carbon starved cultures. Contradictory to earlier findings[4], we saw that Mtb could not consume CO at these levels. Finally, to understand the variations in gene expression under CO exposed conditions in vitro, we performed RNA sequencing, and transcriptomic analysis is currently underway.

Abstract ID: 585 / Poster Number: 267.17P

Carrot co-products valorization: potential for fermentation of leaves and scrap carrots to create innovative and sustainable food ingredients

Presenting author: Ms. Sara Simões, LEAF – Linking Landscape, Environment, Agriculture and Food Research Centre, Lisboa, Portugal

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Several studies predict an accentuated population growth for the upcoming years that will increase the food demand and, thus, the pressure on natural resources[1]. Hence, more than increasing productivity, the agri-food systems must optimize the consumption of resources. Considering the carrot (*Daucus carota*) value chain, not all edible parts are used because either consumption is not common, the case for leaves[2] and stalks, including stems and leaf petioles, or they do not fulfil the strict retail standards being rejected for their non-normative appearance (edible scrap carrots). This work aims to explore these carrot co-products as food ingredients[3], using fermentation to improve their nutritional and preservation properties, increasing their value.

Scrap carrots and carrot' leaves and stalks were scanned for their natural microbiota. Yeast and bacteria isolates were identified by DNA sequencing. Lactic acid bacteria (LAB), such as *Leuconostoc* spp. and *Weissella* spp., and yeasts, including *Candida oleophila* and *Debaryomyces hansenii*, were found. After this screening, drop tests were performed to optimize fermentation conditions. Several NaCl concentrations and microorganism consortia were tested. Different sets of LAB and yeasts were selected for each co-product, given their different compositions namely in sugars. Inocula consortia were selected from the natural and commercial strains. All the tested inocula were able to grow in plates of scrap carrots and carrot leaf solidified pastes, but not on carrot stalks pastes. Thus, two of the three tested co-products present potential for fermentation and to be transformed into new food ingredients, promoting a more circular and efficient agri-food chain.

Abstract ID: 4016 / Poster Number: 457.15P

Catalogue of Antimicrobial Resistance Genes in Species of Bacillus used to Produce Food Enzymes and Feed Additives

Presenting author: Silvia Peluso, PhD, European Food Safety Authority, Parma, Italy

A key step in the characterisation of microbial production strains of food enzymes and other fermentation products is the identification of acquired antimicrobial resistance (AMR) genes, as this has a direct impact on the European Food safety Authority (EFSA) risk assessment process and outcome. Acquired AMR genes, as opposed to intrinsic AMR genes, are favoured by selective pressure and stabilised when horizontally transferred through bacteria. The presence of acquired AMR genes influences important aspects of the risk evaluation such as the applicability of the QPS (Qualified Presumption of Safety) approach. or the need to request analysis for the possible presence of DNA from the production strain in the final product.

There are different definitions of intrinsic AMR genes. The EFSA BIOHAZ QPS Working Group has elaborated a document in which AMR genes are defined for the purposes of the EFSA risk assessments, providing indications about how to analyse AMR genes found in genomes to determine in which of those two categories certain genes fall. Depending on the bioinformatic strategy followed, the applied parameters and the databases used, results may vary, which has occasionally led to uncertainties and to divergences in the outcome of the assessment of very similar substances by different identities.

With the aim of supporting the evaluation of AMR genes found in bacterial production strains we developed a bioinformatic pipeline that implements the criteria set by the QPS WG, where genes found in the genome of the production strains can be analysed consistently and harmonically.

Abstract ID: 3615 / Poster Number: 429.16P

CATALYSE Action: Collaborative Networks Transforming Food Safety Innovative Solutions

Presenting author: Maria Agustina Sarquis, PhD, Catholic University of Sacred Heart, Cremona, Italy

Co-authors: *Diana Patricia Fonseca, Paula Teixeira, Isabelle Guelinckx, Ruchi Shah, Daniela Bassi*

Introduction: Food safety is a critical concern across the entire food chain. According to the WHO, contaminated food causes 600M cases of foodborne diseases and 420k deaths annually, resulting in significant negative impacts on economies, productivity, and food waste. The EU is committed to maintaining high food safety standards from Farm2Fork. Research, innovation, and effective knowledge exchange among food system actors—researchers, industries, innovators, and policymakers—are essential to ensure food safety. However, several barriers often delay knowledge sharing, limiting collaboration opportunities and hindering progress in food safety. This situation highlights the need for a collaborative platform to bridge these gaps among food safety stakeholders.

Objective: The CATALYSE project aims to establish a strong, sustainable, and inclusive community—both online and in practice—that addresses the needs of all stakeholders along the food chain to ensure food safety.

Method: The CATALYSE platform was developed as a collaborative space where food industries, innovators, researchers, and regulators can discuss and cooperate to address food safety concerns through innovative solutions and by translating knowledge into practice. A Stakeholder Engagement Strategy was implemented, involving surveys and interviews, to identify the main needs and priorities related to food safety, with the ultimate goal of creating a Community of Practice (CoP). The platform also includes a knowledge database and educational materials designed to foster collaboration, connectivity, and discussion within the CoP.

Findings: The first step was the Stakeholder Engagement Strategy to define stakeholder groups (Industry, Policymakers, Researchers, and Civil Society) and identify their priorities and needs concerning food safety innovation. The responses collected through the survey and interviews, were analysed, enabling the identification of the main needs and the emerging issues in food safety. The second step involved mapping startups, spinoffs, and innovative solutions to enhance connections and collaborations via the

CATALYSE platform. Furthermore, thematic categories were created within the platform to classify and organize a scientific knowledge database and educational resources, such as courses and training materials, to address gaps in food safety awareness and foster discussion and connectivity within the CoP.

Conclusion: The CATALYSE platform represents a step forward in providing win-win solutions throughout the food chain. It facilitates tailor-made and co-created innovations with identified market potential by fostering interactions and collaborations among food safety stakeholders.

Abstract ID: 3132 / Poster Number: 217.16P

Cathelicidins as antibacterial and antibiofilm agents against multidrug-resistant Pseudomonas aeruginosa

Presenting author: Mr. Frederik Volný, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Co-authors: *Marko Šefranko, Lukáš Vacek, Antonín Pavelka, Lubomír Janda, Filip Růžička*

Introduction: *Pseudomonas aeruginosa* is a multidrug-resistant (MDR) pathogen, posing a significant challenge to conventional antimicrobial therapies. Its ability to develop resistance to a wide range of antibiotics, coupled with its biofilm-forming capacity, often leads to treatment failures. This highlights the urgent need to explore alternative therapeutic strategies.

Objective: This study aimed to evaluate the *in vitro* antibacterial and antibiofilm activities of cathelicidins, a group of naturally occurring antimicrobial peptides.

Methods: The antibacterial activity of five cathelicidins (LL-37, chicken CATH-2, PMAP-23, protegrin-1, and SMAP-29) was evaluated against 19 MDR *P. aeruginosa* strains obtained from clinical samples. MIC_{50} was defined as the minimum inhibitory concentration that results in a 10-fold decrease in bacterial numbers in at least 50 % of the strains tested. The antibacterial and antibiofilm activities of cathelicidin combinations were subsequently assessed against selected strains and fraction inhibition concentration was determined.

Findings: All tested strains were susceptible to at least one cathelicidin. The MIC values ranged from 0.63 to 12.5 mg/ml. The cathelicidin combinations exhibited both synergistic and antagonistic effects. The LL-37/SMAP-29 combination was the most effective one. The peptide concentrations required to reduce bacterial populations were lower in combination than when used alone. For LL-37, the concentrations ranged from 1.56 to 3.13 mg/ml, while for SMAP-29, they ranged from 0.08 to 0.16 mg/ml. The cathelicidin combinations were also capable of significantly disrupting bacterial biofilms.

Conclusion: Cathelicidins not only inhibit bacterial growth and disrupt biofilms but also modulate immune responses, making them a potential solution to combat MDR infections.

Abstract ID: 1490 / Poster Number: 092.17P

Cefiderocol's dark side: The Challenge of Accurate In Vitro Testing

Presenting author: Mr. Zian Asif, Humanitas University, Milan, Italy

Co-authors: *Maria Antonello, Raffaella Renzulli, Riccardo Bollini, Miryam Bonvegna, Cristina Scuderi, Eletta Cavedoni, Maria Calabrò, Daniela Cirasola, Cristina Daleno, Laura Peiretti, Giorgio Da Rin, Erminia Casari, Sara Carloni, Valeria Cento*

Introduction: Cefiderocol, a novel cephalosporin active against a broad spectrum of aerobic Gram-negative bacteria. In-vitro testing of cefiderocol remains challenging. Aim of this study is to establish an in-depth phenotypic and genomic integrated workflow for reliable estimation of cefiderocol resistance, using an in-house BMD method, commercial kits, disk-diffusion (DD) and whole genome sequencing (WGS).

Materials and Methods: Cefiderocol susceptibility of 19 strains of carbapenem-resistant *Klebsiella pneumoniae* (CR-KLPN) and 4 of CR *Pseudomonas aeruginosa* (CR-PSAE) was determined using ComASP Cefiderocol (Liofilchem, Italy), UMIC Cefiderocol (Bruker, USA), an in-house optimised BMD assay (run in triplicate), and DD. WGS was performed using Illumina technology. Bioinformatic analysis identified resistome (Kleborate, RGI), and SNPs (Snippy).

Results: The in-house BMD assay demonstrated 100% reproducibility (41/41) in internal quality controls (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, *K. quasipneumoniae* ATCC 700603), that fell within EUCAST specified range. ComASP identified one resistant isolate, UMIC and BMD detected 3/23, while DD detected 4/23. Categorical agreement with BMD as the reference was 100% for CR-PSAE with all assays, while decreased to 79% (ComASP), 78% (UMIC) and 65% (DD) for CR-KLPN. Notably, 3 CR-KLPN classified as resistant at BMD and susceptible at DD and commercial assays were found to have a missense mutation on TonB iron channel gene, compatible with cefiderocol-resistance.

Conclusion: Cefiderocol susceptibility results varied between approaches, especially for CR-KLPN. Resistance assessment with WGS resolved discordant cases, supporting BMD results over commercial tests and DD while identifying a missense mutation in the TonB transporter as a cefiderocol resistance determinant.

Abstract ID: 3581 / Poster Number: 031.15P

Century-Scale Soil Warming alters Microbial Community Composition in a Subarctic Forest

Presenting author: Mx Anne Peter, Johann-Heinrich Thünen Institute, Braunschweig, Germany

Co-authors: *Damien Finn, Jana Kehr, Christopher Poeplau, Christopher C. Tebbe*

The composition and function of soil microbial communities are central to biogeochemical cycles, and can contribute either positively to climate change through mechanisms such as carbon sequestration, or negatively e.g., carbon dioxide respiration. Soil microbial community composition is sensitive to temperature, and therefore understanding its response to long-term warming is essential for predicting how climate change may alter biogeochemical cycles. This study investigates how century-scale soil warming shapes microbial and plant communities in a subarctic deciduous forest near Takhini Hot Springs, Yukon Territory, Canada. By leveraging a natural geothermal gradient, we examine shifts in microbial and plant community composition, using metagenomic and qPCR analysis to profile fungi, bacteria, archaea, and green plant residues in the soil.

Our approach involves a specialised protocol for DNA extraction from humic rich soils, to assess microbial diversity more comprehensively. High-throughput whole-genome sequencing enables taxonomic profiling, revealing microbial adaptation to warming-induced changes in soil physical properties. qPCR analysis quantifies the relative abundance of key microbial groups and plant-derived DNA, offering insights into the interaction between microbial diversity and plant inputs.

This research advances our understanding of the microbial community adaptation to long-term warming. By integrating soil properties with sequencing data, this study offers critical insights into the ecological impacts of climate change on subarctic soil ecosystems and informs strategies for mitigating its effects on carbon cycling. These findings can advance the prediction of climate-driven changes in biogeochemical cycles related to soil health and ecosystem function.

Abstract ID: 3712 / Poster Number: 464.15P

Challenges in optimizing the use of CRISPR/Cas tool for precision gene editing in the hybrid and organic acid tolerant yeast Zygosaccharomyces parabailii

Presenting author: Prof. Doc. Paola Branduardi, University of Milano Bicocca, Milano, Italy

Co-authors: *Riccardo Milanesi, Pietro Butti, Camilla Magnani, Valeria Mapelli*

Zygosaccharomyces parabailii is a hybrid yeast with a complex genome and heterosis (hybrid vigor) towards the tolerance of organic acids at low pH. This phenotype can be of particular interest for the industrial production of organic acids in the protonated form, reducing the costs in buffering the growth media and product purification. Nevertheless, genomic complexity and a low efficiency in homologous recombination (HR) are hindering the valorisation of this yeast as *chassis* for industrial purposes.

Recently, a CRISPR/Cas9 tool for gene deletion and directed chromosomal integration in *Z. parabailii* was established despite its low efficiency. In the present work, novel approaches aimed to reduce the activity of non-homologous end joining (NHEJ) have been applied. Besides, integration cassettes and gRNA were re-design in order to improve mutant stability and integration precision.

Taken together, these tools will facilitate the engineering of *Z. parabailii*, accelerating the construction of novel strains and paving the way for the use of this yeast as synthetic biology *chassis*.

Abstract ID: 1329 / Poster Number: 053.16P

Characterisation of genes involved in xylo-oligosaccharide metabolism in B. longum subsp. longum NCIMB 8809

Presenting author: Ms. Lisa Friess, APC Microbiome Ireland, Cork, Ireland; University College Cork, Cork, Ireland

Co-authors: *Francesca Bottacini, Fionnuala McAuliffe, Paul Cotter, Ciaran Lee, Jose Munoz-Munoz, Douwe Van Sinderen*

Bifidobacteria are beneficial bacteria found in the human gut that can promote the health of its host. *Bifidobacterium longum* subsp. *longum* is one of the most prevalent species across all ages and is known to be able to utilise complex plant-based glycan, indigestible by its human host. In order to be able to fully break down these complex carbohydrates, such as xylo-oligosaccharides (XOS), the bacteria required a range of different carbohydrate-active enzymes. To gain more insights into the XOS-metabolising pathway we characterised three different enzymes, (i) XosA, (ii) AxoB and (iii) XosC; as well as identified a specific ABC-uptake system. This was done by assessing growth abilities of 27 *Bifidobacterium longum* subsp. *longum* strains; performing substrate assays with synthetic and natural substrates; and site-specific mutagenesis. The results together with *in silico* analysis show that the utilisation of XOS is strain specific. Furthermore, it was shown that XosA is an intracellular β-xylosidase; AxoB is an intracellular α-arabinofuranosidase; and XosC is an extracellular β-xylosidase. Analysis of an adjacent ABC-transporter system revealed that *Bifidobacterium longum* subsp. *longum* can import XOS with a low degree of polymerisation, which is required for growth on these substrates. These findings enhance our understanding of how gut bacteria interact with these soluble dietary fibres, which have the potential to contribute to the development of more effective prebiotics and probiotics, ultimately promoting improved gut health.

Abstract ID: 1104 / Poster Number: 406.15S

Characterising heterogeneity of LPS organisation in colistin persistent bacteria

Presenting author: Ms. Fengyi Wang, Newcastle university, Newcastle upon Tyne, United Kingdom

Co-authors: *Mark Geoghegan, Chienyi Chang*

Antimicrobial resistance (AMR) is a critical global health threat. Colistin serves as a last-resort antibiotics against Gram-negative bacteria by targeting lipopolysaccharides (LPS) to destabilise the cell membrane and induce cell lysis. However, bacterial persistence, a phenotypic survival strategy where a subpopulation of persisters, withstands antibiotic treatment without acquiring genetic resistance, compromises therapeutic efficacy leading to recurrent infections and prolonged treatment failures. The morphological and molecular mechanisms enabling persistence remains poorly understood. Direct Stochastic Optical Reconstruction Microscopy (dSTORM) was employed to investigate the nanoscale organisation and dynamics of LPS, the primary target of colistin, in colistin-susceptible *Escherichia coli* and *Pseudomonas aeruginosa*. Colistin persisters were examined as their parental bacteria cells which were treated with high concentration of colistin after 24 hours. The location of each estimated fluorophore on each single bacteria cell was extracted as X and Y coordinates to perform localisation analysis such as nearest distance of fluorophore, aggregation variance, and molecules density along cell membrane. A heterogeneous distribution and aggregation of membrane lipids and LPS along the bacterial envelope were observed through reconstructed visual images and statistical analysis (N=20 single cells). Figure 1 presents an example of single cell PA14 stained by FM4-64 to label membrane lipid, and by Rhodamine B labelled polymyxin B to stain LPS under dSTORM. Interestingly, LPS exhibits greater spatial variability compared to the membrane lipid. In both colistin persistent *E. coli* and *P. aeruginosa*, LPS increased spatial clustering and aggregation further leading to greater spatial variability, compared to parental colistin-sensitive bacteria. This study highlights the morphological characteristics of LPS organisation in bacterial persistence.

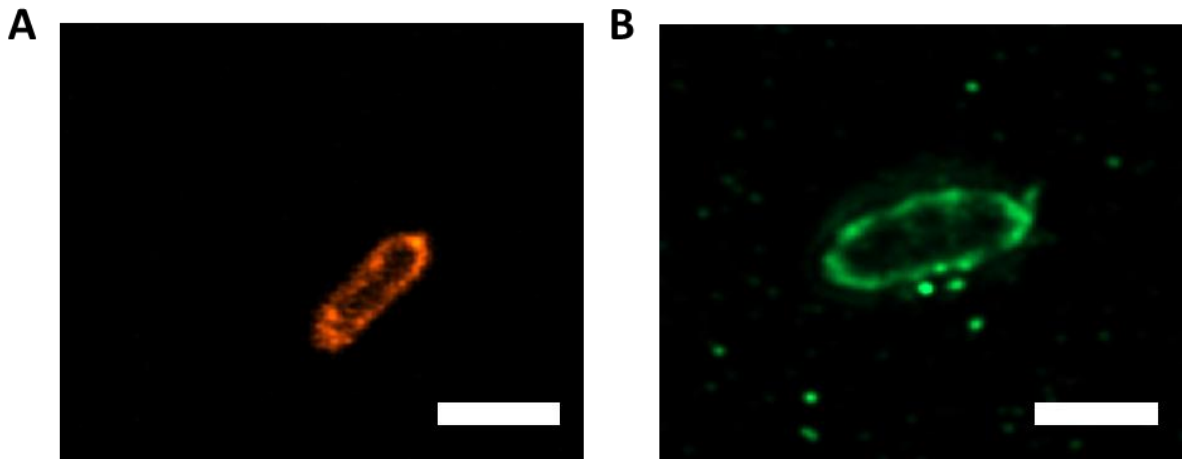


Figure 1: Single cell of PA14 with (A) FM4-64 and (B) Rhodamine B labelled polymyxin B staining under dSTORM. Reconstructed images were obtained from 6000 raw frames. Scale bar (white rectangle) is 1µm.

Abstract ID: 694 / Poster Number: 072.17P

Characterization and engineering of bacteriophage against vancomycin-resistant Enterococcus faecium

Presenting author: Mr. Mohamed El-Telbany, Kyushu University, Fukuoka, Japan

Co-authors: *Yoshimitsu Masuda, Ken-ichi Honjoh, Takahisa Miyamoto*

Vancomycin-resistant E. faecium (VREfm) is the leading species responsible for human infections. E. faecium is listed among the ESKAPE pathogens responsible for most deaths globally. The limitation of the therapeutic options makes VREfm a public health problem. Bacteriophage, a virus that infects bacteria, is a promising treatment for several bacterial infections. This study aimed to isolate, characterize, and engineer phage against VREfm. We isolated phage, named vB_Efm_M29, from compost. The phage vB_Efm_M29 lysed 3 of 10 E. faecium strains. The inhibitory effect against VREfm using the killing curve method revealed the ability of phage vB_Efm_M29 to inhibit bacterial growth for 24 h. The antibiofilm efficacy of phage vB_Efm_M29 was tested using the crystal violet and total viable count methods. The phage vB_Efm_M29 removed more than 80 % of the preformed biofilm of VREfm. The full genome sequencing confirmed that the phage vB_Efm_M29 does not contain any virulence or harmful genes. These basic characterization studies are the foundation upon which we have established a reproducible platform for genome engineering to improve host range and lysis efficacy. We started by editing phage vB_Efm_M29 using CRIPSER-Cas9 to construct a novel bacteriophage-producing leaderless bacteriocin. The plasmid pcrisper/spacer was built and introduced into competent E. faecalis JH2-2 cells to generate the target strain. The pcrispr/spc plasmid is then further modified to harbor a donor DNA (lacticin Q), and the modified plasmid is called pcrispr/spc-donor. In conclusion, engineering coupled with biological characterization will support phage use as an alternative treatment for VREf, and enhance lysis efficiency.

Abstract ID: 3321 / Poster Number: 419.17P

Characterization and Exploitation of the SCO1731 DNA Methyltransferase of Streptomyces Coelicolor M145 A(3)2 Strain

Presenting author: Ylenia Di Leto, Università degli studi di Palermo, Palermo, Italy

Co-authors: *Rosa Alduina, Fanny Claire Capri, Alessia Maria Sampino, Umberto Genchi, Margherita Sosio, Martina Cappelletti*

Streptomycetes are multicellular, Gram-positive bacteria with a GC-rich genome and are characterized by complex morphological and physiological differentiation. They are among the most important strains for biotechnological applications due to their ability to produce a wide range of clinically useful antibiotics, as well as other industrially significant secondary metabolites, including antitumor agents and immunosuppressants.

Streptomyces coelicolor M145 A(3)2 is widely used as a model organism to study the complex morpho-physiological differentiation of the *Streptomyces* genus. Antibiotic production in these bacteria is tightly regulated by a hierarchy of multi-level regulators, including global, pleiotropic, and cluster-situated regulators, the latter often referred to as pathway-specific regulators. Recently, we demonstrated that 5-methylcytosine plays a role in the differentiation of *Streptomyces coelicolor* A(3)2 M145. Furthermore, we identified the SCO1731 DNA methyltransferase as the key enzyme responsible for cytosine methylation (Pisciotta et al., 2018; Pisciotta et al., 2023). However, the target genes regulated by SCO1731 and the effects of its activity on transcription remain unknown.

5-methylcytosine is one of the most widespread epigenetic modifications found in the DNA of both prokaryotic and eukaryotic genomes. However, its role in regulating gene expression in bacteria has been less extensively investigated compared to eukaryotic systems.

The main objectives of this study were the identification of the target genes regulated by SCO1731-mediated methylation and the evaluation of the role of SCO1731 in modulating antibiotic production yields in other industrially relevant strains.

The challenge was to elucidate the molecular mechanism by which DNA methylation controls antibiotic biosynthesis, thereby enhancing our understanding of these fascinating bacteria and increasing the yields of well-known antibiotics. To address this,

we performed methylome and transcriptome analyses of the ΔSCO1731 mutant strain and compared the results to those of the wild-type strain. A SCO1731 overexpression plasmid was constructed and introduced into *Streptomyces* strains, where 5-methylcytosine levels and antibiotic yields were subsequently quantified.

Our findings demonstrate that cytosine methylation significantly influences morphological differentiation and antibiotic production in *S. coelicolor*. These results enhance our understanding of this model bacterial system and present new opportunities to exploit this genetic tool in other bacterial systems.

Abstract ID: 1430 / Poster Number: 202.15P

Characterization of a Gram-positive strain that possess an AHL lactonase

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Co-authors: *Chikaho Sano, Toshiki Nagakubo, Nobuhiko Nomura, Masanori Toyofuku*

Quorum sensing (QS) is one of the mechanisms of bacterial cell-to-cell communication that is driven by signalling molecules. QS regulates the expression of various genes, some of which are known to be related to bacterial pathogenicity and biofilm formation. The signalling molecules used in QS differ between bacterial species. Typically, Gram-negative bacteria use small molecules such as acyl-homoserine lactones (AHLs) while Gram-positive bacteria mainly use peptide signals. Although there is accumulating knowledge on how the QS system is regulated within the community of the same species, our knowledge on the cross-talks between different groups of bacteria is limited.

To gain insights into interspecies interactions, we screened for strains that will affect AHL-driven QS, using Chromobacterium violaceum as a reporter strain. As a result, we isolated a Gram-positive bacterium from a field in University of Tsukuba. We found that this strain can degrade AHLs when they were added in the medium. The degraded AHL could be recovered in acidic solution suggesting that lactonase is involved, which result was supported by whole genome sequence analysis where a putative AHL lactonase gene was identified. We further constructed a lactonase overexpressing strain which showed enhanced quorum quenching activity against C. violaceum. Our results may provide insights into how QS is interfered in natural settings by other species.

Abstract ID: 2144 / Poster Number: 115.17P

Characterization of a multidrug-resistant virulent E. marmotae hints towards zoonotic potential

Presenting author: Dr. Ulrike Binsker, German Federal Institute for Risk Assessment, Berlin, Germany

Co-authors: *Aida Bajraktarevic, Tanja Skladnikiewicz-Ziemer, Annemarie Käsbohrer, Ralf Ignatius*

Introduction: The intensified interconnection between the human, animal, and environmental sector facilitates transmission of zoonoses and antimicrobial resistances (AMR). Reliable detection and accurate information are essential to uncover emerging pathogens and AMR transmission. *Escherichia marmotae*, formerly *Escherichia* cryptic clade V, was considered an environmental bacterium due to its isolation from primarily aquatic sources. However, this species has also been isolated from human clinical samples. *E. marmotae* is phenotypically identical to *E. coli* and can only be identified by MALDI-ToF and whole-genome sequencing (WGS).

Objective: Here, we describe a multidrug-resistant *E. marmotae* isolate from a patient with a urinary tract infection.

Method: Antimicrobial susceptibility testing and short- and long-read WGS were performed to identify phenotypic resistances and responsible antibiotic resistance genes as well as virulence traits. In addition, filter mating assays were performed to elucidate the transferability of the antibiotic resistance.

Findings: Isolate 23-MO01035 belonged to ST133 and carried six resistance genes, i. e., *aph(6)-Id*, *aph(3'')-Ib*, *blaTEM-1*, *sul2* and two *dfrA8* genes on a previously undescribed conjugative 74,450 bp IncFII plasmid conferring phenotypic resistance to ampicillin, sulfamethoxazole and trimethoprim. The plasmid was transferable to other Enterobacterales species. In addition, the isolate carried multiple virulence factors involved in adherence (curly fibres, type I fimbriae), invasion (ibe), effector delivery systems (T6SS), biofilm formation (antigen 43) and nutritional/metabolic factors (heme uptake, yersiniabactin siderophore).

Conclusion: *E. marmotae* is adapted to different biological niches and holds zoonotic and human pathogenic potential highlighting the need for improved methods to distinguish this and other novel *Escherichia* species from *E. coli*.

Abstract ID: 1932 / Poster Number: 248.17P

Characterization of bacteriocins from thermophilic bacteria Introduction:

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Introduction: The endospore-forming thermophilic bacilli hold significant promise due to their ability to produce valuable metabolites, including bacteriocins.

Objective: We aimed to asses evaluate bacteriocin production from six thermophilic bacteria isolated from thermal areas in Türkiye.

Method: Thermophilic isolates were identified through 16S rRNA sequence analysis. Cell-free culture supernatants (CFS) were obtained from growing cultures in 2XYT medium after centrifugation at 8000 x g and filtration using a 0.45 mm membrane filter. CFSs were cross-tested against thermophilic strains using the agar-well diffusion method, and HBB244, which exhibited the strongest inhibitory activity, was selected for further characterization.

The sensitivity of the bacteriocins to temperature and hydrolytic enzymes was evaluated. The samples, were incubated at temperatures of 30 °C, 60 °C, 90 °C, and autoclaved at 121°C for 20 then tested against HBB25. The samples were treated with proteinase K at final concentrations of 1 and 10 mg/ml for 1 and 24 hours. Untreated CFS samples served as the control.

Findings: According to the BLAST analysis results, thermophilic isolates were identified as belonging to the genera *Geobacillus, Aeribacillu*s, and *Parageobacillus*, with homology rates ranging from 87.63% to 97.95%. CFS from HBB244 exhibited inhibitory activity against all tested bacteria and could withstand treatments up to 121 °C for 20 minutes. The proteinaous nature of CFS from HBB244 was confirmed by the loss of activity following proteinase K treatment.

Conclusion: Bacteriocin produced by HBB244 can be utilized against thermophilic spoilage bacteria, particularly in canned foods. Our study proceeds with further characterization of the bacteriocin.

Abstract ID: 2865 / Poster Number: 143.15P

Characterization of biocidal polylactide (PLA) films for active food packaging

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Microbial food spoilage is a significant issue that leads to substantial economic losses. Effective preservation methods are essential to reduce food waste. However, increasing consumer concerns about the safety and health impact of conventional preservatives drive the search for new, preferably natural, antimicrobial compounds. Plant-derived phenolic compounds are promising candidates for use in active packaging. These compounds can be incorporated into packaging materials either as sachets or directly into polymers.

The study aimed to develop an active food packaging material based on polylactic acid (PLA) containing cinnamaldehyde and carvacrol, selected based on their minimum inhibitory concentration (MIC) values against foodborne pathogens. The films were evaluated for biocidal activity according to ISO 22196:2011(E), biodegradability using the OxiTop® system, and antioxidant properties. Additionally, the impact of the materials on soil health was assessed by analyzing their effect on enzyme production by native soil microorganisms.

The prepared materials demonstrated significant biocidal activity against foodborne pathogens, maintained biodegradability, and had no adverse effects on soil health. These findings suggest that the developed materials could be suitable for extending food freshness. However, further studies are necessary, including the evaluation of their impact on the sensory properties of food.

Abstract ID: 1002 / Poster Number: 280.17P

Characterization of DyP and Encapsulin from Cellulose High-Producing Bacteria Komagataeibacter xylinus

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We have been studying DyP for a long time. Recently, DyPs have been reported to be major cargo proteins for encapsulin, which is a nanocompartment in prokaryotic cells. Encapsulin contains a cargo protein inside, suggesting that it supports the role of the cargo protein. Therefore, the physiological role of encapsulation has become a hot topic. So far, a few studies on DyP and encapsulin have been conducted using Mycobacterium tuberculosis. However, this microorganism is a dangerous pathogen, posing a bottleneck to advancing this research.

To address this issue, we have attempted to accelerate the research on DyP and encapsulin by using the non-pathogenic Komagataeibacter xylinus, a high-producing strain of bacterial cellulose.

We surveyed publicly available genomic databases and discovered, for the first time, that K. xylinus possesses both DyP and encapsulin genes. These genes were expressed in E. coli, and their biochemical functions were characterized. Additionally, the structure was observed using transmission electron microscopy. Furthermore, we constructed antibodies for both DyP and encapsulin.

DyP and encapsulin from K. xylinus exhibited typical DyP-type peroxidase activity and nanocompartment structures, respectively. Additionally, immunoblotting with the developed antibodies confirmed that K. xylinus actually expresses both DyP and encapsulin.

Our findings will accelerate research on the relationship not only between DyP and encapsulin but also between general cargo proteins and bacterial nanocompartments, as K. xylinus is a safe microorganism, unlike pathogens such as M. tuberculosis.

Abstract ID: 878 / Poster Number: 146.16P

Characterization of enteroaggregative Escherichia coli (EAEC) with emphasis on isolates producing chain-like adherence (CLA) on HeLa cells

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Introduction: Enteroaggregative *E. coli* (EAEC) is an intestinal pathogen characterized by the formation of the aggregative adherence (AA) pattern on epithelial cells, which is characterized by the organization of bacterial cells in an arrangement resembling stacked bricks. **Objective:** To determine the adherence properties of a collection of 140 EAEC isolates obtained from Brazilian subjects with diarrhea isolated between 2017 and 2023. **Method:** PCR was used to assign the EAEC isolates into the distinct *E coli* phylogroups and detected several adhesin-encoding genes, and *aggR*. Adherence pattern was evaluated using 3-hours assay performed with HeLa cells. Further, chain-like adherence (CLA)-producing isolates were sequenced by Illumina and the draft genomes analyzed. **Findings:** First, we observed that 91.4% of the isolates harbored *aggR*. The majority of the EAEC were assigned into the phylogroups A (57.9%), B1 (18.6%) and D (17.1%); and the adhesin-encoding genes were detected with the following frequencies: *aggA* (37.9%), *agg3A* (14.3%), *agg4A* (13.6%), *agg5A* (13.6%), *aafA* (9.3%) and *afpA* (8.6%); besides 4 isolates (2.9%) harboring *agg3A* and *agg5A* concomitantly. A total of 80.7% of the isolates produced the AA pattern on HeLa cells and/or on the glass coverslips, while 3 isolates (2.1%) produced CLA. The genome analysis of the sequenced CLA-producing EAEC isolates revealed that all isolates were from the phylogroup B1, ST1136, serotype O59:H19 and harbored the *aggR* and *agg4A* genes. **Conclusion:** Our data revealed that EAEC is a heterogenous group of isolates and pointed out the existence of a subgroup of EAEC producing CLA circulating in Brazil.

Abstract ID: 3705 / Poster Number: 047.17P

Characterization of Klebsiella pneumoniae strains from Ukrainian patient infections and phages isolated against them

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Introduction: Multidrug-resistant bacteria (MDR) cause thousands of deaths annually in Europe and millions worldwide. *Klebsiella pneumoniae*, a gram-negative bacteria readily acquires resistance and virulence genes, posing a threat to vulnerable patients. Phage therapy—a promising treatment using viruses that target bacteria, offers a potential solution for combating this critical pathogen.

Objective: The war in Ukraine has led to a rise in MDR pathogens, particularly *K. pneumoniae*. Our lab analyzed bacterial isolates from antibiotic-resistant infections in a conflict-area hospital using whole genome sequencing (WGS) and virulence assays to identify circulating strains (1). New phages were isolated and characterized to target these pathogens from different environmental samples (2).

Method: Hybrid assemblies from short-read (Illumina) and long-read sequencing (Nanopore) were used to analyze plasmid content, sequence type (ST), capsule type, resistance, and virulence genes. Virulence was assessed using the *Galleria melonella* model. Phages were also characterized via WGS and their activity was assessed by killing curves.

Results: This subset of strains reflects the European MDR landscape with common ST23, ST395, and ST39, and capsule types K23 and K57. Despite high genome-assigned virulence scores, model data suggests most strains are not hypervirulent. The phages isolated showed high effectivity in liquid cultures, though regrowth occurs within 24 hours, a growth delay was observed post-infection. No genetic markers for lysogeny, toxicity, virulence, or antibiotic resistance were found in the phages, making them potential candidates for therapy.

Conclusion: Phages can be used to address these war situations to save lives and prevent the possible dissemination of MDR strains worldwide.

Abstract ID: 2325 / Poster Number: 044.17P

Characterization of lytic bacteriophages and development of phage cocktail infecting clinical strains of Klebsiella spp

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The Antimicrobial Resistance (AMR) crisis, worsened by the COVID-19 pandemic, poses an urgent global health threat. Klebsiella pneumoniae, particularly multidrug-resistant (MDR) strains, is a major cause of difficult-to-treat infections. Bacteriophages were once prominent treatments against bacterial infections. The rise in MDR strains has renewed interest in phage therapy, which now includes phage cocktails and phage enzyme applications. This study characterized 10 bacteriophages isolated from clinical strains of Klebsiella spp., assessing their biological and genomic potential as therapeutic agents. Seven phages exhibited myovirus morphology, belonging to the Straboviridae, Ackermannviridae, and Stephanstirmvirinae families. Two were siphoviruses from the Drexlerviridae family, and one was a podovirus of the Autographiviridae family. Genomic analysis revealed high similarity among some phages, such as those of Jiaodavirus (96.3%) and Slopekvirus (93.6%) genus. Biological characterization revealed that some phages showed a narrow host range, infecting only their isolation host, while others had a broad host range. Interestingly, Slopekvirus phages Kp02 and Kp15 showed intriguing differences: Kp02 infected only its isolation host, while Kp15 caused lysis in three strains and disturbances in four others, despite their high genetic similarity. To investigate the genomic differences that might explain this discrepancy, an alignment between these two phages was performed. Genomic alignment revealed significant variability in genes encoding baseplate and tail fiber proteins, critical for host recognition. Our data suggest that our phages have significant potential for controlling virulent strains of K. pneumoniae, including those classified as highly virulent.

Abstract ID: 2999 / Poster Number: 161.17P

Characterization of lytic bacteriophages to combat multidrug-resistant and chronic infections caused by Pseudomonas aeruginosa

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Pseudomonas aeruginosa is an opportunistic bacterium responsible for many sorts of infections, particularly in immunocompromised patients and those with chronic diseases.

The growing increase in antimicrobial resistance has boosted the search of new therapies, like the use of lytic bacteriophages as expanded access drugs to treat this kind of infections. Phage therapy has several advantages, like high specificity for definite bacterial species or strains and the reversion of antimicrobial resistance in some multidrug-resistant strains, so it seems a good alternative or a supporting feature for common antibiotics.

In this investigation, bacteriophages were isolated from environmental and sewage water samples from different sources and using multidrug-resistant *P. aeruginosa* strains recovered from patients of Hospital Universitari i Politècnic La Fe as bacteriophages hosts.

Bacteriophages genetic and morphological characterisation was carried out by genome sequencing and electron microscopy, respectively, and sequence analysis was performed to check their optimal use for phage therapy demonstrating the lack of virulence and lysogeny-related genes. Besides, a comparative genomic analysis was executed, infection kinetics were determined, and heat and pH stability were evaluated. 200 clinically relevant multidrug-resistant *P. aeruginosa* strains were used to examine bacteriophages host range.

Finally, bacteriophages effectiveness in preventing biofilm was tested, as well as capability to eliminate preformed biofilm, improving its effectiveness after encapsulation.

These isolated bacteriophages have potential for treating *P. aeruginosa* infections and characterisation results bring useful data for further investigations about the development of bacteriophage cocktails, expanding the range of target bacteria and preventing antimicrobial resistance emergence.

Abstract ID: 188 / Poster Number: 058.17P

Characterization of Methicillin-resistance Staphylococcus aureus isolated from utensils used for dairy processing and Nasal cavity of pastoralist in Ado-Ekiti, Nigeria

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Methicillin-resistant Staphylococcus aureus (MRSA) present major health risk for human causing serious nosocomial and community-acquired infections. In the present study, MRSA strains isolated from utensils used for dairy processing and nasal cavity of apparently healthy pastoralist in Ado-Ekiti were analyzed. Antimicrobial susceptibility profile was done using the Kirby-Bauer disk diffusion method and genotypically by PCR targeting the mec A gene. 426 samples (230 nasal swab samples and 196 utensils samples) were screened, 172 isolates (100 from nasal samples and 72 from utensils) were identified as Staphylococcus aureus using vitek 2 system. 20 (12%) out of 172 isolates were MRSA. Methicillin resistance isolates was higher in the nasal swab 16 (80%) than 4 (20%) utensils. All MRSA isolates were multidrug-resistant strains. The isolates showed varied resistance patterns to different antibiotics used in this study. They were resistance to vancomycin, cefotaxime, cephradine, cefixime and meropenem at varying percentage and susceptible to tetracycline, cotrimoxazole and chloramphenicol. A total of 14 (70%) harbored MecA gene. The findings showed high prevalence of mecA-positive MRSA in nasal samples and utensils used for dairy processing in Ado-Ekiti. There is therefore need for improved hygienic practices and prudent use of antibiotics in order to mitigate the spread of resistant strains among the pastoralists within the community.

Abstract ID: 3364 / Poster Number: 391.17P

Characterization of microbial communities under optimal methane yield conditions during dry anaerobic digestion of food waste

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Co-authors: *Keunje Yoo*

Introduction: According to the World Bank, food waste (FW) accounts for 25-60% of the world's municipal solid waste, causing environmental problems due to landfills. As a result, renewable energy production using FW is gaining attention.

Dry anaerobic digestion (AD) is receiving increasing interest to overcome the economic limitations of traditional wet AD.

To consistently achieve maximum methane yield in a dry AD process of FW, three major parameters play a significant role: inoculum-to-substrate ratio, leachate recirculation rate, and inoculum type.

These parameters can have direct and indirect effects on the microbial communities involved in methane production.

This study aims to understand the characteristics of microbial community involved in methane production during the dry AD process of FW under three different parameters.

Method: The microbial communities were analyzed based on 16S rRNA gene amplicon sequencing with respect to three different parameters: inoculum-to-substrate ratio, leachate recirculation rate, and type of inoculum.

Findings: Increasing the inoculum addition and elevating the leachate recirculation rates improve methane yield and shorten the reaction time. Key genera such as *Jeotgalibaca* and *Sphaerochaeta* contribute to enhancing AD efficiency, while *Methanosarcina* has emerged as the dominant methanogen in the inoculum.

Conclusion: These findings will help to the role of microbial communities in contributing to methane production in the dry AD process of FW.

Abstract ID: 2355 / Poster Number: 373.17P

Characterization of oleaginous clones derived from adaptive laboratory evolution of Rhodosporidum toruloides

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The production of vegetable oils, an ever-increasing need for the planet's growing population, requires extensive agricultural activity, negatively affecting the environment. Single cell oils (SCO) from oleaginous microorganisms represent a promising alternative for easing this ecological burden. Maintaining economic feasibility is essential for developing SCO production processes, requiring strains capable of efficient substrate conversion. Adaptive laboratory evolution (ALE) strategies can be used for obtaining improved oleaginous phenotypes. We implemented an ALE experiment aiming to improve the lipogenic machinery of *Rhodosporidium toruloides* NRRL-Y 27012, based on repeated subculture of the most oleaginous cells, which had lower specific gravity. For selecting strains from the evolved populations, a method of plating lipid-rich cells on carbon free, but otherwise complete, media, was tested (Daskalaki et al., 2019). Following an evolutionary round, washed lipid-rich cells were plated on carbon-free media to observe growth capacity using lipid reserves. Lipid droplet number and morphology were investigated using epifluorescence microscopy. Strains were tested through shake-flask cultivations in high C/N ratio medium (containing 5% glucose, w/v) to induce lipid accumulation. Substrate assimilation was monitored until exhaustion, then biomass was subjected to compositional analysis (lipid, protein, and polysaccharide content). Evolved clones were successfully isolated from colonies formed on carbon-free media, while storage lipid was consumed in the cells during colony formation. The evolved strains demonstrated improved lipogenesis capabilities, increasing over 10% (w/w) compared to the parental strain. We conclude that the above-described ALE strategy and selection method are suitable for obtaining strains with improved lipogenesis and, consequently, greater biotechnological potential.

Abstract ID: 2514 / Poster Number: 244.15P

Characterization of Pathogenic Traits and Identification of Biomarkers to Differentiate Bacillus cereus and Bacillus thuringiensis Isolated from Agricultural Products and Environments

Presenting author: Mr. Kwang-kyo Oh, National Institute of Agricultural Sciences, Wanju-gun, South Korea

Introduction: *Bacillus thuringiensis (B. thuringiensis)* is a natural entomopathogen closely related to the human pathogen *Bacillus cereus (B. cereus)*. Although *B. thuringiensis*-based biopesticides have been used safely for decades, concerns remain regarding the potential production of enterotoxins by certain strains. This study aimed to characterize the pathogenic traits of *B. cereus* and *B. thuringiensis* isolated from agricultural products and environments in Korea and to identify biomarkers to differentiate the two species.

Objectives: The objective was to compare and analyze the distribution of pathogenic and insecticidal genes in *B. cereus* and *B. thuringiensis* strains isolated from agricultural products and cultivation environments and to identify diagnostic markers for their differentiation.

Methods: Fifty strains of *B. cereus* and *B. thuringiensis* were isolated from peppers, chives, lettuce, soil, and agricultural water in Korea. The distribution of enterotoxin genes (*hblCDA*, *nheABC*, *cytK*) and insecticidal *cry* genes was analyzed using the BtToxin pipeline. Comparative genomic and phylogenomic analyses were conducted on 219 high-quality whole genome sequences retrieved from NCBI. Pangenome analysis using Panaroo identified core genes and potential biomarkers.

Results: Enterotoxin genes (*hblCDA*, *nheABC*, *cytK*) were ubiquitously detected in both species, while *cry* genes were irregularly distributed. Pathogenicity genes were insufficient to distinguish the two species. However, comparative genomics identified *WxL domain-containing proteins* as potential diagnostic markers for *B. thuringiensis*.

Conclusion: This study enhances understanding of the pathogenic gene profiles of *B. cereus* and *B. thuringiensis* in agricultural contexts and provides candidate biomarkers for the accurate identification of *B. thuringiensis* in food safety and agricultural applications.

Abstract ID: 1343 / Poster Number: 347.16P

Characterization of plant grown promoting microorganisms isolated from winter wheat and canola root rhizosphere

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Co-authors: *Eglė Lastauskienė, Audrius Gegeckas*

Introduction: Salinity and heavy metal stress hinder plant growth by limiting nutrient availability, disrupting hormonal balance, generating reactive oxygen species (ROS) and inducing osmotic stress. The rhizosphere, the area around plant roots, harbors diverse microorganisms that can alleviate these stresses and support plant health.

Objective: The goal of this research is to explore and evaluate plant growth-promoting microorganisms found in the rhizosphere of winter wheat (*Triticum aestivum*) and winter canola (*Brassica napus*), with an emphasis on selecting those that can survive and function in saline and heavy metal-contaminated conditions.

Methods: Soil samples of wheat and canola rhizosphere were suspended in physiological solutions and spread on solid LB media enriched with 0.5%, 5%, 10% and 20% sodium chloride with different concentrations of copper, nickel and zinc. Grown visually distinct colonies were separated from the plates that contained the highest concentrations of NaCl, Cu, Ni and Zn. Moreover, their ability to produce siderophores and ammonia, solubilize phosphate and potassium, and display catalase and ACC deaminase activities were analyzed.

Findings: A total of 30 isolates from the canola rhizosphere and 50 isolates from wheat roots that could tolerate 10% NaCl were selected. Of these, 29 isolates demonstrated growth at 100 ppm Cu, 28 at 50 ppm Ni, and 16 at 100 ppm Zn. Based on their abilities to promote plant growth, 11 highest activity strains were selected for detailed analysis.

Conclusion: Our findings indicate that microorganisms obtained from the root rhizosphere of winter wheat and canola have a positive effect on plant growth.

Abstract ID: 3984 / Poster Number: 366.17P

Characterization of putative rubber-degrading bacterial strains: enzymatic profiling and growth assessment

Presenting author: Dr. Daniela Bucchieri, University of Milano-Bicocca, Milano, Italy

Co-authors: *Immacolata Serra, Valeria Mapelli, Cecilia Geijer, Paola Branduardi*

Biopolymers, including natural rubber (NR), are a significant component of Earth's organic biomass. Understanding the microbial degradation of NR is essential due to its widespread use and resultant waste production. While some bacterial strains capable of degrading rubber have been identified, detailed insights into their enzymatic activities and growth conditions remain limited.

This study aimed to characterize putative rubber-degrading bacterial strains, including *Paenarthrobacter nicotinovorans*, *Paenarthrobacter nitroguajacolicus*, and *Olivibacter soli*, assessing their enzymatic activities and growth behavior under varied environmental conditions.

High-throughput screening techniques were employed to evaluate enzymatic activities using API ZYM tests and *p*-nitrophenyl fatty acid assays. Growth profiling was conducted in basal salts media supplemented with different carbon and nitrogen sources at varying pH levels and temperatures.

The enzymatic assays revealed diverse profiles among strains, with *Olivibacter soli* demonstrating the broadest enzymatic activities, including high phosphatase and glycosidase activities. Growth experiments indicated that glucose was the preferred carbon source, with optimal growth at pH 6.5. Notably, none of the strains grew at pH 4, and variable growth was observed with alternative sugars.

This study provides the first detailed characterization of these strains, highlighting their enzymatic potential and growth preferences. The findings suggest these bacteria may rely on microbial consortia for complex carbohydrate degradation and lay the groundwork for future investigations into their roles in rubber biodegradation and environmental adaptability.

Abstract ID: 3173 / Poster Number: 270.15P

Characterization of Ralstonia pickettii isolated from potable water used in the International Space Station

Presenting author: Takehiko Kenzaka, PhD, Setsunan University, Neyagawa, Japan

Co-authors: *Yuuto Masai, Tomoaki Ichijo, Takashi Yamazaki*

The drinking water supplied from the Portable Water Dispenser (PWD) on the International Space Station (ISS) contains bacteria commonly found in terrestrial PWDs. The bacterial abundance can exceed 10^5 cells/mL, with over 70% of these being *Ralstonia pickettii*. This study aimed to enhance knowledge regarding water safety in space living environments by isolating *R. pickettii* from the ISS PWD and analyzing its characteristics. From 2021 to 2023, drinking water samples were collected from the ISS PWD and transported to Earth via SpaceX. Strains of *R. pickettii* were isolated using R2A medium. The bacteria were cultured in MH liquid medium under normal gravity (1G) or simulated microgravity (SMG). Scanning electron microscopy revealed that the number of exopolysaccharide (EPS) particles in the samples was approximately 45 times greater than the bacterial count, with EPS biomass accounting for about 60% of the total biomass. The EPS contained proteins and β-polysaccharides. The isolates cultured under SMG exhibited several differences compared to those cultured under 1G: 1) higher bacterial and EPS particle counts, 2) smaller cell volume and larger EPS volume, and 3) greater total biomass for both bacteria and EPS. The ISS isolate formed red colonies on Congo Red agar, confirming EPS production. Compared to terrestrial strains, biofilm formation was over six times higher in the ISS isolates, and the minimal inhibitory concentration for iodine was also elevated. The high EPS production ability and biofilm formation capacity of *R. pickettii* may play significant roles in its adaptation to the water environment in space.

Abstract ID: 950 / Poster Number: 389.16P

Characterization of the biocontrol potential of species of the genus Bacillus and related genera from different soil types

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Plant growth-promoting (PGP) and biocontrolling bacterial species from the genus Bacillus and related genera are increasingly used in agriculture as an alternative to synthetic biocides and fertilizers that have a positive effect on plant growth and development without compromising soil quality. This study characterizes and compares the specific bacterial diversity and their plant growth-promoting enzymatic and bioremediation potential in nutrient-depleted (ND) and overloaded (OL) soils over two seasons at four sites and eight fields. Using different growth media, comparative morphological analysis and molecular identification, we isolated 242 isolates of Bacillus and related genera. The following genera related to Bacillus were identified: Lysinibacillus, Peribacillus, Paenibacillus, Priestia, Domibacillus, Teriibacillus, Alkalicoccobacillus, Brevibacillus, Metabacillus, Psychrobacillus and Cytobacillus. There were 27 unique strains in spring, 14 in summer, while 24 strains were present in both seasons. The following strains showed the best results in terms of various properties: Alkalicoccobacillus gibsonii (indole acetic acid producer), Peribacillus simplex (siderophore producer), Paenibacillus xylanexedens (phosphate slobilizer), Bacillus haynesii (nitrogen fixer), Lysinibacillus pakistanensis (bioremediator of selenium), P. simplex and Priestia aryabhattai (exopolysaccharide producers) and Bacillus mojavensis (enzymatic activities). The isolates (most were collected in spring) from the second site, which contained higher water content, potassium oxide, phosphorus pentoxide, aluminum, calcium, nickel, carbonates and organic matter, showed the best properties. It can be concluded that the OL isolates had better enzymatic activity than the ND isolates, while the ND isolates had the best PGP properties.

Abstract ID: 3676 / Poster Number: 110.16P

Characterization of the clinical isolate and exopolysaccharide of the Flavonifractor plautii PCM 3108

Presenting author: Daria Artyszuk, PhD, Institute of Immunology and Experimental Therapy PAS, Wroclaw, Poland

Co-authors: *Wojciech Jachymek, Marcelina Pyclik, Paweł Migdał, Jolanta Łukasiewicz*

Microbiota has a great influence on the maintenance of homeostasis or the development of several diseases, such as inflammatory bowel diseases (IBD). IBD include Crohn's disease and ulcerative colitis which pathology is characterized by chronic inflammation of digestive tract. It is known that IBD patients have a greater amount of certain specific species of bacteria compared to healthy individuals.

Flavonifractor plautii is an anaerobic, Gram-variable bacterium belonging to the Clostridiales[2,3]. It is a component of the human gut microbiome well known for ability to metabolize a wide range of flavonoids, however, that is the only one clearly defined property of this species. *F. plautii* is characterized by slow, minimal growth, thus its phenotypic identification is a challenge for microbiologists. Until now, six cases of infections due to *F. plautii* have been described. The enrichment of the *F. plautii* has been indicated in patients with colorectal carcinoma, irritable bowel syndrome and other gastrointestinal tract disorders.

The purpose of this study was to further analyze the biological properties of clinical isolate *F. plautii* PCM 3108. The complete structure of the exopolysaccharide (EPS) was determined using chemical analyses and nuclear magnetic resonance spectroscopy as the repeating unit of →2)-α-Rha*f*-(1→4)-β-Rha*f*-(1→. Moreover, the impact of EPS on cytokine production and TLR receptors recognition were investigated. Bacterial cultures were also analyzed by transmission electron microscopy showing the morphology of cells and cross-sections presenting the structure of the cell wall being responsible for the Gram-variation during staining. The ability to produce membrane vesicles by was also demonstrated.

Abstract ID: 989 / Poster Number: 043.16P

Characterization of the cross talk between PrrA-PrrB and MprA-MprB Two Component Systems in *Mycobacterium spp.*

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Co-authors: *Deepak Kumar Saini*

Mycobacterium tuberculosis mainly uses Two component system (TCS) for its signaling which comprises of a Sensor Kinase (SK) and a Response Regulator (RR). Earlier studies have shown an extensive cross communication between TCS proteins in *M. tuberculosis in-vitro* including PrrAB and MprAB. PrrAB is an essential TCS in *Mycobacterium tuberculosis* but not in *Mycobacterium smegmatis*, an avirulent soil mycobacterium often used as a surrogate model to study *M. tuberculosis*. This prompted us to know whether this crosstalk between MprAB and PrrAB TCSs is also present in a non-pathogenic mycobacterium like *M. smegmatis* and is studied using phosphotransfer assay with cognate and non-cognate RRs individually to observe the signal flow. The cross talk between these TCS is found to be unidirectional and bidirectional between *M. tuberculosis* and *M. smegmatis* recombinant proteins respectively i.e., MprB transfers phosphate to PrrA of *M. smegmatis* but not in *M. tuberculosis*. Thus, it is important to know whether the directionality of this cross talk has any evolutionary significance and is related to the virulence of the mycobacterium. Transcriptomic analysis of *ΔmprAB* and *ΔprrAB M. smegmatis* was performed to find the regulon of these TCSs and the similarities assumed to have in the set of target genes because of the presence of crosstalk between them. The regulon of these TCSs will also be compared across *M. tuberculosis* and *M. smegmatis* to find the essentiality PrrAB only in *M. tuberculosis*. Further, the interaction between these proteins is also being examined at both *in-vitro* and *in-vivo* conditions.

Abstract ID: 750 / Poster Number: 221.17P

Characterization of the wastewater resistome and mobilome at community scale

Presenting author: Pamela Jael Colman Vega, PhD, Institut Català de Recerca de l'Aigua, Girona, Spain; Universitat de Girona, Girona, Spain

Co-authors: *Anna Pico Tomàs, Carles M. Borrego, Lluís Corominas, José Luis Balcázar*

Introduction: Due to rapid urbanisation, significant changes in living standards, lifestyles, and social behaviour have led to public health challenges, including concerns about water and food quality, air pollution exposure, and the spread of infectious diseases. Traditionally, human biomonitoring (HBM) has been the standard approach for assessing population health. However, methodologies such as wastewater-based epidemiology (WBE) have been proposed as innovative tools for evaluating community-level exposure to chemical and biological agents.

Objectives: This study aimed to characterise wastewater microbial communities collected from different neighbourhoods in Girona with contrasting socioeconomic status. It specifically analysed the resistome (the complete set of antibiotic resistance genes, ARGs) and the mobilome (the complete set of mobile genetic elements, MGEs) to assess the potential influence of socioeconomic factors on the presence and distribution of ARGs and MGEs.

Method: Wastewater sampling was conducted at ten locations within the Girona sewerage system, selected to represent neighbourhoods with contrasting socioeconomic levels (low vs. high). Automatic samplers were used to capture temporal variability in the microbial communities. The collected samples were analysed to characterise the resistome and mobilome, focusing on differences in the abundance and diversity of ARGs and MGEs associated with socioeconomic disparities.

Findings: No significant differences were observed in the overall abundance of the resistome and mobilome between samples from neighbourhoods with different socioeconomic levels. However, specific patterns were identified in the distribution of ARGs and MGEs. Notably, samples from lower socioeconomic areas showed a higher abundance of genes conferring resistance to tetracyclines and fluoroquinolones, as well as a greater presence of MGEs such as transposases and integron integrases. In contrast, wastewater from higher socioeconomic areas exhibited a predominance of genes associated with resistance to sulphonamides and β-lactams.

Conclusion: The observed variations in the resistome and mobilome between neighbourhoods with different socioeconomic status suggest that socioeconomic factors play a key role in shaping the distribution of ARGs and MGEs in urban wastewater. These findings underscore the potential of wastewater-based monitoring for evaluating the influence of socioeconomic disparities on antimicrobial resistance, contributing to the development of targeted public health strategies.

Abstract ID: 2206 / Poster Number: 230.15P

Characterization of thermophilic bacteria associated with the surfaces of the International Space Station

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Co-authors: *Anastasiya Sidarenka*

Introduction: The International Space Station (ISS) is an extreme environment, both for humans and for microorganisms. In order to maintain viability in harsh space conditions, microorganisms must acquire different types of adaptation. The assessment of microbial diversity and physiology on the ISS is of particular interest.

Objectives: The objective of this study is to examine the diversity of thermophilic bacteria isolated from ISS surfaces and to provide their comprehensive characterization.

Method: Microbiological, biochemical and molecular methods were used in this study.

Findings: Thermophilic bacteria were isolated from nine out of twelve samples collected in different locations across the Russian segment of the ISS in April 2024. The average number of bacteria in these locations differed from 2.4×104 to 10-80 CFU/10 cm2. The isolates were spore-forming bacteria belonging to the species Geobacillus thermoleovorans, Geobacillus thermodenitrificans, Geobacillus stearothermophilus, Geobacillus sp., Caldibacillus hisashii, Ureibacillus thermosphaericus, Bacillus subtilis and Laceyella sacchari. The resistance of thermophilic bacteria to antibiotics, heavy metals, UV radiation and disinfectants, alone with their biotechnological characteristics, were evaluated. In particular, proteolytic activity was observed in 62.5% of the isolates, amylolytic activity in 50% of the isolates, and lipolytic activity on Tween-20 and Tween-80 in 6.25% and 31.3% of the isolates, respectively. None of the isolates exhibited antagonistic activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes and Candida albicans.

Conclusion: In general, thermophilic spore-forming bacteria are well-adapted to stress factors ensuring their survival in the ISS environment.

Abstract ID: 1512 / Poster Number: 419.15P

Characterization, Heterologous Production and Mutagenesis of Pumicyclin, a Novel Circular Bacteriocin from Bacillus pumilus with Bio-preservation Potential

Presenting author: Dr. Fangfang Liu, University College Cork, Cork, Ireland

Co-authors: *Oscar Kuipers, Paul Ross*

Introduction: Food safety is a critical global concern due to the risks posed by foodborne pathogens like *Listeria monocytogenes* and *Bacillus cereus*. The demand for natural preservatives has driven interest in bio-preservation, utilizing beneficial microorganisms and their metabolites. Circular bacteriocins are characterized by having N- and C-termini ligated together. They are particularly promising due to their potent antimicrobial properties in addition to having increased stability undoubtedly enhanced by their circular nature.

Objective: This study explored the structure/function relationships, biosynthesis and properties of pumicyclin, a novel circular bacteriocin produced by *Bacillus pumilus*.

Methods: Genome analysis identified a six-gene cluster involved in pumicyclin biosynthesis, including a precursor peptide with a 32-amino acid leader and 64-amino acid core peptide. Antimicrobial activity was tested against Gram-positive pathogens, and mutational analysis was performed to study key biosynthetic features.

Findings: Pumicyclin demonstrated potent inhibitory activity against *Listeria* species and *Bacillus cereus*, highlighting its strong potential as a natural food preservative. The genes encoding the bacteriocin were heterologously expressed in *Bacillus subtilis* PG10. Bacteriocin production persisted when the leader was shortened to a single residue (methionine), while mutations near its circularisation site affected maturation. A second downstream peptide, potentially involved in self-immunity, was inessential for production.

Conclusion: Pumicyclin shows significant potential as a natural food preservative due to its broad antimicrobial spectrum, heat stability, and robustness in food processing conditions. Insights into its biosynthesis provide a foundation for rational peptide engineering, enhancing its applicability in the food industry as a safe and effective alternative to synthetic additives.

Abstract ID: 3081

Charting the complexity of the global glacier-fed stream microbiome

Presenting author: Dr. Gregoire Michoud, Ecole Polytechnique Fédérale de Lausanne, Sion, Switzerland

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Glacier-fed streams (GFS) are among Earth's most extreme aquatic ecosystems due to their oligotrophic nature and very dynamic conditions. Furthermore, they are strongly affected by glacier retreat due to climate change. Despite these challenges, they host diverse microbial communities dominated by biofilms. These biofilms form the basis of the food web and thus fulfill critical ecosystem functions. While some studies have started to explore the functional potential of the GFS microbiome, we currently lack a systematic understanding of key energy acquisition strategies and functional traits likely contributing to the biofilms' success in changing GFS worldwide.

Here, the study aimed to investigate the taxonomic and functional complexity of GFS microbiomes worldwide and to identify microbial adaptations to both environmental conditions and to other members of the biofilms including bacteria and algae. Furthermore, we aimed to assess how changing conditions of the GFS ("greening") will affect the functionality of the microbiome.

To address these questions, we analyzed 156 metagenomes from benthic biofilms collected across 86 GFS in nine global mountain ranges. Using advanced bioinformatics pipelines, we have generated 2,868 prokaryotic, 42 eukaryotic, and 4,872 viral metagenome-assembled genomes (MAGs) and conducted functional and taxonomic analyses in relation to environmental data such as chlorophyll-a.

Our study showed that despite the harsh environmental conditions, GFS hosts a diverse and complex microbiome that can sustain itself through cross-domain interactions that efficiently use and recycle resources. We noted a high taxonomic diversity of bacteria (Gammaproteobacteria and Bacteroidia) and eukaryotes (Algae, and fungi). Many prokaryotes were mixotrophs and able to use various energy sources, we thus observed high functional redundancy of metabolism including carbon, nitrogen, sulfur, and hydrogen across the community allowing its members to adapt to fluctuating resources of the GFS. Many of those prokaryotes also possessed genes for light energy harvesting and UV protection, indicating adaptation to high solar radiation. We also observed that

bacteria possessed many genes putatively involved in alga-bacteria interaction such as vitamin B12 biosynthesis, iron uptake (e.g., siderophores), thiamine and biotin biosynthesis, strongly suggesting mostly synergistic relationships between algal and bacteria. Finally, using chlorophyll-*a* as a marker for GFS greening, we observed that the microbial community may shift from a chemoautotrophic towards a more heterotrophic lifestyle due to increased benthic primary production (e.g. algal biomass).

This study highlights the diversity, adaptability, and ecological roles of GFS microbiomes. As glaciers shrink, GFS biofilms transition to more heterotrophic systems, underscoring the urgency of studying these vulnerable ecosystems in the face of climate change.

Abstract ID: 1694 / Poster Number: 299.17P

Chemical and genomic insights into extracellular polymeric substances by Brevibacillus thermoruber strain Tatev 9-4 isolated from an Armenian geothermal spring

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Introduction: Geothermal springs harbor thermophilic microorganisms capable of synthesizing extracellular polymeric substances (EPS) with significant biotechnological potential.

Objective: To identify the best microbial fermentation conditions, in terms of both duration and carbon source utilization for the production of EPS of the strain Tatev 9-4, isolated from the geothermal source of Tatev, Armenia. Chemical composition of the EPS produced from Tatev 9-4 strain was studied and putative genes encoding biosynthesis and assembly of EPSs were revealed.

Methods: EPS production was assessed in minimal media supplemented with sucrose (2% w/v), galactose (2% w/v), and molasses (4% w/v), at time. EPS composition was analyzed using TLC, HPAE-PAD, and GC-MS. Genome analysis employed RAST, KBase, BV-BRC, antiSMASH and Proksee tools.

Findings: The strain Tatev 9-4 was identified as Brevibacillus thermoruber based on 16S rRNA gene sequencing and genome analysis. The exoproduct (EP) production yields were 940 mg/L, 93 mg/L, and 325 mg/L observed after 72 h incubation with sucrose, galactose, and molasses, respectively, at 55 °C and pH 7.5. EPS was identified as a heteropolysaccharide with a composition comprising glucose, fucose, arabinose, glucosamine, ribose, and mannose. The highest EPS yield was recovered from sucrose (carbohydrate content 72.55%, w/w); in addition, the EP from sucrose showed a low proteins' (8 %) and nucleic acids' amount (0.46%), and a significant uronic acids level (12%). The genome (4.5 Mb, 26 contigs) revealed key genes for sugar transport, polysaccharide biosynthesis, EPS's assembly and export.

Conclusion: B. thermoruber Tatev 9-4 is a promising cost-effective (based on molasses) EPS producer.

Abstract ID: 2239

Chemical signaling between the gut microbiota and enteric pathogens

Presenting author: Caetano Antunes, PhD, University of Kansas, Lawrence, United States

Introduction: Our group studies the role of small molecules in host-microbiota-pathogen interactions. We have thus far focused on the impact of microbiome-derived compounds on enteric pathogen behavior. Using untargeted metabolomics, we previously showed that the human gut harbors thousands of small molecules, most of which are unknown. Antibiotic treatment altered the abundance of most of the metabolites detected, showing that the gut microbiome is involved in production of these compounds. We then hypothesized that some of these compounds may elicit responses in various cell types in the gut. Organic extracts of human feces were then used to determine transcriptional responses of pathogens to the chemical milieu of the human gut through mRNA sequencing. Our results showed that multiple enteric pathogens, such as *Vibrio cholerae* and *Salmonella enterica*, display marked transcriptional responses to the human gut metabolome, and that genes required for host interactions and virulence are modulated.

Objective: Given the broad transcriptional responses observed, our goal was to dissect the effects of microbiome-derived compounds on the behavior *V. cholerae* and *S. enterica*.

Methods: We used a combination of classical methods, such as culturing of gut microbiota, motility, biofilm, and invasion assays, and advanced techniques, such as digital droplet PCR and mass spectrometry metabolomics. This allowed us to determine the effect of compounds produced by the gut microbiome as a whole as well as by specific members of this microbial community on various aspects of *V. cholerae* and *S. enterica* virulence.

Findings: Our *V. cholerae* RNAseq data showed a great overrepresentation of motility and chemotaxis genes as being repressed. Therefore, we used motility assays in agar plates as well as direct observation by fluorescence microscopy to show that *V. cholerae* swimming motility is drastically repressed in the presence of fecal extracts. We also isolated and identified bioactive members of the gut microbiome. Pure cultures of various species of *Enterocloster* produce bioactive compounds that repress *V. cholerae* swimming motility, recapitulating the effect of the fecal extract. Due to the repressive effect on motility, we predicted that biofilm formation would also be affected by bioactive

commensals. Indeed, *V. cholerae* produced significantly more robust biofilms in the presence of *Enterocloster*-derived compounds. Host cell interaction assays using cultured colonic epithelial cells showed that small molecules produced by *Enterocloster* also modulate toxin-mediated host cell death induced by *V. cholerae*. Previously, we have been able to identify microbiome-derived bioactive compounds that affect *S. enterica* virulence, the most active of which was 3,4-dimethylbenzoic acid (DMB). Interestingly, DMB is not responsible for the effect on *V. cholerae*. Metabolomics of *Enterocloster* culture supernatants have revealed several other small aromatic compounds, and these are currently being tested for their ability to regulate motility and other properties.

Conclusion: Our results suggest that a new bioactive compound produced by a member of the gut microbiome modulates *V. cholerae* behavior. Ongoing work is focused on identifying the bioactive compound produced by *Enterocloster* and revealing the molecular mechanisms behind bioactivity against *V. cholerae* and *S. enterica*.

Abstract ID: 805 / Poster Number: 103.16P

Chitin and N-acetylglucosamine stimulate equol production in microflora

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Co-authors: *Shin-ichiro Yokoyama, Kohei Nakamura*

Objective: Equol is a compound metabolized from daidzein by gut microbiota. Since equol is an analog of estrogen, it has attracted attention for its potential mitigating effects on diseases caused by hormonal imbalance, such as osteoporosis and menopause.To clarify the effect of dietary fiber supplementation on equol production, we employed ex vivo and in vitro culture studies using human feces and isolated equol-producing bacteria, respectively.

Methods: The fecal suspension was added to sterilized Gifu Anaerobic Medium (Shimadzu Diagnostics Co.) supplemented with 1% (w/v) of three types of dietary fiber [pectin, resistant starch (RS), and chitin], and cultured anaerobically at 37°C. The culture supernatant was collected and used to analyze isoflavones, short-chain fatty acids, and pH. Bacterial DNA was extracted from the slurry and used for quantification of equol-producing bacteria and microbiota analysis.

Results: The equol conversion rates of the pectin and RS groups decreased to 1/10 and 1/3, respectively, compared to the control group. In addition, the relative abundance of lactic acid bacteria and bifidobacteria increased significantly in these groups, accompanied by decreased pH. Conversely, chitin supplementation increased the equol conversion rate by 2.7 times compared to the control group. The in vitro culture experiments showed that the supplementation of N-acetylglucosamine (NAG) increased equol production in some equol-producing bacteria (Eggerthella sp. strain YY7918 and Adlercreutzia equolifaicens JCM14793).

Conclusion: These results suggest that the intake of chitin and its hydrolysed product (NAG) enhances equol metabolism in certain microflora.

Abstract ID: 1739

Chitosan Extracted From Fishery Industry By-Products: an Innovative Antimicrobial Approach for Marine Applications

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In marine environments, natural and artificial submerged structures are quickly colonized by marine organisms in a phenomenon known as marine biofouling. This process is responsible for several economic, environmental, and industrial problems, including the bioinvasion of non-native species, increased hydrodynamic drag resistance, elevated fuel consumption, and enhanced environmental pollution. Although several biocides and cleaning agents have been employed to control biofouling, their negative effects on marine ecosystems highlight the need for sustainable and eco-friendly solutions to prevent biofouling in ship hulls, such as bio-based coatings. Chitosan (CS), a polysaccharide obtained by the deacetylation of chitin, can be sourced from fishery industry by-products. In addition to promoting the valorization of marine waste, CS has garnered significant interest due to its exceptional biological properties, including non-toxicity and antimicrobial activity, which is influenced by factors such as concentration and molecular weight (Mw).

This work aimed to (i) extract and depolymerize CS from *Loligo opalescens* pens, (ii) assess the antimicrobial activity and mechanism of action of CS; (iii) produce and characterize poly(lactic acid) (PLA) surfaces coated with CS of different Mw and concentrations, (iv) evaluate the long-term antimicrobial performance of PLA-CS surfaces against *Cobetia marina* biofilms.

A combination of enzymatic and alkaline treatments was employed to extract CS from *L. opalescens* pens, which was subsequently depolymerized into derivatives of different Mw. The antimicrobial activity of CS against *C. marina* was assessed by determining the minimum inhibitory and minimum bactericidal concentrations, and its mechanisms of action were clarified by flow cytometry through the evaluation of cell membrane potential and integrity after exposing *C. marina* cells to native CS. PLA films were then coated with 0.5% and 1% (w/v) solutions of native CS and its derivatives by dip coating, and the

produced surfaces were characterized for wettability, roughness, and morphology. The antimicrobial efficacy of the CS-based surfaces was assessed over seven weeks at 25 ºC under conditions simulating marine environments, and *C. marina* biofilms were analysed by colony-forming unit counts to quantify culturable cells.

Surface characterization revealed the successful incorporation of native CS and its derivatives into the PLA films, which presented an enhanced hydrophilicity compared to the uncoated PLA control. In terms of CS antimicrobial activity, CS had a minimum inhibitory concentration of 31.3 µg/mL and a minimum bactericidal concentration of 62.5 µg/mL, regardless of Mw. The evaluation of the CS mechanisms of action revealed that native CS induces cell membrane depolarization and causes cell membrane lesions after 24 h of exposure. Regarding the long-term performance of CS coatings, PLA films coated with 0.5% and 1% (w/v) CS exhibited notable antimicrobial activity, reducing the number of culturable cells by up to 58% and 69%, respectively, compared to PLA. The analysis of biofilms suggested a Mw-dependent effect, with surfaces coated with lower Mw CS demonstrating higher antimicrobial activity. No significant differences were observed in the antimicrobial performance between the 0.5% and 1% (w/v) CS-coated surfaces.

Overall, CS presented a strong antimicrobial activity linked to cell membrane depolarization and consequent loss of membrane integrity. CS coatings showed long-term and Mw-dependent antimicrobial activity against *C. marina* biofilms under the marine environmental conditions mimicked in this study. Incorporating CS into marine paints offers a promising eco-friendly strategy to mitigate biofouling while promoting the valorization of fishery industry waste.

Abstract ID: 1152 / Poster Number: 040.17P

Chlorine tolerance regulation by the small non-coding RNA SclT in Salmonella enteritidis

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Co-authors: *Feng Liu, Yingping Xiao, Hua Yang, Wen Wang*

Introduction: Small non-coding RNAs (sRNAs) play a pivotal role in regulating environmental stress response pathways, enabling bacterial cells to transition from a sensitive to a resistant state and adapt rapidly to challenging conditions. A small non-coding RNA (sRNA SclT) was discovered via RNA-seq sequencing, which could promote the chlorine tolerance by repressing the expression of outer membrane protein gene (ompW). The expression level of the sRNA SclT gene in the post-transcriptional tolerant strain was significantly upregulated by 11.8 times. However, whether the sRNA SclT is implicated in regulating chlorine tolerance in *Salmonella enteritidis* remains unclear.

Objective: This study aimed to clarify the effect of sRNA SclT in chlorine tolerance in *Salmonella enteritidis*.

Method: Chlorine tolerance characteristics of test strains were investigated by the Minimum Inhibitory Concentration (MIC) and Logarithmic Reduction. Furthermore, RT-qPCR was performed to determine the critical related gene (*ompW*) in the strains.

Findings: The MIC values for sodium hypochlorite of the ΔSclT strain decreased from 512 to 256 mg/L. The cross-resistance in antibiotics for beta-lactam (cefazolin) and tetracyclines (minocycline) in ΔSclT strain were also decreased. Besides, bacterial reduction for ΔSclT strain significantly ($p < 0.05$) differed when cultured for 10 min with 20 mg/L NaClO resulting in approximately 1 log Log CFU/mL (ΔSclT strain) and 0.2 Log CFU/mL (tolerant strain, control group), respectively. The expression of ompW was upregulated (1.3-fold) in the ΔSclT strain. The sRNA SclT may influence the down-regulation of the ompW, resulting in *Salmonella* chlorine to NaClO.

Conclusion: sRNA SclT promotes the properties of chlorine tolerance in *Salmonella enteritidis*.

Abstract ID: 1961

Circulating vaccine-derived poliovirus type 2 outbreak in Europe: the original wake-up call in Barcelona sewage

Presenting author: Prof. Doc. Albert Bosch, Universitat de Barcelona, Barcelona, Spain

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Introduction: The poliovirus eradication initiative relies on universal vaccination campaigns, with the oral (OPV) and inactivated vaccines. The appearance of vaccine-derived polioviruses (VDPV) after OPV represent a major obstacle to achieve the main goal of eradication. Environmental surveillance has been a key part of the polio eradication program and has proven to be successful in tracking both wild-type and VDPV.

Objective: Environmental surveillance, genomic and molecular analysis of polioviruses found in sewage samples in Barcelona.

Method: Sewage samples from two large wastewater treatment plants in Barcelona, were gathered and analysed in a twice a month basis. Amplicon NGS direct-detection methods were used to generate full VP1 1000-nt sequences using a MiniON platform (ONT). A bioinformatic pipeline based on the VSEARCH software was designed, enabling to compare our sequences with a database of 6396 Enteroviruses (EV) sequences. Additionally, after the detection of a circulating VDPV (cVDPV2) strain, a three-month period of enhanced environmental surveillance was implemented. Multiple RT-qPCR targeting this strain, which bore the key I143A mutation, and the Sabin-like polioviruses 1 and 3 were designed.

Findings: A cVDPV2 belonging to the Nigerian NIE-ZAS-1 cluster was detected in Barcelona wastewater on September 16 and October 28, 2024, but not thereafter. Of note, Sabin-like PV1 and PV3 were detected on September 30.

The sewage mutant swarm of the cVDPV2 isolated revealed the occurrence of over 100 different haplotypes at the nucleotide level and over 10 haplotypes at the amino acid level. Among these haplotypes there are sequences found thereafter across Europe.

Conclusion: Multiple detections of cVDPV2 throughout Europe followed our detection on September 16, including the chronological detection in Poland, Germany, UK and Finland. In the absence of flaccid paralysis cases, detection of sequences in sewage is a

more accurate approach than syndromic surveillance. Direct NGS can be used as a quasispecies tracking tool to elucidate critical information on virus evolution and epidemiology.

Abstract ID: 2832 / Poster Number: 258.15P

Classification of prokaryotic soil communities based on a hierarchical system of macroscopic ecosystem characteristics

Presenting author: Mr. Eugenio Perez-Molphe-Montoya, University of Zurich, Zurich, Switzerland

Co-authors: *Janko Tackmann, Von Mering Christian*

A classification of Earth's ecosystems is essential to structure our understanding of them, guiding research, decision-making, and ultimately supporting human, vegetal and animal welfare. To this end, we are creating a catalogue of the soil prokaryotic part of the terrestrial ecosystems, to identify and describe the main drivers and foundational members of the microbial communities that inhabit the plethora of distinct soil ecosystems around the planet.

We base our approach on the IUCN Global Ecosystem Typology, a hierarchical, multilevel structure that comprises 34 Ecosystem Functional Groups (EFGs) within the terrestrial realm. Its definitions rely on macroscopic ecosystem characteristics, such as the origin of the seasonal water deficit of the pyric tussock savanna and the temperate subhumid grassland, being the seasonal temperatures in the former and the seasonal rainfall in the latter.

We cross-linked EFG definitions with MicrobeAtlas, a broad catalogue of microbial communities (16S-based) with geographical context, resulting in the matching of over 50,000 soil microbiome samples from almost every terrestrial ecosystem. This enables EFG-specific microbiome characterizations and, eventually, may inform the EFG definition process itself by bridging the gap between the macroscopic and the microscopic world.

In our first analyses, we found microbial communities to cluster approximately to EFGs, with significant separation between clusters, and found hints at potential drivers and foundational groups for this separation. For example, the pyric tussock savannas contain a significantly higher proportion of archaeal community members than the temperate subhumid grasslands, revealing an interesting interplay of climatic and soil variables behind this proportion.

Abstract ID: 2058 / Poster Number: 435.15P

CleanBar: A Versatile Demultiplexing Tool for Split-and-Pool Barcoding

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Split-and-pool barcoding involves constructing thousands of unique barcode strings through sequential ligations in a 96-well plate format, making single-cell omics more accessible by eliminating the need for expensive equipment. Building on these advances, we present CleanBar, a flexible tool for demultiplexing reads tagged with sequentially ligated barcodes, accommodating variations in initial barcode positions and linker lengths, compatible with any sequencing method and split-and-pool barcoding platform. It is designed to avoid misclassifying natural barcode-like sequences and effectively handles diverse ligation errors. Importantly, it produces statistics useful for optimizing laboratory procedures. We demonstrate its use with the Atrandi platform for microbial single-cell genomics, coupled with PacBio sequencing, to address the limitations of the traditional bulk metagenomics in identifying phage-bacteria interactions. Four *Klebsiella* strains were infected with their corresponding phages and a control phage. While the resulting global infectivity patterns aligned with plaque assays and bulk sequencing, single-cell analysis revealed infection heterogeneity, with phages detected in 76% of cells. Atrandi whole-genome amplification provided uniform coverage, enabling estimation of phage copy number per cell. The absence of non-specific binding of control phage highlights the technique's potential for application to diverse environmental samples. By combining efficiency, adaptability, and precision, CleanBar, when applied to the Atrandi split-and-pool barcoding platform and PacBio sequencing, represents a transformative high-throughput tool for accelerating microbial single-cell genomics and unlocking new frontiers in understanding microbial ecology and evolution.

Abstract ID: 2115 / Poster Number: 097.15S

Climate change impacts on octocorals: emergent pathogens, symbiont preservation and probiotic solutions

Presenting author: Ms. Matilde Marques, Instituto Superior Técnico (IST), University of Lisbon, Lisbon, Portugal

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Coral reefs are vital ecosystems endangered by climate change, with octocorals (Subclass Octocorallia) being vulnerable to rising temperatures, pathogens, and anthropogenic disturbances too. Interactions between octocorals and their microbiomes are key to maintaining coral health under climate stress, yet the octocoral holobiont response to stressors such as rising temperature and pathogens is not well understood. This work investigates: 1) octocoral holobiont responses to synergistic environmental and pathogenic stressors, 2) the role of aquaria in preserving octocoral microbiomes, and 3) the development of a bacterial probiotic consortium to mitigate climate change-induced stress in octocorals. To evaluate octocoral responses to climate change stressors (Objective 1), the impacts of elevated temperature and the fungal pathogen *Aspergillus sydowii* on *Litophyton* sp. were assessed in controlled aquarium mesocosms. To address the role of aquaria in octocoral microbiome conservation (Objective 2) and select probiotic bacteria for octocorals (Objective 3), specimens of three tropical octocoral species, *Litophyton* sp., *Lobophytum* sp. and *Sclerophytum* sp., maintained in long-term mesocosms at Oceanário de Lisboa were sampled. In addition, wild *Litophyton* sp. and *Sclerophytum* sp. specimens were sampled in the Red Sea. Octocoral-associated bacteria were characterized based on both cultivation-dependent and -independent approaches using 16S rRNA gene sequencing. Cultivated bacterial strains were screened for octocoral beneficial traits, including polysaccharide degradation and pathogen inhibition.

Octocorals exposed to elevated temperatures exhibited a significant decline in the photosynthetic efficiency of their S*ymbiodiniaceae* (microalgae) symbionts. This decline was exacerbated by the presence of the fungal pathogen *A. sydowii*, increasing octocoral mortality.

Our cultivation attempts led to the isolation of 152 octocoral-associated bacteria, representing six classes, 14 orders, 27 described genera, and six unclassified isolates that likely represent novel genera within the *Alteromonadaceae* and *Spongiibacteraceae* families. *Sclerophytum* sp. retained part of its culturable microbiome, including taxa

such as *Ruegeria* and *Roseobacteraceae* spp., in aquarium mesocosms compared to samples from the Red Sea. Based on genus-level diversity, 25 bacterial isolates were selected for phenotypic screenings of host-beneficial properties, with 22, 18 and 9 isolates exhibiting antioxidant, cellulose-degrading, and chitin-degrading activities, respectively. Antimicrobial assays, conducted at both control (26 °C) and elevated (34 °C) temperatures, revealed that 19 isolates possessed antagonistic activity against coral bacterial and fungal pathogens under heat stress conditions. *Pseudoalteromonas*, *Kocuria*, and *Micrococcus* were identified as putative beneficial microorganisms for a probiotic consortium for octocorals.

16S rRNA gene metabarcoding revealed that key genera such as *Endozoicomonas*, and families such as *Terasakiellaceae* and *Flavobacteriaceae*, persisted in aquarium-kept *Litophyton* sp. even after over 25 years in captivity. Alpha and beta diversity analyses showed no significant differences between the bacterial communities of aquarium-maintained and wild octocorals, suggesting a stable microbiome in captivity.

This study highlights the critical impact of environmental changes on interactions among marine organisms, demonstrating that the fungus *Aspergillus sydowii* can act as a disease-causing agent in octocorals under increased temperatures. The preservation of putative beneficial symbionts such as *Endozoicomonas* in long-term captivity suggests that man-made ecosystems could serve as repositories of stable and healthy octocoral microbiomes. This work is paving the way for the first application of probiotics in octocoral conservation, offering a novel strategy to bolster octocoral health and contribute to coral reef restoration efforts.

Abstract ID: 2433 / Poster Number: 196.16P

Clinical Correlation of Laboratory Results in Clostridioides difficile Infections

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Introduction: *Clostridioides difficile* infection (CDI) is a serious healthcare-associated infection associated with increased morbidity and mortality. Current guidelines recommend a two-step diagnostic approach incorporating toxin-A/B and glutamate dehydrogenase (GDH) antigen detection with nucleic acid amplification testing (NAAT) in patients with clinical suspicion (≥3 unformed stools within the last 24 hours).

Objective: We aimed to evaluate the appropriateness of *C. difficile* testing requests and contribution of laboratory results to the diagnosis of CDI.

Methods and Results: Between 01.01.2023-31.12.2024, 1121 stool samples from patients suspected of having CDI were analysed by NAAT to detect *toxA*/toxB genes and NAAT-positive samples (n=141, 6%) were subjected to immunochromatographic testing to detect GDH/ToxinA/ToxinB antigens. Of the NAAT-positive samples, 83/141(58.9%) were negative for GDH/ToxinA/ToxinB antigens (1st group), 55/141(39%) were positive for GDH only (2nd group), and 3/141(2.1%) were positive for all antigens (3rd group).

50/83(60.3%) of the patients in the 1st group, 38/55(69.1%) in the 2nd group and all patients in the 3rd group were treated with metronidazole and/or oral vancomycin with CDI diagnosis. All patients with CDI diagnosis (n=91) had at least one underlying risk factor including prior antibiotic use (90%), hospitalization (85%), advanced age (60%), chemotherapy administration (36.3%), and colorectal cancer (5.5%).

Conclusion: Given that ~90% of the NAAT results are negative, we conclude that *C. difficile* testing is over utilized in our hospital. Clinicians preferably take NAAT results into account when deciding on the initiation of therapy which may lead to overtreatment. Only correlation of laboratory results with clinical presentation can discriminate between colonization and infection and correctly diagnose CDI.

Abstract ID: 2217 / Poster Number: 002.16S

Closing the gaps in diazotrophy across the global freshwater ecospace: from abundance, N2 fixation rates and diversity to their abiotic drivers

Presenting author: Assoc. Prof. Edo Bar Zeev, Ben Gurion University of the Negev, Midreshet Ben Gurion, Israel

New evidence and previous estimations indicate that dinitrogen fixation by diazotrophs may play a central role in the freshwater biosphere along the ecological scales. And yet, little is currently known on freshwater DIAZOTROPHY, namely their abundance N_2 fixation rates and diversity across the freshwater ecospace, while even less is known on the environmental drivers that control them. This global scale study was based on comprehensive international sampling effort (91 partners from 68 countries) applying state-of-the-art analytics. Specifically, each partner received a costume-made sampling kit that was sent back to BGU to quantify the number of diazotrophs via a new immunolabeled flow-cytometry approach, (ii) Identify the diazotrophs via MiSeq of the *nifH* gene and (iii) Measure N_2 fixation using the $^{15}N_2$ technique. Our lates results from the global freshwater ecospace indicate that the number of diazotrophs is highly variable (<5 $x10^{10}$ L^{-1}), yet generally less than 12% of the total bacterial count. N_2 fixation rates were overall low, yet few hotspots were located with rates ranging from 10-50 nmol L^{-1} d^{-1}. These N_2 fixers were highly diverse (α diversity up to 7.5) and often dominated by non-cyanobacterial diazotrophs. Interestingly, it was evident that much of these diazotroph communities were highly affected by changing concentrations of dissolved organic carbon rather N or P. Altogether, it could be deduced that freshwater diazotrophy is highly dynamic and are significantly impacted by the surrounding land use. These new global results and corresponding insights highlight the need to further delve into the spatiotemporal contribution of diazotrophy to freshwater primary productivity across the ecological scales.

Abstract ID: 1188 / Poster Number: 287.16F

CO2MOS: CO2 Metabolism Optimisation Strategy to prevent CO2 loss during methanol assimilation

Presenting author: Mr. Markus Klitgaard Friis, Aarhus University, Aarhus, Denmark

Co-authors: *Fatma Ece Altınışık Kaya, Berna Sariyar Akbulut, Marta Kartazyna Irla*

Methanol is a promising alternative feedstock for bio-production due to its large-scale availability and potential for sustainable synthesis from renewable resources. Among methylotrophic bacteria, *Bacillus methanolicus* shows significant promise for industrial applications. However, approximately 50% of the carbon is lost as CO_2 during methanol-based growth, limiting its efficiency as a bio-production host.

This study aims to enhance carbon utilization in *B. methanolicus* and deepen our understanding of its metabolic regulation. To this end, flux balance analysis (FBA) was performed using the reconstructed genome-scale metabolic model (GEM) of *B. methanolicus* MGA3. During the analysis, three distinct formaldehyde dissimilatory pathways were identified as contributors to CO_2 loss, therefore these pathways were considered as potential targets for strain engineering. Since two of the pathways are NADPH-generating, their deletion during FBA increases flux through the putative isocitrate dehydrogenase in *B. methanolicus*. Through a crude enzyme extract assay, isocitrate dehydrogenase was confirmed to be a NADP-dependent variant with a specific activity of 50.17 U/mg and 201.71 U/mg for forward and reverse reaction, respectively. To further understand methylotrophic gene regulation, a growth experiment with formaldehyde supplementation of *B. methanolicus* was conducted. A logistic decay correlation between formaldehyde concentration and growth rate was observed, with IC_{50} estimated at 1.57 mM. Using this information, the regulation of formaldehyde detoxification in *B. methanolicus* is under investigation through comparative transcriptomic analysis.

In conclusion, deletion targets have been identified to potentially optimize carbon utilization in *B. methanolicus*, and the regulation of genes involved in formaldehyde detoxification is under investigation.

Abstract ID: 3574 / Poster Number: 030.15P

Coastal forested wetland soils are microbially primed for shrub growth

Presenting author: Mx Aeran Coughlin, Duke University, Durham, United States

Co-authors: *Justin Wright*

Introduction: Plants influence soil microbial communities in species-specific ways, resulting in plant-soil feedbacks (PSFs), which play a critical role in structuring plant communities. Coastal freshwater wetland salinization under saltwater intrusion and sea level rise (SWISLR) has been associated with tree decline and subsequent expansion of shrub-dominated vegetation. Plant-microbial interactions represent a critical missing component of our understanding of plant community transitions with wetland salinization.

Objective: We assessed the role of PSFs in coastal vegetation transition under SWISLR. We sought to determine the direction and strength of PSFs of key tree and shrub species and whether microbial community patterns predict PSFs.

Methods: We measured growth of dominant tree (*Pinus taeda*) and shrub species (*Morella cerifera* and *Ilex glabra*) of salt-impacted coastal wetlands of Eastern North America in soils with home, away (forest or shrubland), and sterile inoculum additions. We quantified symbiont colonization and collected soils to sequence fungal (ITS1) and bacterial (16S) community DNA.

Findings: In comparison with tree *P. taeda*, dominant shrub *M. cerifera* experienced a significantly stronger positive growth effect in away soil, suggesting that forest soils are microbially primed for the growth of shrubs. Pathotroph abundance was significantly negatively correlated with plant growth, however, while symbiotrophs were more abundant in plant-home soil pairings, their abundance did not predict growth.

Conclusion: These findings highlight the potential role of microbial interactions in vegetation shifts under global change and demonstrate that microbial community composition can be related to the direction and strength of PSFs for some functional groups, but not others.

Abstract ID: 1624 / Poster Number: 172.15P

CodY-Mediated Control of Srr2, a Critical Adhesin in Group B Streptococcus Infections

Presenting author: Dr. Giulia Barbieri, University of Pavia, Pavia, Italy

Co-authors: *Angelica Pellegrini, Samuele Irudal, Viola Camilla Scoffone, Gabriele Trespidi, Silvia Buroni, Giampiero Pietrocola*

Introduction: Group B Streptococcus (GBS) is an asymptomatic colonizer of the gastrointestinal and genital tracts in 15%–40% of healthy adults. However, it poses a significant threat as an opportunistic pathogen capable of causing life-threatening infections in newborns, the elderly, and vulnerable individuals. Its ability to cause pneumonia, sepsis, and meningitis is attributed to various virulence factors, like the serine-rich repeat glycoprotein Srr2. This protein facilitates bacterial dissemination and aids in adhesion to brain endothelial cells, promoting blood-brain barrier invasion, particularly in newborns. The transition of GBS from a harmless symbiont to an invasive pathogen relies on the controlled expression of virulence factors, driven by the coordinated actions of diverse regulatory and signaling systems.

Objective: We investigated the role of CodY, a global transcriptional regulator dependent on branched-chain amino acids, in controlling the expression of *srr2* in GBS.

Methods: DNA-binding assays, expression studies, and mutational analyses were performed to elucidate the mechanisms of CodY-mediated repression of *srr2* during exponential growth in rich medium.

Results: CodY directly binds within the regulatory region of *srr2*, exerting negative control over its expression. Furthermore, we observed a significant interplay between CodY and CovR, another important transcriptional regulator, in the regulation of *srr2* expression, revealing a complex regulatory network governing GBS pathogenesis.

Conclusions: Understanding how CodY regulates *srr2* and coordinates its activity with other regulators provides insights into the molecular mechanisms of GBS virulence and host interaction. This knowledge could aid the development of targeted therapies aimed at disrupting GBS-host interactions and mitigating GBS-related infections.

Abstract ID: 3768 / Poster Number: 111.16P

Co-Infection with Salmonella typhimurium Elevates cagA Copy Number and Virulence Potential of Helicobacter pylori

Presenting author: Dr. Kavinda Tissera, University of Peradeniya, Peradeniya, Sri Lanka

Co-authors: *Ashansa Ramanayake, Jeong-Heon Cha*

Helicobacter pylori colonizes the stomachs of about 50% of the world's population. This human-specific bacterium causes diseases ranging from chronic gastritis, peptic ulceration to gastric cancer. Development and progress of these gastric disorders are determined by various virulence factors including the bacterial oncoprotein, CagA. Some of the *H. pylori* strains, underwent a dynamic expansion and contraction of *cagA* copy number correlating with disease development.

We explored how *H. pylori* manipulates *cagA* copy number during a concurrent systemic infection by using *Salmonella typhimurium* and *H. pylori* co-infected mouse model. Real-time PCR was performed to quantify the number of *cagA* gene copies in mice re-isolates using *ureA* as a reference gene for relative quantification.

Across three independent mouse experiments, *H. pylori* isolates from co-infected mice exhibited significantly higher *cagA* copy numbers compared to those from control mice, with the mean copy number increasing from 2.64 (±1.03) in the control group to 3.22 (±1.63) in the co-infected group. Three *H. pylori* isolates with high *cagA* copy numbers (6.1, 9.1, and 11.0 copies) were assessed for virulence features. Notably, two of these isolates maintained elevated cell elongation, CagA phosphorylation, and mouse infectivity, supporting their relevance as a model for *H. pylori* infection studies. These findings suggest that co-infection with *Salmonella typhimurium* increases the *cagA* copy number and also enhances the virulence potential of *H. pylori*.

En masse, the multiple *cagA* repeats in *H. pylori* tend to increase their *cagA* copy number during co-infection, possibly provoking the virulence characteristics of this pathogen.

Abstract ID: 3542 / Poster Number: 381.16P

Co-inoculation of Rhizobium tropici and Azospirillum brasilense reduces severity of Fusarium wilt in common bean

Presenting author: Dr. Enderson Ferreira, Embrapa Arroz e Feijão, Santo Antônio de Goiás, Brazil

Co-authors: *Adriane Wendland, Arthur Damaceno*

The common bean plays an important economic and social role in Brazil. However, some factors may limit its production, such as the occurrence of diseases caused by soil fungi. The fungus *Fusarium oxysporum* f. sp. *phaseoli* is one of the main causes of root disease in common bean and an alternative for its control can be through co-inoculation with bacteria capable of inhibiting and reducing the incidence of the disease. In this sense, the objective of this work was to evaluate the effect of co-inoculation with *Rhizobium tropici* and *Azospirillum brasiliense* on the severity of Fusarium wilt in five common bean cultivars. Three field experiments were conducted for two consecutive cropping years in two different locations, Santo Antônio de Goiás (2018 and 2019), and Abadia de Goiás (2019). The experiments were carried out in a randomized block design in a 5x3 factorial scheme, five common bean genotypes (Pérola, BRS Estilo, BRS Notável, BRS FC402, and BRS FC104) and three nitrogen sources (co-inoculation, nitrogen fertilization, and absolute control). The Fusarium wilt severity was determined along the growth cycle and grain yield of the common bean was determined at the physiological maturity. Pérola and BRS FC402 were more resistant to Fusarium wilt than BRS Estilo and BRS FC104. The co-inoculated common bean cultivars were 7% more tolerant to Fusarium wilt than when N-fertilized. The co-inoculated cultivars showed grain yield values similar to the N-fertilized treatment.

Abstract ID: 3427 / Poster Number: 099.15S

Collective Response and Navigation Dynamics of Magnetospirillum gryphiswaldense under Rotating Magnetic Fields

Presenting author: Mr. Danny Villanueva, Universidad del País Vasco (UPV/EHU), Leioa 48940, Spain

Co-authors: *Alicia G. Gubieda, Alfredo García-Arribas, Ana Abad Díaz de Cerio, Jorge Feuchtwanger, David De Cos, M. Luisa Fdez-Gubieda*

Introduction: Magnetotactic bacteria are self-propelled aquatic microorganisms capable of aligning and navigating along geomagnetic field lines. This ability arises from the biomineralization of intracellular magnetic nanoparticles, called magnetosomes. Their unique properties position them as a promising bio-nanorobot agent for biomedical applications. However, achieving precise control over their navigation remains a challenge, limiting the full exploitation of their potential.

Objective: We studied the motility of *Magnetospirillum gryphiswaldense* (MSR-1) under rotating magnetic fields with strengths ($B = 0, 100, 250, 500,$ and $1000\ \mu\mathrm{T}$) and frequencies ($f = 0.1, 0.2,$ and $0.5\ \mathrm{Hz}$). The investigation focused on identifying the critical parameters influencing their navigational response, evaluating their alignment efficiency, and characterising their motility under these conditions.

Method: MSR-1 samples were cultured in FSM medium at 28ºC and collected after 48 hours to ensure the formation of well-defined magnetosome chains. The samples were placed in microfluidic systems to maintain controlled environmental conditions. Transmission electron microscopy (TEM) and optical microscopy were used to characterise MSR-1 and track living samples, respectively. A custom image processing software, *BacMov*, was developed for the detection and tracking of MSR-1 bacteria. Rotating magnetic fields (RMF) were applied using three-axis Helmholtz coils.

Findings: We worked with MSR-1 bacteria swimming both with and against the RMF, north and south seeker bacteria, respectively. We observed a collective MSR-1 response to the RMF, with 250 μT marking the threshold for alignment. Focalisation and alignment improved with increasing field strength *at* $f = 0.1$ and $0.2\ \mathrm{Hz}$, as evidenced by trajectory and navigation analysis. Approximately 70% of the population aligns with the rotating magnetic field, exhibiting variable navigation speeds ranging from 10 to $80\ \mu\mathrm{m}/s$. At $f = 0.5\ \mathrm{Hz}$, represents a threshold for magnetic response, localised circular trajectories were observed, but collective alignment diminished.

Conclusion: The collective motion of MSR-1 depends strongly on the frequency of the rotating magnetic field, occurring at low frequencies, 0.1 and 0.2 Hz, but diminishing at 0.5 Hz. Alignment becomes more focused as the field intensity increases.

Abstract ID: 2743

Combination of Dilution-to-Stimulation and Artificial Selection Methods to Enrich a Plastic-Degrading Microbial Consortium

Presenting author: Lila Aldakheel, PhD, KAUST, Thuwal, Saudi Arabia

Co-authors: *Diego Jimenez avella, Tahira Jamil, Ruben Diazrua, Intikhab Alam, Cecilia Martin, Susann Rossbach, Alexandre Rosado*

According to the latest statistics, plastic production worldwide reached 413.8 million tonnes in 2023, and it will continue to increase year after year. The majority of plastics are resistant to biodegradation, which makes them persist in the environment for many years, thus distributing the ecosystem, especially the terrestrial environment, including mangrove forests. It is reported that there is an accumulation of microplastic in the Red Sea mangrove soils. The mangrove soil is a biodiverse environment full of microorganisms, which is an excellent system for exploring plastic-degrading microbes. In this research, two ecological approaches are used to selectively cultivate microbial communities capable of utilizing PET and cork as the sole carbon sources. The first approach is Dilution-to-Stimulation; in this stage, serial dilution is employed to reduce microbial diversity and promote the growth of microorganisms capable of utilizing PET and cork as primary carbon sources. Followed by the second approach, which is Artificial Selection and Coalescence; in this stage, the enriched microbial communities are subjected to selective pressures and coalescence to enhance the degradation capacity of the consortium.

Our results from metagenomics data showed eleven PETases with M5 motifs that exactly match the *Ideonella Sakainess* PETase gene. Over an eight-month period, we conducted an observational analysis of microbial growth within enrichment cultures, utilizing polyethylene terephthalate (PET) and cork as the sole carbon source. The 16S rRNA sequencing results showed potential microbes that might be involved in PET degradation, such as *Nitratireductor*, *Paenibacillus, Pseudaminobacter, and Brevibacillus*.

In conclusion, this research aims to establish a stable microbial consortium capable of utilizing PET and cork as carbon sources. These findings lay the groundwork for reconstructing synthetic bacterial communities capable of degrading microplastics. Additionally, the study highlights the potential identification of novel plastic-degrading enzymes, which could play a crucial role in transforming plastic waste within biotechnological applications.

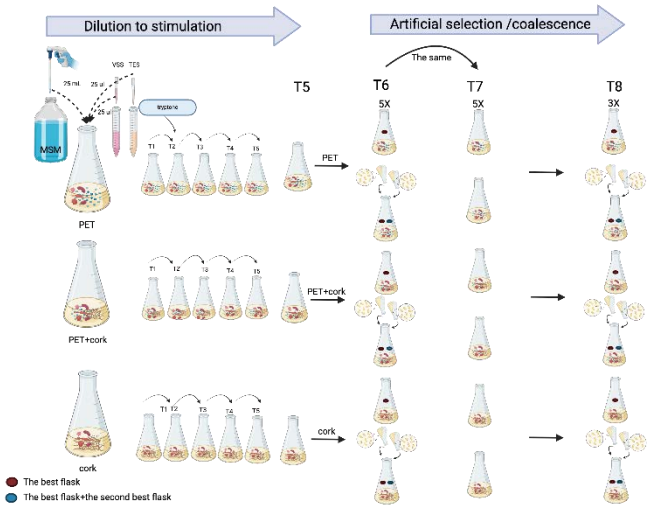


Figure 1: Experimental Design for Microbial Enrichment Culture Using PET and Cork as Carbon Sources. This experiment aims to enrich and study microbial communities capable of degrading PET (polyethylene terephthalate) and cork, utilizing a two-stage approach: Stage 1: Dilution-to-Stimulation. Stage 2: Artificial Selection and Coalescence.

Abstract ID: 3055

Combined population- and single-cell-based approach reveals high metabolic versatility and phenotypic heterogeneity during diazotrophic growth of a marine Non Cyanobacterial Diazotroph

Presenting author: Ms. Pauline Cretin, Nantes University, Nantes, France

The process of dinitrogen (N_2) fixation, or diazotrophy, supports a significant portion of ocean primary production and is now recognized as a key process in the biogeochemical cycles of nitrogen and carbon. N_2-fixation is restricted to a small part of polyphyletic prokaryotes called diazotrophs. Nitrogenase, the key enzyme in diazotrophy, is encoded by the *nif* operon and converts N_2 into ammoniac (NH_3) at the expense of 16 ATP molecules. Due to this high energetic demand, known diazotrophs have evolved genetic regulatory systems to tightly control N_2-fixation. Recent evidences showed that marine Non-Cyanobacterial Diazotrophs (NCDs) are widespread in oceans, yet their physiological responses during diazotrophy remain poorly understood. Our work combines high-throughput sequencing, mutant generation, physiological characterization, and single-cell monitoring of nitrogenase expression to investigate the strategies of *Vibrio diazotrophicus*, a marine NCD model, under nitrogen limitation. Our findings reveal that this marine NCD possesses a highly versatile metabolism, capable of utilizing a broad range of organic and inorganic nitrogen sources (**Fig. B**). Quantitative fluorescence microscopy (**Figure**) further revealed that nitrogenase expression is restricted to a subpopulation of cells and is modulated by ammonium concentrations which influence both the proportion of nitrogenase-expressing cells and the expression level within this subpopulation (**Fig. B and C**). Finally, we demonstrate that this phenotypic heterogeneity in nitrogenase expression is not limited to a single strain, suggesting it is a conserved trait among marine NCDs. These findings provide new insights into the strategies of marine NCDs under nitrogen-limited conditions, significantly enhancing our understanding of this ecologically important yet understudied polyphyletic group.

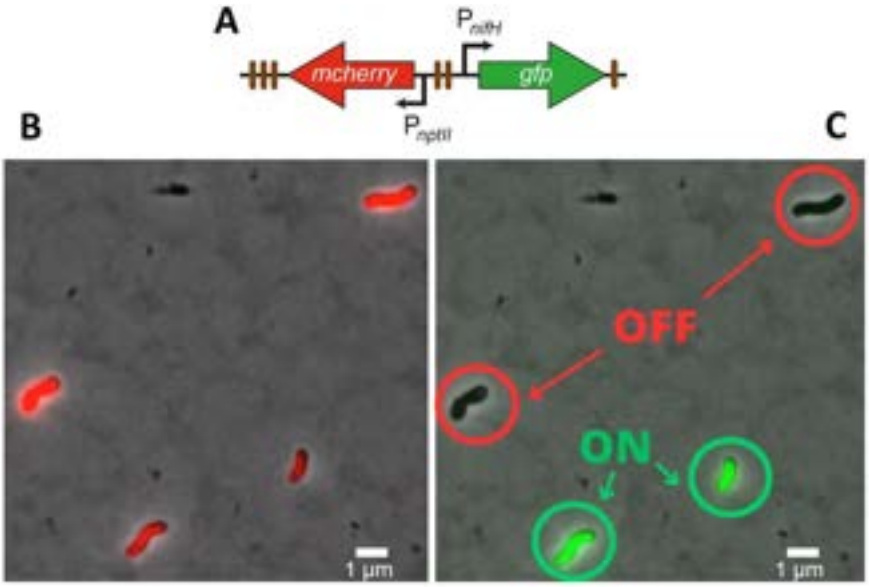


Figure: Single-cell based measurement of P_{nifH} expression by quantitative fluorescence microscopy in single cells. (A) General construction of the bioreporter, with the *mcherry* gene under the control of a constitutive promoter and the *egfp* gene under the control of the P_{nifH} promoter; (B and C) Epifluorescence pictures of the bioreporter constitutively expressing *mcherry* (B) and expressing *egfp* from the P_{nifH} promoter when cultured with N_2 as sole nitrogen source (C).

Abstract ID: 3579 / Poster Number: 184.17P

Combined therapy of Quercetin and N-acetylcysteine against biofilm-forming Staphylococcus spp infections

Presenting author: Mrs. Irene Fernández Babiano, University Institute of Biosanitary Research of Extremadura (INUBE), Badajoz, Spain

Co-authors: *Maria Coronada Fernández Calderón, Javier Ferrín Franco, Belen Mendoza Chamizo, Antonia Calvo Cano*

Infections involving implantable medical devices, generally associated with the biofilm formation by Staphylococcus spp, currently represent a clinical challenge. In combination with the actual increase of multiresistances, it is necessary to approach this emergency with new antibacterial agents.

In this study, the antimicrobial and anti-biofilm effect of a natural compound such as Quercetin (QR) combined with N-acetylcysteine (NAC) was evaluated against Staphylococcus aureus and Staphylococcus epidermidis strains. The inhibitory and bactericidal concentrations of the compounds alone and in combination were determined. The effect of su-inhibitory concentrations on growth and biofilm formation and their regulation was also studied. Furthermore, their possible combined mechanisms of action and the safety of their use on human cells were investigated.

The results showed that QR and NAC significantly reduce bacterial growth and biofilm formation. The combination presents a synergistic effect, especially on mature biofilms. QR alters the integrity of the bacterial membrane, fluidizing it and promoting the production of reactive oxygen species (ROS), while NAC increases membrane depolarization. In combination the effects compensates each other, to increase their antibacterial potential. Furthermore, these compounds were shown to be safe against human cells.

These findings suggest that the combined action of QR +NAC is a promising strategy for the prevention and treatment of biofilm-associated infections. The combination may have potential to be integrated into biomaterials and be useful in the management of Staphylococcus spp. infections.

Abstract ID: 3751 / Poster Number: 015.17S

Commensal skin bacteria produce molecules that affect Staphylococcus virulence

Presenting author: Rosana Ferreira, PhD, University of Kansas, Lawrence, United States

Introduction: The human microbiota plays a crucial role in health and disease. Much of current research focuses on the gut, and the role of the skin microbiota as a primary defense against pathogens remains less understood. *Staphylococcus epidermidis* and *Cutibacterium acnes* are the most abundant species in the human skin microbiome. Both have been shown to have a beneficial role in maintaining skin homeostasis. Our lab is interested in understanding if and how these species affect the virulence of bacterial pathogens. Among them, *Staphylococcus* spp., including *Staphylococcus aureus*, *Staphylococcus hominis* and *Staphylococcus lugdunensis*, are associated with a range of infections and their ability to adhere to surfaces and produce biofilms is a main virulence factor.

Methodology: Our lab uses cell-free supernatants to evaluate and identify bioactive molecules produced by skin commensals that affect the virulence of *S. aureus*, *S. lugdunensis* and *S. hominis*. For that, we collected cell-free culture medium (CFCM) of commensal strains of *S. epidermidis* and *C. acnes*. We evaluated their impact on growth, biofilm, and host cell adhesion and invasion of A549 epithelial cells by both pathogens. We also analysed their impact on global gene expression of *S. aureus*, *S. lugdunensis* and *S. hominis* by RNASeq. Furthermore, we purified and characterized bioactive molecules using treatments with enzymes, molecular weight filters, solvent solubility, High Performance Liquid Chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS). We also evaluated the impact of these on host cell viability using MTT and LDH assays.

Findings: Our results showed *S. epidermidis* secretes a small and polar molecule that significantly inhibits biofilms of methicillin-susceptible and methicillin-resistant *S. aureus* strains, without inhibiting growth. *S. aureus* grown in the presence of these molecules were less able to adhere to and invade A549 cells. Importantly, *S. epidermidis*-diffusible molecules were not cytotoxic at the concentrations used to inhibit virulence, indicating that they can potentially have a therapeutical application. Transcriptome analysis revealed that *S. epidermidis*-diffusible molecules greatly affect *S. aureus* gene expression, with around 30% of its genome being differentially regulated, including several virulence genes. *C. acnes* was also proven to produce molecules that inhibit

virulence in other two *Staphylococcus* species, *S. lugdunensis* and *S. hominis*. These species produced significantly less biofilm and were not able to adhere A549 cells, and no effect on bacterial growth was observed. Purification and analysis of the CFCM indicated that the bioactive molecules produced by *C. acnes* is also a small and highly polar molecule. Transcriptome analysis revealed that the purine synthesis pathway is greatly inhibit in *S. lugdunensis* in response to *C. acnes*-diffusible molecules, which might indicate a mechanism of activity since in *S. aureus* inhibition of this pathway is known to reduce biofilm. Finally, the molecules did not affect the viability or the metabolism of the epithelial cells.

Conclusions: Our findings highlight the potential of skin commensal-derived molecules in combating *S. aureus*, *S. lugdunensis* and *S. hominis* infections and underscore the importance of further exploring the skin microbiota for therapeutic applications.

Abstract ID: 1907 / Poster Number: 438.16P

Communicating microbiology: connecting science and society

Presenting author: Dr. Cristina Sánchez-Porro, University of Sevilla, Sevilla, Spain

Co-authors: *Alicia García-Roldán, Dáša Straková, Cristina Galisteo, Ana Durán-Viseras, Blanca Vera-Gargallo, María José León, Rafael R de la Haba, Antonio Ventosa*

Microorganisms remain largely unknown to many sectors of the population and, therefore, we consider it essential to bring Microbiology closer to society. For many years, the research group "Study of the halophilic microorganisms" from the University of Sevilla (Spain) have been actively engaged in science communication, with the primary goal of introducing the microbial world to diverse audiences.

To date, we have reached out to:

(i) Curious adults, by participating in outreach talks such as *Science in Boulebar* (an initiative where science is discussed informally in a bar setting), contributing to radio programs (*RadiUS*), and recording podcasts (@sosfarmapodcast) about the "beauty" of microorganisms.

(ii) Pre-university students, through activities such as *MicroMundo@Sevilla*, a global initiative in which we conduct school visits and workshops to raise awareness about antimicrobial resistance and the responsible use of antibiotics while also encouraging scientific careers. We also participate in events like the *Student Fair* and the *European Researchers' Night*, where we perform rather simple microbial experiments. Additionally, we collaborate in *Coffee with Female Scientists*, an initiative that promotes the role of women in research.

(iii) Young children, by conducting hands-on workshops where they learn how to grow microorganisms, observe them under a microscope, inoculate Petri dishes, and even inflate a balloon through yeast fermentation.

Our experience demonstrates that the society is unaware of the microbial relevance to protect the life on Earth and the biotechnological capabilities of microorganisms. We actively share all our activities on X (@Crissanpor), promoting engagement in microbiology outreach.

Abstract ID: 1469 / Poster Number: 019.16P

Community-level sporulation dynamics of Bacillota in the human gut

Presenting author: Ursa Miklavcic, PhD, National Laboratory for Health, Environment and Food (NLZOH), Maribor, Slovenia; University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

Co-authors: *Jacopo Grilli, William R. Shoemaker, Sandra Janezic, Maja Rupnik*

Bacterial sporulation is an effective and prevalent survival strategy in and outside of the gut, facilitating within-host persistence and between-host transmission. Approximately 50% of intestinal bacterial species are estimated to possess the capacity to form spores. However, research efforts into sporulation in the gut have primarily focused on pathogenic sporeformers, a miniscule fraction of the microbiota. Which results in a lack of understanding the ecological dynamics of commensal gut sporeformers.

We examined the ecological dynamics of sporeformers in a longitudinal study where microbiota and sporobiota were examined separately. DNA was isolated using 'bulk microbiota' and 'ethanol and EMA treatment' protocols on stool samples collected from healthy male donors every fortnight over six months, enabling the quantification of both spores and cells from the sequence data. We identified OTUs that were part of the ethanol resistant fraction and classified to the phylum Bacillota, the known sporeformers. For those we calculated sporulation frequency as a ratio of normalized abundances (calculated from relative abundance and copy number of the 16S rRNA gene) in bulk and ethanol treated samples.

Sporulation frequency differed from host to host and within host's time-points. The synchronized increase/decrease in sporulation of the ethanol resistant Bacillota within individual, implies a dependance on same environmental signals. Previous research on individual species, demonstrated that bacterial dormancy correlates with environmental stress and resource availability. Here we show that not only individual species but the entire community of spore-formers in the human gut sporulates simultaneously, therefore probably respond to similar signals.

Abstract ID: 1271 / Poster Number: 105.16P

Comparative analysis of fecal microbiota reveals elevated levels of fungi in urban-industrialized cohort – Insights from Madagascar and Germany

Presenting author: Mrs. Johanna Saalfrank, Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany

Co-authors: *Corinna Bang, Lucas Moitinho e Saliva, Malte Rühlemann, Daniela Fusco, Andre Franke*

Intestinal eukaryotes play crucial roles in human health, affecting millions worldwide. However, they are often neglected in human microbiome studies due to challenging technical implementation of high-throughput sequencing methods and underrepresentation of samples from regions with high prevalence of parasitic eukaryotes. Geographic location significantly influences human gut microbiota, but comparisons of fecal Eukaryota across industrialized and non-industrialized regions, and along the urbanization gradient, remain largely unexplored.

Using 18S V4V5 rRNA amplicon sequencing, we analyzed 1,394 human stool samples from Madagascar (Andina, Mahajanga, and Tsiroanomandidy) and Northern Germany (Kiel), spanning gradients of urbanization and industrialization.

Our work reveals distinct regional patterns: Helminths, protists, and environmental fungi were enriched in Madagascar, while in Germany the diversity of food-associated fungi was notably higher, including a higher prevalence of *S. cerevisiae*. We further confirmed known geographical patterns regarding fecal bacteria composition; namely decreasing alpha-diversity with urbanization, and distinct distribution of microbial enterotypes including the predominance of the *Bacteroides* enterotype in Germany and the *Prevotella* enterotype across Madagascar.

In summary, this work demonstrates the importance of integrating urbanization gradients into human microbiome analysis of (non-)industrialized regions and highlights the need for deeper investigating the role of fungi within the human gut microbiota.

Abstract ID: 564 / Poster Number: 032.16P

Comparative Analysis of Transcriptional Response to Inducing SPI-2 Condition in *Salmonella enterica* Serovars Enteritidis and Dublin

Presenting author: Andres Iriarte, PhD, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Co-authors: *Hernan Juan, Virginia Cantera, Laura Betancor, Cecilia Quiroga, José Alejandro Chabalgoity, Lucia Yim,*

Enteritidis and Dublin are closely related non-typhoidal serovars of *Salmonella enterica*. Unlike Enteritidis, Dublin is cattle-adapted and rarely infects humans, but when it does, it often leads to systemic infections. A critical step in the progression of systemic infection is the pathogen's ability to survive within macrophages, enabling it to evade immune defenses and disseminate to extraintestinal tissues. Using transcriptomic and comparative genomic analyses, this study aims to identify differential gene expression between the two serovars that may be associated with intramacrophage survival.

An RNA-seq experiment was conducted to evaluate the pathogen's response to the inducing SPI2 growth condition— a model mimicking the intramacrophage environment—relative to the control condition LB. Around one thousand differentially expressed genes (DEGs) were identified in each strain analysed, two Dublin and one Enteritidis strain. About 65% of DEGs are orthologs showing a conserved expression, including all SPI-2 genes. These conserved DEGs, linked to previously reported functional terms, such as fatty acid beta-oxidation and tricarboxylic acid metabolic process, likely represent part of the common *Salmonella* response to InSPI2.

More than 350 protein-coding orthologs and 41 small non-coding RNAs were identified as DEGs with different transcriptional responses between the serovars, along with strain- and serovar-specific DEGs. These genes may explain the differential responses to the macrophage environment, as well as the varying pathogenicity of Enteritidis and Dublin in humans.

Our results advance the understanding of mechanisms for intramacrophage survival in non-typhoidal serovars of *Salmonella enterica* and shed light on the rapid evolution of this complex trait.

Abstract ID: 3423 / Poster Number: 337.17P

Comparative efficiency of chitosan and inorganic acids to fight lactic acid bacteria contamination in ethanolic fermentation

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Chitosan is a biopolymer with antimicrobial activity utilized in the wine industry to combat undesirable microorganisms. In the bioethanol industry this polymer was not applied yet to verify its efficiency and viability. This issue should be investigated because the most utilized antimicrobials in this industry are strong inorganic acids such as sulphuric acid and more recently hydrochloric acid. Our aim here was to compare the efficiency of chitosan with these inorganic acids in decreasing the contamination by *Limosilactobacillus fermentum*, one of the most important lactic bacterium contaminating the process, in cell-recycled fed-batch fermentation with sugarcane juice and an industrial strain of *Saccharomyces cerevisiae* (PE-2). The results indicated that chitosan (0.49%) was effective in reducing the number of *L. fermentum*, without affecting the yeast viability, reducing the number of bacteria by 4 to 5 log cycles. Infrared spectra showed that chitosan does not accumulate throughout the cycles. However, a reduction in the ethanol production was observed, indicating a possible impact on the fermentative capacity of the yeast. Gradual increases in ethanol production throughout fermentation cycles suggest a possible adaptation of yeast to chitosan treatment. Both acids (pH 2.5) had a similar effect in reducing the number of bacteria by approximately 5 log cycles, with no significant effect on the ethanol production. Chitosan is a prospective alternative to strong inorganic acids in the ethanolic fermentation to reduce bacterial contamination.

Abstract ID: 2124 / Poster Number: 114.17P

Comparative Evaluation of Dry-Off Antibiotic Strategies on Milk Microbiota in Dairy Cows and Goats

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Introduction: The milk microbiota plays a critical role in dairy product quality and safety, as well as in animal and human health. Antibiotic treatments during the dry-off period may influence microbial diversity and promote antimicrobial resistance (AMR). Notably, in the European Union, the routine preventative use of antibiotics in livestock has been banned, making Selective Dry Cow Therapy (SDCT) mandatory unless a specific infection risk justifies treatment. Understanding the impact of dry-off strategies on milk microbiota is essential for improving udder health while minimizing antibiotic use.

Objectives: This study aimed to compare the effects of SDCT and blanket dry goat therapy on milk microbiota composition, udder health, and IMI prevalence, highlighting species-specific differences.

Methods: For cows, SDCT involved intramammary cloxacillin combined with or without internal teat sealants (ITS). In goats, blanket cefazolin treatment was compared to a control group with no antibiotics. Milk samples were collected at dry-off and subsequent lactation stages, with microbiota analyzed via 16S rRNA gene sequencing. Somatic cell counts (SCC) and IMI rates were also evaluated.

Results: In cows, ITS alone effectively maintained udder health in healthy animals, reducing antibiotic usage. Microbiota diversity showed no significant changes between treatment groups. In goats, cefazolin reduced IMI prevalence but did not significantly alter core microbiota diversity. Microbial variations were primarily linked to lactation stages rather than antibiotic use.

Conclusions: Both strategies effectively reduced pathogen loads while preserving milk microbiota diversity. The findings support sustainable antibiotic use to minimize AMR risks, aligning with One-Health principles. Customized dry-off protocols are recommended to optimize outcomes for cows and goats.

Abstract ID: 2691 / Poster Number: 359.16P

Comparative genome analysis of Aspergillus oryzae isolated from Korean fermentation starter and its closely related species within Aspergillus section Flavi

Presenting author: Mrs. Eunji Jeong, Soongsil University, Seoul, South Korea

Aspergillus oryzae is an industrially important fungus used to produce traditional fermentation foods and beverages in Korea and other East Asian countries. *A. oryzae* belongs to *Aspergillus* section *Flavi*, which includes *A. sojae*, used in fermented food production, as well as *A. flavus* and *A. parasiticus,* known for producing carcinogenic mycotoxins. *A. oryzae* is known to share 99.5% of its genome with *A. flavus*. To compare chromosome level differences between two species, we constructed complete genome sequences of two *A. oryzae* strains isolated from the Korean traditional fermentation starter, *nuruk*. Additionally, we obtained draft genome sequences of *A. oryzae* (12), *A. flavus* (11), *A. sojae* (2), and *A. parasiticus* (3) from additional sequencing and GenBank database. Through chromosomal-level pangenome analysis, we found that *A. oryzae* strains from *nuruk* have many enzyme-related genes, especially transcription factors and CAZymes-relatd genes. In addition, 82 conserved secondary metabolite gene clusters were identified across 30 strains of *Aspergillus* section *Flavi* for genome-level comparison of secondary metabolites production. This research aimed to elucidate the genetic diversity and evolutionary dynamics of *A. oryzae* strains isolated from *nuruk*. A comparison of the genomic features of *A. oryzae* and *A. flavus* may indicate that *A. oryzae* was a domesticated species derived from *A. flavus* as a result of selection pressure in the fermentation environment. Our study presents two high-quality genome assemblies of *A. oryzae*, along with a chromosome level comparison to its closely related species, which will provide valuable genetic insights into *A. oryzae* and relatives.

Abstract ID: 1150 / Poster Number: 210.17P

Comparative genomic analysis of generic Escherichia coli from broiler chickens in Canada: Elucidating differences in pathotype diversity, antimicrobial resistance and virulence

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Introduction: *Escherichia coli* is a Gram-negative bacterium of the *Enterobacteriaceae* family and is a normal inhabitant of the gastrointestinal tract of animals and humans. Pathogenic strains of *E. coli* can cause a variety of diseases in humans ranging from diarrheagenic to extraintestinal infections due to the presence of specific virulence factors and the production of various toxins [1]. In the 2012-2013 National Microbiological Baseline Study (MBS) in Broiler Chicken in Canada, generic *E. coli* was isolated from broiler chickens at slaughter or from retail outlets as a means to measure the faecal contamination in abattoirs [2].

Objectives: The objectives of the present study were to use a comparative genomic approach on the *E. coli* obtained from the MBS to: 1) describe the serotypes, Sequence Types (STs) and pathotypes recovered from poultry farms across Canada, 2) characterize antimicrobial resistance determinants and virulence factors, and 3) evaluate the concordance between whole genome sequencing (WGS)-based AMR predictions and phenotypically determined antimicrobial susceptibility.

Methods: *Escherichia coli* isolates were recovered from broiler chicken samples collected from federally registered poultry slaughter establishments from six regions across Canada as described previously [2]. All *E. coli* isolates (n = 2,732) obtained from the MBS were subjected to WGS on an Illumina MiSeq or NextSeq platform. Assembled genomes were analyzed using the TORMES pipeline [3]. MOB-suite was used to characterize plasmids and determine if resistance genes were of chromosomal or plasmid origin [4].

Results: These *E. coli* isolates were highly diverse, representing 376 serotypes, 236 Sequence Types and 21 pathotypes, of which 19 were hybrid pathotypes. In total, 63 resistance genes and 10 point mutations were identified. The most frequently detected resistance genes included *mdf*(A) (100%) which encodes for a multidrug efflux pump,

followed by *sul2* (44.5%), *sul1* (35.9%) and bla_{CMY-2} (35.4%). Over 95% of the β-lactam, fluoroquinolone, and phenicol resistance genes were predicted to be plasmid-borne. Only two toxin genes, *astA* and *sat,* were identified among the 2,732 *E. coli* genomes and were found in 54 and 0.07% of isolates, respectively. A high concordance (>85%) between resistance phenotype and the presence of antimicrobial resistance genes and point mutations observed for 13/15 antimicrobials.

Conclusion: This study highlights that sequencing data from a large number of bacterial genomes can be screened relatively easily with existing databases to identify pathotypes and high-risk strains with increased virulence potential which can be compared to clinical data to identify linkages. This is particularly important when characterizing *E. coli*, as not all strains are human pathogens, but all strains can serve as reservoirs of resistance genes and virulence factors which could pose an increased risk of emerging into novel strains that warrant attention.

Abstract ID: 2007 / Poster Number: 061.16P

Comparative genomics and gene-trait matching of members of the Bifidobacterium dentium taxon reveals the presence of a strain-specific fucosyllactose utilization cluster

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Bifidobacteria are beneficial commensals of the human gastrointestinal tract and their presence in the gut has been associated with positive health effects on the host. They account for a large proportion of the infant gut microbiota, in particular when the infant is fed on a milk-only diet, with their relative abundance progressively decreasing in adult and elderly. In contrast to other bifidobacteria, B. dentium was initially considered an opportunistic pathogen as its presence in the oral cavity was associated with the development of dental caries. While B. dentium has frequently been isolated from the oral cavity of children with caries, recent microbiome investigations and preliminary genomic analyses have suggested that this species is also adapted to colonise the gastrointestinal tract, where could exert potential beneficial effects for the host. To gain a better understanding of B. dentium genomic diversity and metabolic potential, the current study presents the genomic analysis and characterisation of ten novel B. dentium isolates from human fecal samples, obtained by next-generation sequencing (NGS). We investigated the genomic diversity of genetic loci predicted to be involved in host interaction and gut colonization in this species by means of comparative and pan-genome analyses. Through a combined genotypic and phenotypic characterisation in terms of its carbohydrate metabolism we were also able to identify a fucosyllactose utilization cluster in two representative strains.

Abstract ID: 2889 / Poster Number: 229.17F

Comparing the diversity of animal and human strains and their antimicrobial resistances in Staphylococcus aureus isolated from rabbit farming

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Introduction: *Staphylococcus aureus* is an opportunistic bacterial pathogen causing various diseases in rabbits and is considered a multi-host pathogen. Humans play a central role in its transmission between species. Through genetic changes, *S. aureus* can adapt to new hosts, posing challenges due to its rapid acquisition of antimicrobial resistances.

Objectives: This study aims to examine the interaction between human and animal *S. aureus* isolates from Spanish rabbit farms, identify predominant strains, compare their identities, and investigate the presence and transmission potential of antimicrobial resistance genes.

Methods: Samples were collected from humans and rabbits on 37 Spanish farms, cultured in Columbia CNA agar, and sequenced to obtain MLST profiles. A generalized mixed model evaluated the presence of resistance genes, while a generalized linear model analyzed the number of resistance genes.

Findings: In 45.95% of the farms, the same sequence types (STs) were isolated in humans and rabbits, indicating potential strain transmission. The clonal complexes CC121 and CC96 were the most frequently detected in both hosts, with isolates within these complexes displaying a high degree of genetic similarity, as reflected by an average nucleotide identity (ANI) >99.8%. Remarkably, strains from different hosts in two of the sampled farms exhibited ANI=100%.

No significant differences in resistance genes were detected for aminoglycosides, bleomycin, lincomycin, macrolides, or quaternary ammonium compounds, suggesting potencial gene transfer.

Conclusions: The close relationship between rabbits and farmers is a common source for the trade of microorganisms with zoonotic potential and, with them, antimicrobial resistance genes.

Abstract ID: 3425 / Poster Number: 255.16P

Comparison of protective efficacy of eight DNA vaccines against Klebsiella pneumoniae virulence factors

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Introduction: *Klebsiella pneumoniae* represents an important clinical problem in the community and hospital settings given the increase of infections caused by hypervirulent and resistant to multiple antibiotics strains, respectively. The development of vaccines against these species may help to prevent infections and reduce the associated mortality and morbidity. A promising approach would be DNA vaccines given their stability and flexibility.

Objective: The aim of this study is to develop DNA vaccines using *K. pneumoniae* virulence factors belonging to fimbriae, porins and siderophore receptors, confirm their expression in eukaryotic cell culture, and evaluate the vaccines' immunogenic capacity and protective efficacy against infection.

Methods: Eight virulence factors of *K. pneumoniae* (4 porins, 3 siderophores, and a fimbrial subunit) were rationally selected for the study. These proteins were bioinformatically analysed to predict immunogenicity, B- and T-cell epitopes using the VaxiJen 2.0, ABCpred and ProPred-I servers, respectively. An *in silico* prediction of the immune response profile was obtained using the C-IMMSIM server. The coding sequences of the selected proteins were adapted for expression in humans, fused to immunostimulatory elements, and cloned independently on the pVAX1 plasmid. The resulting vaccine candidates were validated by transfection into HEK-293 eukaryotic cell culture and antigen expression was assessed by RT-qPCR. Vaccines were tested *in vivo* by an immunization assay in mice to analyse antibody levels elicited, and efficacy of protection against sepsis was recorded.

Findings: Immunogenic capacity scores ranged from 0.60 to 0.78. The antigens with the most predicted immunogenic capacity were a siderophore, a porin, and the fimbrial subunit. B-cell epitopes were predicted for all antigens with a score range of 0.51–0.95. The antigens with the highest number of B epitopes relative to size were a porin and the fimbrial subunit. Regarding T-cell epitopes, siderophores were the immunogens with a

predicted higher number of epitopes and the highest score. The eight potential antigens identified were successfully cloned into pVAX1and expression in eukaryotic HEK293 cells was confirmed for all constructs, with levels of $2^{-\Delta ct}$ ranging between 1.7–17. Porin vaccine candidates induced the highest levels of total IgG, IgG1 and IgG2a subtypes and were the best candidates to protect against *K. pneumoniae* infection (p=0.024).

Conclusion: The porins tested can elicit better protection against *K. pneumoniae* infection compared to the siderophore and fimbrial subunit selected as antigen. DNA platforms can be a good tool for the development of vaccines against multidrug-resistant bacteria.

Abstract ID: 468 / Poster Number: 401.15P

Comparison of three methods for generating the coccoid form of Helicobacter pylori and proteomic analysis

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Co-authors: *Gyu Min Kong*

Introduction: Helicobacter pylori thrives in the acidic stomach environment and is a major cause of peptic ulcers and gastric cancer. Under stress, it can transform into a coccoid, viable but non-culturable (VBNC) form, retaining pathogenic genes while reducing metabolic activity. This transformation is associated with treatment resistance, false-negative test results, and an ongoing risk of gastric cancer.

Objectives: This study aimed to induce H. pylori's coccoid form using three methods—starvation, antibiotic treatment, and exposure to Streptococcus mitis supernatant—and compare morphological changes, viability, culturability, and protein expression among these methods.

Method: Coccoid forms were induced in vitro through starvation, amoxicillin treatment, or S. mitis supernatant exposure. Morphology and viability were assessed via fluorescence microscopy with SYTO9/propidium iodide staining. Culturability was evaluated by counting colony-forming units on chocolate agar plates. Proteomic analysis compared protein expression between spiral and coccoid forms and among coccoid forms generated by the three methods.

Findings: Starvation induced VBNC cells after 7 days, whereas amoxicillin formed VBNC cells within 3 days. S. mitis supernatant prolonged culturability, likely due to factors supporting spiral form maintenance. Proteomic analysis revealed significant differences in protein expression between spiral and coccoid forms, as well as among coccoid forms induced by different methods.

Conclusion: H. pylori transformed into coccoid forms via starvation, amoxicillin, and S. mitis supernatant, with the latter preserving some viable spiral forms. Proteomic analysis showed distinct protein profiles between spiral and coccoid forms, underscoring the need for further research on their roles in treatment failure.

Abstract ID: 618 / Poster Number: 268.17P

Complete Genome Sequence of Bacillus licheniformis Isolated from Traditional Korean Soybean-fermented Food for Probiotics Application

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Introduction: Fermented foods have long been appreciated as major sources of probiotics. In particular, Cheonggukjang, a traditional Korean soybean-fermented product, is expected to provide various health benefits due to the presence of beneficial bacteria. Among these microorganisms, Bacillus species are prevalent in fermented soybean products, and certain strains are already commercially utilized as human probiotics, further underscoring their importance.

Objective: This study aimed to perform complete genome sequencing of a beneficial bacterium isolated from Cheonggukjang and to assess its genomic features that could support human probiotic functionality.

Method: Genomic DNA was extracted using a phenol–chloroform protocol and sequenced on an Oxford Nanopore Technologies MinION platform. ResFinder detected acquired antibiotic resistance genes, and BLASTn searches of the Virulence Factor Database (VFDB) identified potential virulence factors.

Findings: Bacillus licheniformis (B. licheniformis) was isolated from Cheonggukjang. The complete genome of B. licheniformis contains one circular chromosome (4,577,775bp) with a GC content of 46.0%. Annotation revealed 5,182 protein-coding sequences, 24 rRNA, and 81 tRNA. VFDB analysis identified genes associated with poly-gamma-glutamate (γ-PGA) synthesis and bacillibactin and confirmed the presence of the hlyIII gene. Additionally, ResFinder results confirmed that no antibiotic resistance genes were identified.

Conclusion: B. licheniformis is recognized by EFSA as having Qualified Presumption of Safety (QPS) status. Although genes for γ-PGA and bacillibactin were detected, no reports indicate direct human toxicity. Moreover, despite the presence of hlyIII gene, no hemolytic activity was observed on blood agar. These findings suggest that B. licheniformis has potential for commercial application as the human probiotics.

Abstract ID: 1996 / Poster Number: 353.15F

Complex formation of a trimeric autotransporter adhesin with a peptidoglycan-binding periplasmic protein

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Introduction: Trimeric autotransporter adhesin (TAA) is an outer membrane (OM) protein that is widely distributed in gram-negative bacteria and is involved primarily in adhesion to biotic and abiotic surfaces, cell agglutination, and biofilm formation. TAAs are secreted onto the OM by the type Vc secretion system (SS). Because the interactions between TAAs and chaperones or special assistant proteins during secretion are short-lived, it is thought that TAAs reside on the OM without forming complexes with other proteins after secretion.

Objective: In this study, we aimed to clarify the interactions between an *Acinetobacter* TAA, AtaA, and a peptidoglycan (PG)-binding periplasmic protein, TpgA.

Method and Findings

Pull-down assays using recombinant proteins identified the interacting domains. X-ray crystallography revealed the A3B3 heterohexameric complex structure of the N-terminal domain of TpgA with the transmembrane domain of AtaA and showed that both electrostatic and hydrophobic interactions contribute to stable complex formation. Bioinformatic analysis suggested that the TAA-TpgA complex is formed in a wide range of species that harbor the *taa-tpgA* gene cassette in their genome.

Conclusion: We revealed the structure of a PG-binding periplasmic protein that forms a stable complex with the transmembrane domain of a TAA. Our findings will contribute to a better understanding of the type Vc secretion system and the control of TAA-mediated adhesion, which is important for medical and biotechnological applications.

Abstract ID: 1401 / Poster Number: 314.15P

Complex of adaptations for propane and n-butane assimilation by Rhodococcus ruber actinomycetes, the most important biooxidizers of C2–C4 gaseous alkanes

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Introduction: Microorganisms that oxidize C_2–C_4 gaseous alkanes are responsible for introducing carbon from these compounds into global biogeochemical cycles. This unique and specific ecotrophic group is not properly studied. Propanotrophic *Rhodococcus ruber* actinomycetes are of particular interest. They can adapt to extreme conditions, have potential for feed protein biosynthesis, biocatalysis and bioremediation, and are the most relevant bioindicators of propane and *n*-butane.

Objective: To elucidate adaptations of *R. ruber* for the assimilation of propane and *n*-butane.

Methods: Culture-based techniques for isolation and growth of propane and *n*-butane biooxidizers. Identification of isolates using polyphasic taxonomy. Illumina sequencing to obtain draft genomes. Standard biochemical methods to study the composition of bacterial cell walls. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to study ultrastructural changes of the cells. Combined atomic force microscopy (AFM) and confocal laser scanning microscopy for in-depth studies of cell surface properties. Real-time polymerase chain reaction with reverse transcription to analyze the expression of functional genes.

Findings: Several hundred thousand water, soil, rock, bottom sediment, snow and wastewater samples from contrasting ecological-geographical regions were analyzed and a total of 216 *R. ruber* strains were isolated. The strains were deposited in the Regional Specialized Collection of Alkanotrophic Microorganisms (acronym IEGM, #285 WFCC, http://www.iegmcol.ru). All strains used propane and *n*-butane as sole growth substrates and the association of *R. ruber* with oil and gas deposits was confirmed. *R. ruber* were persistent and dominant components of the microbiocenosis in the zone of the gas dispersion over gas and oil fields. Physiological responses of *R. ruber* to propane and *n*-butane included increased pigmentation, cell aggregation, adhesion to solid surfaces and overproduction of extracellular amino acids. Increased cell surface area, appearance of multiple cell appendages, growth and invagination of the cell wall,

formation of electronically dense inclusions containing oxidative enzymes, lipid droplets and volutin granules (Figure 1) were the basic adaptive modifications of *R. ruber* cells for efficient and facilitated assimilation of C_3 and C_4 alkanes. The involvement of alkane-1-monooxygenases AlkB and soluble di-iron monooxygenases MmoA was demonstrated. The expression of both types of enzymes in *R. ruber* cells was $2.6 \cdot 10^4$–$6.0 \cdot 10^4$ times higher in the presence of gaseous alkanes than in nutrient broth or medium with glucose.

Conclusion: *R. ruber* actinomycetes have a unique set of adaptations that make them the most important biooxidizers of C_2–C_4 gaseous alkanes and promising biotechnological agents.

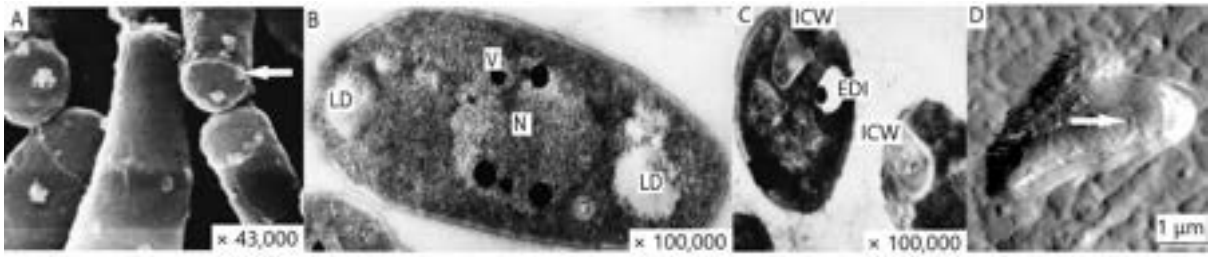


Figure 1: SEM (A), TEM (B, C) and AFM (D) images of R. ruber cells grown in the presence of propane and n-butane. LD – lipid droplet, V – volutin, N – nucleoid, ICW – invagination of the cell wall, EDI – electronically dense inclusion. Arrows show cell appendages.

Abstract ID: 2685 / Poster Number: 181.15P

Compost extracts as sustainable tools for plant disease suppression and soil health promotion: findings from the Tomato–Phytophthora parasitica pathosystem

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The mechanisms driving the suppressive effect of plant pathogens and the biofertilizing capacity of compost and its derivatives depend largely on the biological activity of indigenous microbiota. These microorganisms interact with soil organic matter and plant roots, exerting regulatory effects on the rhizosphere microbial community. This study aimed to quantify rhizosphere microbial activity and diversity in the tomato-*Phytophthora parasitica* pathosystem after applying a compost a compost extract via fertigation, as well as to establish its ability to suppress the disease caused by *P. parasitica* under greenhouse conditions. For this purpose, rhizospheric enzyme activities related to C, P and N cycling were measured, as well as microbial metabolic functionality was assessed using Biolog EcoPlates. Metabarcoding analyses were performed to investigate soil microbial biodiversity. Finally, plant health status and plant development parameters in the tomato-*P. parasitica* pathosystem were evaluated by bioassay.

Results showed that compost extracts significantly reduced the severity of *Phytophthora parasitica* disease by 60%, while improving tomato plant agronomic traits. Both facts could be related to possible alterations in rhizospheric enzyme activity after treatment with the extract. Compost extract application increased the activity of enzymes involved in P and C cycling, as well as the metabolic profile and relative abundance of rhizosphere microorganisms. However, these effects were strongly influenced by the presence of the phytopathogen, disclosing the existence of potential biomarkers related to plant disease suppression. Therefore, this study revealed the potential of compost extracts as a sustainable tool for soil-borne disease management and soil health promotion.

Abstract ID: 3617 / Poster Number: 346.17P

Compost metagenome as a source of industrially relevant CAZYmes

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Co-authors: *Viktor Zupančič, Ljubomir Radic, Anna Alessi, Neil Bruce*

Introduction & objective: Second-generation biofuels are suffering from a lack of efficient and cost-effective hydrolytic enzymes which are crucial for lignocellulose pre-treatment. These enzymes are also gaining interest in textile, paper and chemical industries as environmentally friendlier alternative to harsh chemicals. Metagenomics is a powerful approach to explore the diversity and potential of lignocellulolytic microbial communities in natural environments, revealing novel enzymes that may not be detected by traditional methods.

Methods: In our study, a complement of bioinformatics and experimental approaches was used to characterize *in silico* predicted carbohydrate active enzymes (CAZymes) targets and evaluate their potential for lignocellulose pre-treatment in comparison to commercially available counterparts.

Results: Among 96 target genes which were cloned and expressed, 12 novel thermophylic xylanases were succesfully isolated and characterized. One of the promising targets was approximately 40 kD enzyme with a GH10 catalytic domain, active against beechwood xylan and arabinoxylan. The enzyme was optimally active at 50 °C and pH=8. Its xylanolytic activity increased in the presence of 5 mM Co^{2+}, Fe^{3+}, Mn^{2+} or Ca^{2+} ions or reducing agents such as 2 mM DTT or 10 mM ß-mercaptoethanol. Apart from its stability in different environmental conditions, the target enzyme also proved efficient in the degradation of natural hemicellulose-rich substrates, such as wheat or barley bran as well as considerable robustness in the presence of the most common chemicals used in lignocellulose pretreatments.

Conclusion: The results highlight promising potential of newly characterized enzyme for use in industrial applications, such as the production of biogas and bioethanol.

Abstract ID: 835 / Poster Number: 040.16P

Comprehensive analysis of histamine biosynthesis in cheese using qPCR and HPLC techniques

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Introduction: Biogenic amines (BAs) are organic compounds formed by microbial metabolism in fermented foods like cheese. While they indicate microbial activity and contribute to flavor development, high levels of BAs, such as histamine, could present food risk, requiring monitoring.

Objective: This study develops an in-house method using quantitative PCR (qPCR) to detect and quantify the 'hdcA' gene copies, linked with bacterial histamine production in cheese. High-performance liquid chromatography (HPLC) validates qPCR findings by measuring histamine levels in tested samples.

Method: Standard curves were performed for the marker gene (16S rRNA) and the non-marker gene ('hdcA') by correlating known concentrations of DNA with corresponding Ct values for qPCR normalization. DNA from twenty cheese samples was analysed by qPCR to amplify 'hdcA' and 16S rRNA genes. The number of copies of 'hdcA' was normalized against the 16S rRNA gene for accurate measurement and microbial load. To validate qPCR findings and assess histamine production, HPLC analyses were performed on the same cheese samples.

Findings: The results present that the number of copies of 'hdcA' gene obtained by qPCR ranges from 154 to 1459121. From a specific Ct value, the copy number of 'hdcA' gene shows a positive correlation with histamine levels quantified by HPLC. Conversly, in cases where histamine is not detected by HPLC, the values obtained through qPCR are variable.

Conclusion: The combination of qPCR and HPLC assays provides a reliable method for detecting and quantifying histamine in cheese, allowing the control of BAs generation in cheese production to ensure food safety.

Abstract ID: 2881 / Poster Number: 428.16P

Comprehensive skill set development for teaching microbiology and genetics to future scientists

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The life sciences research field offers unique opportunities for young scientists to explore the benefits of direct involvement in research and teaching for their cognitive and psycho-emotional development. We consider a successful scientist one who can transfer their knowledge onto others. To this extent, for the past three years, we have participated in a new approach to teach students the basics of biology. This involved performing laboratory experiments (microbial community analysis; UV mutagenesis; forensic genetics) with graduate students (BSc, MSc) and then helping them to transfer the acquired knowledge to high school students in an extracurricular science club entitled "The Young Researcher". Starting from the idea that both graduate and high school students need to understand the real-life applicability of the studied biology topics, we designed a program through which graduate students become teachers for younger students under the supervision of university and high school faculty members. This approach exposes future scientists to basic microbiology, molecular biology, and experimental design concepts, improving their communication skills through explaining in such a manner high school students can also understand. Involving students in both research and teaching activities raised their awareness on the practical applicability of life sciences. Thus, these learning by teaching activities stimulate students' interest, providing numerous benefits for their psycho-affective development and for European key competences improvement. Therefore, our involvement as coordinator of dual teaching systems represented a great opportunity to obtain comprehensive skills regarding the education of young researchers and help them inspire the younger ones.

Abstract ID: 2349 / Poster Number: 089.15P

Computational models of Live Biopreservatives and Bacteriophages on food safety and the dynamics of antimicrobial resistant genes in foodborne pathogens

Presenting author: Pere Puigbò, PhD, Autonomous University of Barcelona, Bellaterra, Catalonia, Spain; University of Turku, Turku, Finland

Co-authors: *Tuomas Tall, Marjo Helander, Miho Nakamura,*

Foodborne pathogens have been a problem for all human societies since their inception. Although standards in food treatment and production have improved in many countries, it still remains a global problem according to the World Health Organization (WHO). According to the WHO, there are 31 types of foodborne infections that result in 600 million illnesses annually and more than 400,000 deaths. Moreover, the growing presence of antimicrobial resistant (AMR) strains —i.e., microorganisms able to withstand current antimicrobial treatments, leads to harder treatments of foodborne pathogens and a to a potential transfer of AMR genes from the food chain into host-associated microorganisms.

Here, we present a theoretical model on the use of live biopreservatives (LBs), defined as microorganisms that produce antimicrobial substances and bacteriophages to control the growth and spread of foodborne pathogens. We hypothesize on the potential impact of LBs and bacteriophages i the spread of antimicrobial resistance. Moreover, biodegradable plastic packing is exposed to the action of environmental plastic-degrading microorganisms, leading to potential interactions with LBs and bacteriophages.

Counting models of pathogen growth can be written as a general birth-death process with internal dynamics as a function of the population size. Modelling interactions among a bacterial pathogen, LB, bacteriophage and the physicochemical properties of the food production and storage can be achieved by combining aspects of competitive growth models and predator-prey dynamics and logistic growth models. Furthermore, stochastic versions of the equations can account for real-world randomness. We provide a simulation dynamic of microbial growth based on three equations: equation 1) to determine pathogen growth in the presence of LB, bacteriophages, and environmental properties; equation 2) to determine LB growth in the presence of a pathogen, and environmental properties; and equation 3) grown to the pathogen-specific

bacteriophage. All equations take into account microbial interactions, as well as the carrying capacity. Furthermore, we show a micro-evolutionary dynamic of antibiotic resistant genes in bacterial strains of foodborne pathogens based on bacterial strains from the alignable tight genomic clusters (ATGC) database.

Biopreservation with LBs and bacteriophages is an emerging field in food processing and research, that provide potentially precise and strain-specific solutions for controlling bacterial populations. Adding LBs can extend shelf life through bacteriostatic mechanisms, whereas bacteriophages offer direct lytic action against pathogens. However, effective food preservation with these approaches requires further empirical and theoretical research. Furthermore, biopreservation will be combined with heat, refrigeration, or reduced water activity, and these physicochemical strategies should be considered.

Abstract ID: 2288 / Poster Number: 121.17P

Concanavalin A-Rose Bengal Conjugate for Targeted Antimicrobial Photodynamic Therapy Against Gram-Positive and Gram-Negative Bacteria

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The increasing prevalence of antibiotic-resistant bacteria underscores the need for innovative antimicrobial strategies. This study introduces a novel photodynamic therapy (PDT) approach using a Concanavalin A-Rose Bengal (ConA-RB) conjugate to target Gram-positive and Gram-negative bacteria. ConA, a lectin with a specific affinity for bacterial surface carbohydrates, was conjugated with Rose Bengal, a potent photosensitizer, yielding a conjugate with enhanced antibacterial efficacy.

Microscopic analysis demonstrated a strong binding affinity of ConA to Gram-positive bacteria, attributed to their carbohydrate-rich peptidoglycan layer. In the case of Gram-negative bacteria significant interactions with ConA were also detected, suggesting that ConA can overcome or bypass their protective outer membrane. The ConA-RB conjugate demonstrated significant production of reactive oxygen species (ROS) under LED light illumination, eradicating both Gram-positive (e.g., Staphylococcus aureus) and Gram-negative bacteria (e.g., Escherichia coli, Pseudomonas aeruginosa, and Salmonella paratyphi B). The conjugate showed superior antibacterial activity, compared to free Rose Bengal, achieving total eradication of bacteria at lower concentrations. Additionally, the ConA-RB conjugate exhibited no dark toxicity, emphasizing its safety profile.

These findings highlight the ConA-RB conjugate as a promising candidate for PDT, addressing critical challenges in combating resistant bacterial infections.

Abstract ID: 3764 / Poster Number: 195.17P

Conjugation-driven horizontal gene transfer in bacteria on antimicrobial surfaces

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It is known that unfavorable environments, such as exposure to antimicrobials may induce various stress response pathways in bacteria. One of the outcomes could be enhanced exchange of genetic information often enabling a fast spread of resistance mechanisms towards the experienced unfavorable environment, such as the presence of an antimicrobial.

Antimicrobial surfaces as passive means to decrease microbial touch transfer potential are gaining popularity in healthcare sector but also in highly populated public areas. Although in theory meant to kill bacteria, most of such surfaces leave behind survivors that have been exposed to quite significant antimicrobial stress. Our current understanding on the behaviour of surface-surviving microbes is however rather limited.

In this work we studied the frequency of conjugation-driven exchange of plasmid-borne antibiotic resistance genes between Escherichia coli donor and Pseudomonas putida recipient on copper, silver and quaternary ammonium compound (QAC)-based antimicrobial surfaces. We demonstrate that bacterial conjugation was enhanced on copper and QAC surfaces, however rather limited on silver surfaces. When conjugation was studied in the presence of the active agents of antimicrobial surfaces, copper, silver ion and QAC in liquid format, no clear evidence on conjugation-enhancing effect of copper was seen.

In this study we evidenced the possibility of enhanced conjugation-driven horizontal transfer of plasmid-borne resistance genes on copper- and QAC-based antimicrobial sufaces and suggest that this issue could be overcome by increasing the efficacy of antimicrobial surfaces to minimize the amount of survivors.

Abstract ID: 2475 / Poster Number: 374.17P

Construction of a simplified synthetic nitrifying microbial ecosystem through co-culture of isolated nitrifying bacteria

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Crop cultivation relies on the nitrogen cycle driven by soil microorganisms. Constructing an artificial microbial ecosystem involved in the nitrogen cycle could enhance the crop productivity. However, the microbial community in the nitrogen cycle is composed of different functional groups of microorganisms, including heterotrophic bacteria decomposing organic matter and nitrifying bacteria converting ammonia into nitrite and nitrate. Due to its complexity, it has been difficult to control and reconstruct a simplified nitrifying ecosystem. Moreover, since autotrophic nitrifying bacteria grow slowly, and are highly sensitive to their environment, it has been difficult to isolate and to cultivate. This study focused on evaluation of optimal conditions for isolating nitrifing bacteria and constructing a synthetic nitrifying microbial ecosystem. First, representative nitrifying bacteria were used to optimize colony formation, revealing that condition with 0.4% CO_2, 0.6% gellan gum, medium volume of 60 mL with 90 x 20 mm depth, and supplemention of trace elements significantly shortened the colony forming time for isolation. Second, nitrifying bacteria were enriched using soil as microbial seed for several times by synthetic inorganic nitrogen medium under 37 °C. Liquid culture analysis confirmed the activity of nitrifying isolates, identifying 10 ammonia-oxidizing bacteria (AOB) and 30 nitrite-oxidizing bacteria (NOB) strains through 16S rRNA sequencing. The isolated AOB and NOB were then co-cultivated with a heterotrophic bacterium *Bacillus badius* NBRC 15713 in an organic nitrogen-containing medium. This study demonstrated a simple method for isolating nitrifying bacteria and successful construction of a simplified synthetic microbial ecosystem offering a model for soil nitrification process.

Abstract ID: 1200 / Poster Number: 041.17P

Construction of multi-tailfiber phage against enterohemorrhagic Escherichia coli

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Introduction: Lytic bacteriophages are effective tools for controlling bacterial infections in humans and foods, although there are still some challenges such as their narrow host ranges.

Objective: This study aimed to expand phage host range by introducing random multiple tailfibers into a single phage particle without genetic modification in phage genome.

Method: First, we constructed the host strains which heterologously express the structural genes of long tailfibers (LTF) of enterohemorrhagic E. coli (EHEC) phage PE37. Then, EHEC phage ECP52 were converted into multi-tailfiber (MTF) phages by infecting the LTF expressing host strains in which ECP52 would randomly utilize heterologous LTFs for phage particle construction without genetic modification. Finally, the lytic activity of constructed MTF ECP52s against planktonic or biofilm cells were evaluated using EHEC host or non-host strains.

Findings: Three MTF ECP52s were constructed and termed as ECP52-LTFr, -LTFe or -LTFre which involves the PE37 LTF protein locating at the root connection with its main tail structure, LTF protein locating at the edge side of LTF structure, or both root and edge proteins, respectively. Of course, parental phage ECP52 could not exhibited lytic activity against non-host strain E. coli No.166. On the other hand, all MTF ECP52s showed significant lytic activities against strain No. 166 but still did not form any plaques on agar plate. ECP52-LTFre especially exhibited remarkable activity as strong as by PE37 which could originally infect strain No. 166.

Conclusion: This study demonstrated the potential of MTF phage approach to expand the phage host ranges.

Abstract ID: 2114 / Poster Number: 113.17P

Construction of quorum sensing AI-3 pathway silenced uropathogenic Escherichia coli

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Uropathogenic Escherichia coli (UPEC) causes most community-acquired urinary tract infections (UTIs) commonly treated with antibiotics. The rise in antimicrobial resistance warrants new approaches for treatment, such as blocking bacterial quorum sensing (QS). Effective silencing of QS could revive the efficacy of antibiotics and serve as a preventive measure in recurrent UTIs. Autoinducer 3 (AI-3) is a recently discovered and less-researched QS pathway known to regulate virulence in over 25 pathogens including UPEC. AI-3 key gene, qseC, controls virulence traits vital for UTI initiation and progression, such as biofilm production, motility, and adherence and invasion of bladder epithelial cells (BECs).

Our objective was to construct a qseC-silenced clinical E. coli isolate CFT073 with CRISPRi technology and to characterize the silenced strain in order to validate qseC as a new antivirulence drug target. It is believed that CRISPRi inducible silencing is superior to deletion as it does not remove the genes from their native regulon.

The CRISPR machinery with a deactivated Cas9 (dCas9) protein finds and binds targeted gene with a gene-specific spacer preventing transcription. We designed six different spacers as the spacer sequence and binding site affects its silencing efficacy. The incubation time and mating strain ratio was optimized. The silencing efficacy was assessed with RT-qPCR. To study the effect of qseC-silencing on different virulence traits we utilized crystal violet assay for biofilm production, motility agar assay, and evaluated adhesion and invasion of bacteria to BECs.

Abstract ID: 2637 / Poster Number: 319.17P

Construction of the advanced riboflavin producers in the yeast Candida famata

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Introduction: Riboflavin is water-soluble vitamin serving the precursor of flavin nucleotides FMN and FAD. This vitamin is important commodity used in agriculture, food industry and medicine and is produced using microbial overproducers. The yeast *Candida famata* belongs to the most active riboflavin producers, still needs to be improved to compete with current industrial producers *Bacillus subtilis* and *Ashbya gossypii*. To meet this goal, the stable riboflavin overproducer AF-4 was isolated by classical selection and further improved by overexpression of genes *RIB1, RIB6, SEF1, PRS3* and *ADE4*.

Objectives: The objective of this work aimed in construction of the mutants of *C. famata* overproducing riboflavin on cheap substrate like cheese whey and lignocellulosic hydrolysates.

Methods: The standard methods of molecular genetics were used.

Findings: The riboflavin overproducing strains of *C. famata* were characterized by robust growth and riboflavin oversynthesis on cheese whey supplemented with nitrogen source. Riboflavin synthesis on whey was activated due to introduction of transcription activator gene *SEF1* under control of lactose-induced promoter *LAC4* as well as due to overexpression of gene *GND1* coding for 6-phosphogluconate dehydrogenase responsible for synthesis for ribulose-5-phosphate. C. famata is characterized by robust growth and riboflavin synthesis on major sugars of lignocellulosic hydrolysates, glucose, xylose and L-arabinose. Overexpression of genes *XYL1* and *XYL2* improved riboflavin synthesis on xylose and lignocellulosic hydrolysates. Further enhancement of riboflavin synthesis was achieved by simultaneous overexpression of genes *RIB6, GND1* and *RFE1*.

Conclusion: The flavinogenic yeast *C. famata* appeared to be the robust organism for riboflavin oversynthesis on standard media with glucose as carbon source and on the cheap media obtained from residues like cheese whey or lignocellulosic hydrolysates.

Abstract ID: 2885 / Poster Number: 151.17P

Continuous antibiotic prophylaxis has long-lasting effect on the gut microbiome structure and functionality in infants with vesicoureteral reflux

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Introduction: The development of the gut microbiome (GM) during childhood can be influenced by several factors, including antibiotic treatment, which may have long-term consequences for host health (e.g., development of allergy, asthma, and obesity). Vesicoureteral reflux (VUR) is a typical disorder of early childhood, which is treated with continuous antibiotic prophylaxis (CAP) to limit the development of urinary tract infections and subsequent damage to the renal parenchyma. However, the use of CAP is controversial.

Objective: In this context, the aim of this study was to investigate the long-term effects of CAP on the GM of children with high-grade (III-V) VUR.

Method: Specifically, the GM of 122 children with VUR treated or not with CAP was longitudinally characterized from enrolment to 72 months by 16S rRNA amplicon sequencing and targeted metabolomics (e.g., short-chain fatty acids, amino acids, sugars). Moreover, shotgun metagenomics was performed in a subset of samples for resistome profiling over time.

Findings: Comparative subgroup analysis revealed temporal changes in GM diversity in CAP-exposed infants compared to the non-CAP group. From the taxonomic standpoint, CAP-related GM was characterized by an initial dysbiosis, i.e., an increased relative abundance of opportunistic pathogens and decreased proportions of health-associated taxa. The dysbiosis tended to resolve over time, also from a functional point of view, but some potentially harmful signatures persisted.

Conclusions: This study demonstrates that even a short exposure to CAP in children with VUR can lead to long-term GM alterations, with potential risks to host health.

Abstract ID: 1293 / Poster Number: 326.15P

Contribution of diazotrophs to the N cycle by free-living and biofilm-associated lifestyles in the Jordan River subsurface

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Aquatic heterotrophic N_2 fixation plays an important role in primary and secondary production. However, freshwater environments pose a challenge in determining the role of heterotrophic diazotrophy due to extreme ecological heterogeneity. Here, we investigate diazotrophy along a steep oxygen gradient found within the Jordan River subsurface. Distinct focus was given to two diazotrophic lifestyles: biofilm-associated cells adhered to sediment and planktonic cells suspended in porewater. Samples were collected from aerobic and anaerobic strata of the Jordan River streambed and incubated in microcosms for 24h with dissolved $^{15}N_2$ under ambient conditions. Samples were analyzed for diversity of the different lifestyles, abundance, and N_2 fixation rates. Confocal scanning laser microscopy unveiled the isochronal presence of phototropic (cyanobacterial) diazotrophs in aerobic biofilms and a more abundant and pervasive dispersal of diazotrophs in anaerobic biofilms. Immunolabelling the nitrogenase enzyme coupled with flow-cytometry indicated that aerobic sediment had a greater quantity of diazotrophs in the magnitude of an entire order and a higher proportion of diazotrophs to total bacteria than the anaerobic layer (10%, 4%, respectively). Nevertheless, most proportions of diazotrophs to bacteria ranged from 1% to 11%. Furthermore, significantly higher N_2 fixation rates were measured in anaerobic porewater in the summer than the winter with a mean difference of 0.4 nmol N $L^{-1}d^{-1}$ and in anaerobic sediment along this same trend with a mean difference of 18 nmol N $g^{-1}d^{-1}$. *nifH* sequencing identified the dominancy of *Pseudomonadota, Desulfobacterota*, and even *Cyanobacteria* (possibly mixotrophs) in the anaerobic pore water. These new findings suggest that the diazotrophic community within these anaerobic layers are coupling N_2 fixation to sulfate reduction and active metabolizing of organic matter. Altogether, our results and corresponding insights intimate the immense importance of subsurface diazotrophy to the N cycle in freshwater environments considering its seasonal, temporal, and eco-stoichiometric vicissitudes.

Abstract ID: 2709 / Poster Number: 253.15P

Contribution of FocA to pH Homeostasis and ATP generation during E. coli fermentation

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Introduction: Formic acid is a major product of enterobacterial mixed-acid fermentation. During exponential-phase growth, formic acid passes from the cytoplasm to the periplasm via the pentameric channel, FocA. Re-uptake of formate into the cytoplasm during the late exponential and stationary phases of growth, or upon a decrease in external pH below 6.5, allows its disproportionation into the gaseous products CO_2 and H_2 by the membrane-associated formate hydrogenlyase (FHL) complex. Uptake of formate by FocA is dependent on an active FHL complex, suggesting coupling between both systems and this depends on conserved histidine (H209) and threonine (T91) residues. A H209N variant of FocA has a serious fermentative growth deficiency caused by to rapid efflux of formic acid from the cell and the inability to take up formate from the growth medium.

Objectives: Our aim was to determine mechanistically the reason for the growth defect of a cell excreting formic acid. This could be due to a deficiency in balancing pH, a loss of cellular carbon or a deficiency in ATP generation.

Methods: We use a formate-responsive reporter system, together with defined *E. coli* mutants to assess changes in various physiological parameters during fermentation, including changes in H_2 production, intracellular pH, CO_2, and ATP levels.

Results: A cell producing the $FocA_{H209N}$ variant generates less ATP than wild type. The intracellular pH also remains at a near-neutral value during growth. Mutants that lack an FHL complex do not exhibit the same phenotype.

Conclusion: Together, our findings suggest that formate production coupled with H_2 production are together important in balancing intracellular pH, controlling carbon flow through glycolysis, and thus contribute to optimize energy conservation during mixed-acid fermentation. Restricting carbon flux to formate appears to be important in optimizing ATP generation during fermentation.

Abstract ID: 3176 / Poster Number: 333.15P

Contributions of Non-Essential Sec-Pathway Components and a Cell Envelope-Associated Protease to Conjugation of plasmid pLS20 in Bacillus subtilis

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Introduction: Bacterial conjugation is a key mechanism of horizontal gene transfer, driving the dissemination of genes associated with antibiotic resistance, virulence, and metabolic traits. This process involves the transfer of DNA from a donor to a recipient cell, promoting genetic diversity and evolutionary adaptability. The Type IV secretion system has been shown as the essential machinery for conjugation in most bacterial species. To determine whether other genes also play a role in conjugation, the roles of non-essential components of the general Secretory (Sec) pathway and cell envelope-associated proteases of the model organism *Bacillus subtilis* were investigated.

Method: Homologous recombination was employed to delete the genes of interest in both donor and recipient cells in *B. subtilis*. The resulting mutant strains were subjected to conjugation assays to evaluate the effects of the deletions on conjugation efficiency.

Findings: Deletion of individual Sec-dependent signal peptidases (SipS, SipT, SipU, SipV, and SipW) in donor cells resulted in a reduced conjugation efficiency, underscoring the importance of proper protein localization and activation. Conversely, deletion of the gene *yqeZ*, which encodes a membrane-bound serine protease, in recipient cells unexpectedly enhanced the conjugation efficiency. This suggests a role for this protease in maintaining cell envelope integrity during conjugation.

Conclusion: These findings reveal novel aspects of the molecular mechanisms underlying bacterial conjugation and highlight the significance of membrane protein dynamics and cell envelope integrity in this process.

Abstract ID: 1654 / Poster Number: 383.15P

Convergent evolution in Nucleococcaceae fam. nov., a novel lineage of Opitutales (Verrucomicrobiota), and other endosymbionts of termite gut flagellates

Presenting author: Mrs. Undine Sophie Mies, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

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Cellulolytic flagellates perform a key role in lignocellulose digestion in termite guts. Previous studies have documented that each flagellate species hosts a diverse assemblage of symbionts from various bacterial phyla. From the metagenomes of 78 termite species, we recovered 82 metagenome-assembled genomes (MAGs) from a novel, family-level lineage of endosymbionts in the order *Opitutales* (phylum *Verrucomicrobiota*), comprising previously reported endonuclear bacteria of the genus *Nucleococcus*. Fluorescence in situ hybridization confirmed that the new symbionts are exclusively localized within the nucleus. Comparative genome analysis revealed that all family members experienced a severe genome reduction compared to other *Opitutales* (average genome size 1.68 vs. 2.98 Mbp). Substantial gene losses in carbon and energy metabolism were accompanied by the acquisition of a nucleotide triphosphate transporter and numerous transporters for the uptake of sugar phosphates, amino acids and vitamins. Comparing the *Nucleococcaeceae* MAGs with other established or putative endosymbionts of termite gut flagellates from the phyla *Actinomycetota* (*Ancillulaceae*), *Desulfobacterota* (*Adiutricales*), *Elusimicrobiota* (*Endomicrobiaceae*), *Bacillota* (*Acutalibacteriaceae*), and *Pseudomonadota* (Rickettsiales, RUG11792, UBA3830, UBA9339) revealed strong patterns of evolutionary convergence. The acquisition of gene functions for the uptake and subsequent conversion of alternative substrates, such as sugar phosphates and hexuronic acids, and in many cases, an ADP/ATP antiporter, is driven by horizontal gene transfer and compensates for functional losses due to an ongoing genome erosion.

Abstract ID: 2500 / Poster Number: 106.16P

Co-occurrence network analysis reveals the alterations of gut microbiome in infants born to obese mothers

Presenting author: Dr. Muhammad Arshad, New York University, Abu Dhabi, United Arab Emirates

Co-authors: *Yvonne Vallès, Nizar Drou, Kristin Gunsalus, Abdishakur Abdulle*

Introduction: Most gut microbiome studies have primarily concentrated on bacteria. However, non-bacterial microorganisms are also common inhabitants of the gut ecosystem. Our understanding of the composition and potential interactions among these microorganisms is still limited, particularly in the context of the infant gut microbiome and obesity.

Objective: Whether mother's microbiome contribute to the "priming" phenomenon contributing to the early onset of childhood obesity?

Methods: We used whole genome shotgun sequencing data from the stool samples at five timepoints (1-week, 2, 4, 6 and 12 months) from infants born to obese (n=23 participants, n=104 samples) and normal-weight (n=23 participants, n=99 samples) mothers.

Findings: A pairwise comparison showed a lower diversity in infants born to obese mothers compared to normal-weight with significant differences at 4-month timepoint. Taxonomic classification and abundance analysis revealed that the Methanoregulaceae, Schizosaccharomycetaceae and Geminigeraceae were the predominant families of archaea, fungi and protozoa, respectively, in the infants' gut metagenomes. Co-occurrence analysis revealed that number of interactions were lower in cases (obese) compared to controls (normal-weight) in the entire network as well as when compared between prokaryotes and eukaryotes. Moreover, a reduced number of interactions was observed between bacteria and other prokaryotes (archaea & virus), but the total number of interactions were increased between bacteria and eukaryotes (fungi & protist) in cases (obese) from birth to one year.

Conclusion: Microbial abundance profile and co-occurrence network analysis offer valuable insights into microbial composition and interactions, particularly in the context of mother's pre-pregnancy status and child's early onset of obesity.

Abstract ID: 685 / Poster Number: 319.16F

Coordination of responses in complex chemotaxis systems: Phosphorylated CheV interacts only with a subset of chemoreceptors in Pseudomonas aeruginosa

Presenting author: Dr. Mario Cano-Muñoz, Estacion Experimental del Zaidin, Granada, Spain

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Introduction: Chemotaxis signaling pathways are among the most sophisticated and well-studied signal transduction pathways. Central to these pathways is the ternary complex formed by chemoreceptors, the CheA autokinase and coupling proteins. Intriguingly, there are two types of coupling proteins: CheW and the phosphorylatable CheV. Whereas CheW is omnipresent in chemosensory pathways, CheV is only found in bacteria that contain an elevated number of chemoreceptors, such as in our model, Pseudomonas aeruginosa, for which the signal recognised by half of its 26 chemoreceptors is known.

Objective: Of all chemosensory proteins, CheV is the one that is least understood. The objective is to identify the physiological relevance of CheV and the functional role of its phosphorylation.

Methods: Quantitative capillary chemotaxis assays of wt and ΔcheV P. aeruginosa, isothermal titration calorimetry (ITC) of purified proteins.

Findings: Chemotaxis of a cheV mutant to chemoeffectors with known cognate chemoreceptor was strongly reduced in some cases, like nitrate, but unaltered for other compounds, like amino acids, suggesting that CheV interacts only with a subset of chemoreceptors. ITC experiments showed that unphosphorylated CheV failed to bind to the cytosolic fragments of the McpN nitrate receptor and the PctA amino acid receptor. In contrast, a CheV phosphorylation mimic bound with high affinity to McpN but not to PctA, which agrees with the chemotaxis analyses.

Conclusion: CheV is required for chemotaxis mediated by only a subset of chemoreceptors, and its phosphorylation is essential for binding to these receptors. This may permit the coordination of responses in complex chemotaxis systems.

Abstract ID: 3694 / Poster Number: 118.15F

Core Fungal Microbiome of Peach Palm (Bactris gasipaes): Diversity Across Islands and Leaf Sections

Presenting author: Ms. Angelis Marbello-Santrich, Boyce Thompson Institute, Ithaca, NY, United States

Co-authors: *Silvia Restrepo*

Plant-associated microbial communities include beneficial, neutral, and pathogenic microorganisms. The endophytes are essential for plant health, contributing to growth and resilience. However, their diversity and community structure in many plants remains understudied. Describing these microbial communities is a critical first step to understanding their potential roles and interactions. The peach palm (*Bactris gasipaes*), a crop of significant cultural and economic importance in Colombia, forms part of the food heritage of the Pacific and Amazon regions. Beyond its cultural value, peach palm holds great potential for sustainable agriculture and local economies. In this study, we used a metabarcoding approach to describe the fungal communities associated with peach palm leaves, focusing on spatial and structural factors. Samples were collected from multiple islands and different sections of the large leaves to assess the potential influence of leaf position on community structure. Our findings revealed a core microbiota that remains stable across spatial scales (islands) and within the sections of the leaves, suggesting a conserved community structure. This work provides a comprehensive description of peach palm-associated fungal diversity, offering a valuable foundation for future studies on these communities' ecological roles and interactions. This research highlights the potential of culture-independent tools for studying plant-associated microbiota and positions peach palm as a model for understanding stable microbial associations in sustainable agricultural systems, while celebrating its cultural and ecological significance in Colombia.

Abstract ID: 3273 / Poster Number: 450.15P

CRISPR Interference Screening Using Shortened sgRNA Libraries for Novel Target Gene Identification

Presenting author: Ms. Song Hee Jeong, Chung-Ang University, Anseong, South Korea

Co-authors: *Nan Ju Kim, Yong Seong Choi, Sang Jun Lee*

CRISPR interference (CRISPRi) can regulate the expression of target genes by binding dCas9/sgRNA complex to specific target sequence. CRISPRi has been developed as a screening method for genotype-phenotype mapping. However, the library size of sgRNA containing a random 20nt target recognition sequence (TRS) is large (~10^{12}). To identifying target genes associated with a specific phenotype, we developed novel CRISPR screening method. Here, we demonstrated that the dCas9/sgRNA with significantly shortened TRS could repress the expression of target genes, regardless of binding to a promoter or coding region. When screened with 5'-shortened and random sgRNA libraries, we selected 17 target genes to enhance violacein pigment production in synthetic *Escherichia coli* cells. All 17 strains with a single deletion of each target gene showed an increase in violacein production of up to 3.4 times compared to the wild-type strain. Our results indicate that microbial target genes can be identified by reducing the library size using a 5'-shortened random TRS library.

Abstract ID: 3564 / Poster Number: 092.15P

Crossing borders: *Fusarium musae* travelling from banana to hospitals

Presenting author: Prof. Matias Pasquali, University of Milan – DEFENS, Milano, Italy

Co-authors: *Valeria Tava, Luca Degradi, Marco Saracchi, Paolo Cortesi, Matthias Brock, Greetje Vande Velde*

Fusarium musae causes crown rot in bananas and clinical infections in humans. A collection of 19 *F. musae* isolates—11 from bananas across Ecuador, Costa Rica, Panama, and Colombia, 8 from human patients in Europe and the USA—was analyzed. Multilocus phylogenetic studies confirmed *F. musae*'s distinctiveness from its sister species *F. verticillioides*. Genome sequencing of strain F31 resulted in 12 chromosomes and an N50 of 4.36 Mb, making it one of the most complete assemblies in the *Fusarium fujikuroi* species complex. Mitogenome haplotype analysis revealed the genetic similarity across plant and human isolates, supporting potential cross-host transmission. Infection models demonstrated that *F. musae* could grow at 24°C and 37°C, with mortality rates in *Galleria mellonella* larvae, used as a proxy for animal infection, exceeding 70% at 37°C, comparable to human-pathogenic strains. In bananas, *F. musae* caused extensive crown rot symptoms within 14 days. Sensitivity assays showed that *F. musae* is less susceptible to azole antifungals than *F. verticillioides*, highlighting potential challenges for treatment in clinical and agricultural contexts.

RNA sequencing on blood and banana peel medium highlights a diverse set of effectors involved. RNAseq data during *Galleria melonella* infection shows the crucial role of specific effectors in the colonisation of the animal model.

These findings demonstrate *F. musae*'s transkingdom pathogenicity, genetic adaptability, and resistance to antifungal agents and identify putative genes involved in transkingdom pathogenicity. This research provides a robust framework for addressing emerging fungal threats to human health and food security by integrating genomics, transcriptomics, and infection biology.

Abstract ID: 3193

Cross-species quorum sensing interference: Lactiplantibacilli as potential anti-virulence agents against Staphylococcus aureus

Presenting author: Dr. Stephanie Fulaz Silva, University of Copenhagen, Copenhagen, Denmark

Co-authors: *Martin S. Bojer, Weizhe Wang, Hanne Ingmer*

Quorum sensing (QS) is a bacterial communication system that regulates collective behaviors, including virulence, via signaling molecules. In Gram-positive bacteria, these signals are auto-inducing peptides (AIPs). *Staphylococcus aureus*, a major human pathogen, presents a growing challenge due to rising antibiotic resistance, including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant strains (VISA, VRSA), alongside the lack of effective vaccines. Anti-virulence therapies, which target virulence rather than killing bacteria, offer a promising alternative. In *S. aureus* this approach is based on the accessory gene regulator (*agr*) QS system.[1] *S. aureus* strains are divided into four *agr* allelic subgroups, each producing unique AIPs that selectively activate or inhibit QS within and across strains. Oher staphylococcal species naturally inhibit *S. aureus agr*, showcasing interspecies communication as a mechanism for QS interference.[2]

Inspired by this interspecies interaction, we hypothesized that lactic acid bacteria (LAB), which encode *agr*-like systems,[3,4] could similarly interfere with staphylococcal QS. This study investigates their potential as anti-virulence agents through QS disruption. To test this, we performed genomic analysis on LAB strains, identifying *agr*-like systems and predicting their AIP sequences. *Lactiplantibacillus plantarum* was found to possess three distinct AIP variants, expanding upon the single previously reported AIP, while new AIPs were identified in L. *paraplantarum* and *Carnobacterium divergens*. Synthetic versions of these AIPs and their stabilized analogs were tested for their ability to inhibit QS in *S. aureus*, using reporter strains and measuring virulence factor expression via hemolysin activity.

Our findings demonstrate that LAB-derived AIPs effectively inhibit QS in *S. aureus* without affecting bacterial growth. Among the tested AIPs, *L. paraplantarum*'s AIP exhibited the strongest inhibitory effect, significantly reducing virulence factor expression. Notably, the peptide sequence and chemical structure were critical to their activity. As shown in Figure 1, variations in chemical structure, even with the same AIP sequence, influenced their effectiveness, with Lp-AIP 3 exhibiting the highest activity across all *agr*-subgroups.

These results highlight the potential of LAB as interspecies QS disruptors and their application in mitigating pathogenic virulence through probiotic-based strategies.

In conclusion, the ability of LAB to interfere with *S. aureus* QS highlights the therapeutic potential of probiotics as anti-virulence agents. This study advances our understanding of microbial interactions and opens new avenues for developing innovative therapies that target bacterial communication systems.

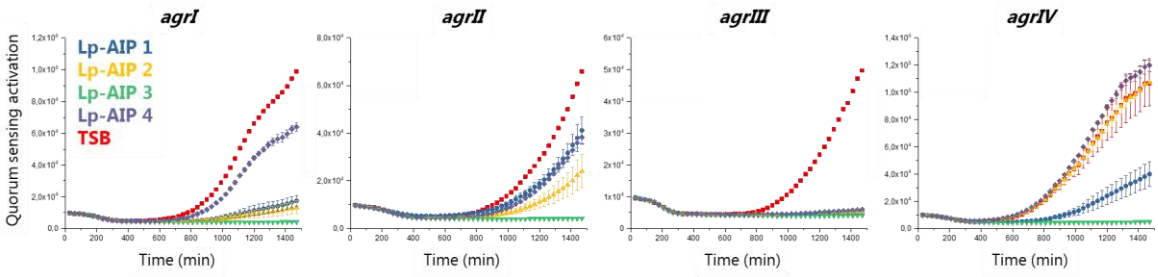


Figure 1: Lactiplantibacilli AIP structure impact its ability to interfere with *S. aureus* quorum sensing system. The activity is assessed by QS-regulated expression of Yellow Fluorescent Protein (YFP) in P3-*yfp* reporter systems in the four-*agr*-allelic groups measured as YFP accumulation overtime.

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Abstract ID: 1214 / Poster Number: 359.17P

Culturable mycobiome of nutrient-depleted and overloaded soils in Serbia: from diversity to designed microbial probiotics

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Co-authors: *Milica Ljaljević Grbić, Ivana Đokić, Katarina Kruščić, Aleksandar Knežević, Tamara Janakiev, Maja Radulović, Milan Gavrilović, Pedja Janaćković, Ivica Dimkić*

Unsustainable agricultural practices, such as intensive tillage, monocropping, and overuse of artificial nutrients, have degraded soil health and fertility. During spring and summer seasons culturable mycobiomes of nutrient-depleted (ND) and overloaded (OL) soils in maize cropping systems in Serbia, were analized via ITS sequencing and classified into ecological guilds: saprobes, plant pathogens, and animal/human pathogens. In both seasons, OL soils showed greater taxonomic diversity, with only 10% species overlap: spring – Penicillium raperi and Talaromyces purpureogenus; summer – Clonostachys rosea, Dichotomopilus funicola, Trichoderma brevicompactum. Ascomycota dominate both soil types, while OL soils were additionally characterized by the presence of Zygomycota and numerous cellulolytic fungi. Within saprobe guild, plant-growth-promoting Ascomycota, such as Aspergillus, Clonostachys, Penicillium, Talaromyces, and Trichoderma, dominated. Plant pathogen guild contains only small number of Fusarium, Pyrenochaetopsis, and Neosetophoma species, with Fusarium solani documented in OL soils in both seasons, and Fusarium longifundum isolated from ND soils in summer. Animal and human pathogens were scarce with only isolated species being entomopathogenic Bauveria bassiana (OL) and Metarhizium robertsii (ND) – well known biocontrol agents. Chrysosporium keratinophilum (OL) and Scedosporium dehoogii (ND) were the only documented human pathogens. It can be concluded that OL soils, as nutrient enriched, are taxonomically more diverse ensuring greater association complexity and making it an excellent source of benefical fungi to be utilized in designed microbial probiotics for reshaping of soil microbiome, aimed at improving soil health in agricultural ecosystems.

Abstract ID: 2839 / Poster Number: 325.17P

Cyanobacteria as a valuable tool in the biocatalytic production of high value compounds

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Cyanobacteria are the largest and most diverse group of photosynthetic prokaryotes, with the ability to grow in extreme habitats and fix atmospheric nitrogen, which makes them major contributors to global biogeochemical cycles [1]. Cyanobacteria, as a rich source of biologically active compounds, have found application in many branches of industry, such as pharmaceutical, cosmetic, and food industries [2,3]. Their extraordinary catalytic abilities have also been observed, but this area is still unexplored. Biocatalysis has emerged as a more sustainable and environmentally friendly alternative to conventional chemical synthesis.

Six cyanobacteria strains were tested towards epoxymethyl dimethyl phosphonate, which is a representative of organophosphorous compounds characterized by stable P-C bond. The results clearly showed that all tested cyanobacteria strains were active to 1 mM epoxymethyl dimethyl phosphonate, which resulted in pure isomer of dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate with a high conversion degree. Complete conversion was observed with *Limnospira* strains after one day of biotransformation and with *Synechococcus bigranulatus* after 3 days of bioconversion.

The most active strains were used in a 1 L bioreactor to explore the possibilities of scaling up the process and also to check the effect of a high substrate concentration on the cyanobacteria cell. Phosphonates are known for their inhibitory activity so increasing the scale of biotransformations is challenging. The results obtained show the ability of the cyanobacteria to convert epoxyphosphonates and the potential to use it in large-scale processes.

Abstract ID: 2100 / Poster Number: 418.16F

CYAnobacteria- based consolidation TECHnology (CYA-TECH): biomineralization on demand

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Co-authors: *Federica Villa, Francesca Cappitelli*

Organic and inorganic carbon fluxes in cyanobacteria lead to efficient capture of CO_2 and take place during photosynthesis and microbially induced carbonate precipitation (MICP). Photosynthetically active microorganisms, capable of producing calcium carbonate ($CaCO_3$), have great potential for the development of environmentally friendly, sustainable biotechnologies. The aim of the project CYAnobacteria- based consolidation TECHnology (CYA-TECH) is taking advantage of the cyanobacterial responses to different physical switches (environmental conditions) to modulate calcite precipitation on heritage stone, while sequestering anthropogenic CO_2. CYA-TECH is designed to maintain cell viability, efficient photosynthetic activity and to induce biomineralization associated with a delivery system innocuous to the monumental and ornamental calcareous stones.

The CYA-TECH system is a great model for in depth understanding of the organic and inorganic carbon fluxes in cyanobacteria, the carbonate mineralization process, the interplay between calcification and photosynthesis, and how this interaction may be influenced by climate. As CYA-TECH will provide a scientific basis for envisioning strategies to manipulate biomineralization on-demand, it will be also critical for future research on ecology and climate change and the design environmentally friendly construction materials.

CYA-TECH is a project funded by the Horizon Marie Skłodowska-Curie Actions (MSCA) Postdoctoral Fellowships Program (2024-2026). The cyano-based consolidation technology, CYA-TECH, will be a novel solution overcoming the drawbacks of the chemical approaches and improving the performance and sustainability of the currently available biotechnologies.

Abstract ID: 2019 / Poster Number: 354.15F

Cyanobacterial Membrane Vesicles: Composition and Roles

Presenting author: Mr. Ilia Tapelzon, Bar-Ilan University, Ramat Gan, Israel

Co-authors: *Eleonora Sendersky, Rakefet Schwarz*

Among bacterial cellular processes, membrane vesicle (MV) production has been widely recognized for its roles in nutrient delivery, communication, and biofilm formation. However, while MVs in heterotrophic bacteria have been extensively studied, cyanobacterial MVs remain largely underexplored. Due to their photosynthetic capabilities, cyanobacteria, such as Synechococcus elongatus, are crucial participants in the global carbon cycle. This research focuses on characterizing the composition and functions of MVs produced by *S. elongatus* PCC 7942 under various physiological conditions, including nutrient starvation and biofilm development.

Experiments using density gradient ultracentrifugation and mass spectrometry reveal that MVs from *S. elongatus* carry a diverse array of proteins linked to photosynthesis, membrane structures, and cytoplasmic functions. This suggests the presence of multiple vesicle types with, possibly, distinct origins. MVs from nitrogen-starved cells included various cytoplasmic proteins; however, GFP – highly expressed under a nitrogen-responsive promoter – was undetectable in MVs. This suggests a selective packaging process for cytoplasmic proteins. Additionally, the proteome of MVs of cells isolated from biofilms comprise a large set of unique proteins compared to MVs from planktonic cells. The role of MVs in biofilm development and fitness will be discussed.

This study aims to expand our understanding of cyanobacterial MVs' physiological roles and their adaptive significance under environmental stress. The findings have potential implications for biotechnology, e.g. in biofuel production, where cyanobacterial MVs could be harnessed to release valuable bioproducts, enhancing sustainability and productivity.

Abstract ID: 2944 / Poster Number: 007.16S

Cyanobacterial production of the key environmental sulfur compound dimethylsulfoniopropionate

Presenting author: Dr. Andrew Curson, University of East Anglia, Norwich, United Kingdom

Co-authors: *Keanu Walsham, Jinyan Wang, Xiaoyu Zhu, David Pearce, Beth Williams, Xiao-Hua Zhang, David Lea-Smith, Jonathan Todd*

Introduction: Dimethylsulfoniopropionate (DMSP) is a hugely influential organosulfur molecule that is produced in very large amounts by marine algae and bacteria, primarily as an osmoprotectant but with other possible roles. Through its conversion to the gas dimethyl sulfide (DMS), it has a critical role in the global sulfur cycle, returning large amounts of sulfur from the oceans to the land. DMS is also a signalling molecule for a range of organisms from bacteria to seabirds, and may affect climate through its role in cloud formation.

Although previous work had shown that cyanobacteria can produce or import DMSP[1,2,3], little was known about the range of organisms that can produce it and nothing was known about the genes or enzymes involved in DMSP production in cyanobacteria.

Objectives: To identify genes and enzymes involved in DMSP biosynthesis in cyanobacteria and use this information to determine which organisms are producing DMSP and how much contribution they make to global DMSP.

Methods: Bioinformatic analysis of cyanobacterial genomes to look for candidate DMSP synthesis genes. Cloning of candidate genes and enzyme assays to determine function. Culturing of cyanobacteria to determine how much DMSP they produce and how production is regulated in response to changing environmental conditions.

Findings: We have identified two distinct cyanobacterial methylthiohydroxybutyrate methyltransferases, termed DsyC and DsyG, that catalyse a key step in DMSP biosynthesis. The *dsyC* gene is found in *Trichodesmium* and the *dsyG* gene in *Zarconia*, and both genes are predominantly found in genera of the Oscillatoriales order of cyanobacteria. By culturing cyanobacteria that have homologues of these genes, as well as other cyanobacteria, it was shown that they produce DMSP and that in some cases DMSP production was regulated by environmental factors such as salinity or nitrogen availability, in common with other types of DMSP-producing organisms.

Conclusion: Identification of novel genes for DMSP biosynthesis in cyanobacteria combined with direct measurement of DMSP production in cultured species can allow us to predict which organisms are likely to be producing it and estimate how much they may produce. The gene sequences can also be used as markers, alongside those genes already identified in eukaryotic algae and heterotrophic bacteria, to assess the abundance of DMSP-producing enzymes and organisms in different environments so that we can better understand the contribution of this important sulfur compound in biogeochemical cycling and marine ecosystems.

Abstract ID: 3174 / Poster Number: 271.15P

Cyanobacterial sigma factor controls biofilm-promoting genes through intra- and intercellular pathways

Presenting author: Dr. Eleonora Sendersky, Bar-Ilan University, Ramat-Gan, Israel

Co-authors: *Shiran Suban, Rakefet Schwarz*

Cyanobacteria frequently constitute integral components of microbial communities known as phototrophic biofilms, which are widespread in various environments and hold significant industrial relevance. Previous studies of the model cyanobacterium *Synechococcus elongatus* PCC 7942 revealed that its planktonic growth habit results from a biofilm-suppression mechanism that depends on an extracellular inhibitor, an observation that opens the door to investigating cyanobacterial intercellular communication.

Here, we demonstrate that the RNA polymerase sigma factor SigF1, is required for this biofilm-suppression mechanism and suggest that *sigF1*-inactivation impairs secretion of the biofilm inhibitor. The *S. elongatus* paralog SigF2, however, is not involved in biofilm regulation. Comprehensive transcriptome analyses identified distinct regulons under the control of each of these sigma factors. Additional data indicate that SigF1 regulates biofilm through its involvement in transcriptional induction of genes that include those for the primary pilus subunit: *sigF1* inactivation both prevents pilus assembly and abrogates secretion of the biofilm inhibitor. Consequently, expression is significantly upregulated for the *ebfG*-operon that encodes matrix components and the genes that encode the corresponding secretion system.

Thus, this study uncovers a basic regulatory component of cyanobacterial intercellular communication, a field that is in its infancy. Elevated expression of biofilm-promoting genes in a *sigF1* mutant supports an additional layer of regulation by SigF1 that operates via an intracellular mechanism.

Abstract ID: 3730 / Poster Number: 236.16P

Cytotoxicity and antibiofilm effect of smart thermosensitive hydrogels containing antibiotic combinations for dental applications

Presenting author: Dr. Cristiane Duque, Universidade Católica Portuguesa, Viseu, Portugal; Universidade Estadual Paulista, Araçatuba, Brazil

Co-authors: *Gabriela Pacheco de Almeida Braga, Lucas Da Silva Ribeiro, Rafaela Laruzo Rabelo, Karina Sampaio Caiaffa, Vanessa Rodrigues dos Santos, Gabriel Pereira Nunes, Georgia Rondó Peres, Anna Carolina Volpi de Mello Moura, Renata Toledo Alves, Emerson Rodrigues Camargo*

Introduction: Conservative therapeutic approaches have been studied aiming to develop an intracanal medication with antimicrobial effect and harmless to remaining pulp cells to be used for the treatment of infected root canals in permanent teeth with incomplete root formation.

Objective: To evaluate the cytotoxicity and antibiofilm effect of a thermosensitive hydrogel containing a triple antibiotic combination for the treatment of immature permanent teeth.

Methods: Antibiotics were tested alone and in double or triple combinations against oral bacteria and *Candida albicans* in planktonic and biofilms conditions. Metronidazole (ME), ciprofloxacin (CI), and fosfomycin (FO) were incorporated into poly(N-vinylcaprolactam) (PNVCL) hydrogels, after physicochemical characterization, and their antibiofilm activity was compared to PNVCL containing chlorhexidine (CHX) or calcium hydroxide (CH) as controls. Cytotoxicity of the hydrogels was assessed on odontoblast-like cells using MTT assays. The data were submitted to ANOVA and Tukey test $p < 0.05$.

Findings: The ME+CI+FO combination demonstrated synergism and higher effect on mono- and dual-species biofilms and on biofilms formed inside root canals, comparable to CHX. PNVCL hydrogels with ME+CI+FO significantly reduced *E. faecalis* biofilms in root canals, showing higher efficacy than PNVCL+CH. Antibiotics and PNVCL, with or without antibiotics, minimally affected cell viability.

Conclusion: Thermosensitive PNVCL hydrogel combined with ME+CI+FO demonstrates potent antibiofilm activity and low cytotoxicity, highlighting its promising potential as an

alternative intracanal medication, particularly for the treatment of immature permanent teeth.

Abstract ID: 464 / Poster Number: 185.15P

Dam-mediated epigenetic control of prophage stability via host-derived integrase in Salmonella Typhimurium

Presenting author: Ms. Jihye Yang, Korea university, Seoul, South Korea

Co-authors: *Yongjun Son, Woojun Park*

Despite being in a golden age of bacterial epigenomics, no systematic examination has addressed the plasticity of the DNA methylome during the temporal progression of environmental transitions. In our in vitro reconstitution of Salmonella infection cycle [the sequential stages (pre-mature-post) of Salmonella-containing vacuole (SCV)], the pathogen exhibits various phenotypic adaptations including morphological changes, membrane instability, and perturbation of prophage dynamics. Upon transition to the SCV condition, reduced host-derived Dam levels simultaneously promote enhanced excision of the cryptic prophage ST64B and its subsequent extracellular leakage. Combined approaches of single-molecule, real-time sequencing (SMRT-seq) and methylated DNA immunoprecipitation sequencing (MeDIP-seq) revealed the regulatory region of intA, which encodes for host-derived putative integrase, is hypomethylated during the SCV phase. Lambda red-based chromosomal deletion and CRISPR interference (CRISPRi)-based transcriptional silencing of dam showed the expression of intA is modulated by the Dam-mediated methylation of its regulatory region. Integration Host Factor (IHF) binding to this region is contingent upon the methylation status, indicating that IHF dissociation from the intA promoter during the SCV phase enables Dam-mediated methylation, establishing a regulatory circuit of prophage stability. Our findings reveal a novel epigenetic switch that coordinates prophage behavior with bacterial stress responses, providing insights into the complex interplay between host-derived regulatory factors and prophage maintenance.

Abstract ID: 848 / Poster Number: 272.16F

De novo assembly and functional annotation of 84 bacterial genomes using Nanopore sequencing technology

Presenting author: Mr. Sokratis Zekkas, Democritus University of Thrace, Alexandroupolis, Greece

Co-authors: *Dimitra Basdani, Athanasios Kylonis, Dimosthenis Tzimotoudis, Tamás Felföldi, Károly Márialigeti, Giannoulis Fakis, Sotiria Boukouvala*

Antimicrobial resistance is a major challenge to public health, compromising the efficacy of available antibiotics and highlighting the urgent need for new natural products. Microorganisms are pivotal to antibiotic biosynthesis, generating a diverse array of secondary metabolites with significant biological activity and therapeutic potential. The discovery of such novel compounds has been enhanced through microbial genomics, enabling the identification of uncharacterized biosynthetic gene clusters. In this study, a taxonomically representative collection of 84 bacterial isolates was sequenced using Nanopore technology. Raw reads generated by the MinION device were basecalled using Guppy, with the SUP model preferred over FAST as it significantly enhances accuracy, with the majority of reads exceeding Q15. After basecalling, quality assessment and read filtering, Flye *de novo* genome assembly was preferred because it more efficiently incorporates diverse genomic regions. Assemblies were then polished using Medaka to generate the final consensus sequences, the majority of which exceeded the 90% gene completeness threshold. Through the Prokka functional annotation process, 81 of 84 genome assemblies were successfully annotated, revealing an average of 7,546 coding, 17 rRNA and 88 tRNA genomic sequences. Potential contaminants were assessed using the ContEst16S tool. The genomic dataset and the complete analysis workflow will be made public for researchers who wish to expand the current knowledge of microbial biosynthetic potential, evolutionary history and biotechnological significance. The research project was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the “2nd Call for HFRI Research Projects to support Faculty Members & Researchers” (Project Number: 3712).

Abstract ID: 3923 / Poster Number: 096.16P

Deciphering Gut Bacterial Metabolism of Diet-Derived Polyphenols

Presenting author: Ms. Arianna Giurleo, McGill University, Montreal, Canada

Co-authors: *Reilly Pidgeon, Bastien Castagner*

The microbial community in the human gut varies in composition among individuals and has been causally linked to many diseases. Polyphenols are natural diet-derived molecules that impact the composition of the gut microbiota by mechanisms that remain elusive. Castalagin, a polyphenol found in the camu-camu berry, has been shown to enhance the effects of immune checkpoint inhibitor (ICI) therapy in cancer. Camu camu extracts are therefore being tested in clinical trials in combination with ICI therapy but also in metabolic diseases. The gut microbiota is capable of metabolizing polyphenols like castalagin to downstream bioactive metabolites known as urolithins, via the production of ellagic acid. There has been significant interest in these metabolites given their proposed beneficial effects on human health such as antioxidant, anticancer and cardioprotective effects. The mechanisms by which castalagin, ellagic acid and urolithins are metabolized remain unknown, including many of the bacterial species involved. We aim to identify the genes, species and enzymes involved in the metabolic conversion of castalagin. Using a multi-omics approach and in vitro metabolism assays, we have identified previously undescribed bacterial metabolizers, and a putative decarboxylase linked to ellagic acid breakdown, as well as oxidoreductases capable of converting downstream urolithins. By identifying the gut bacterial genes and species responsible for the metabolism of polyphenols we can better understand their impact on the gut microbiome and on human health, providing an avenue for a personalized approach to polyphenol supplementation.

Abstract ID: 2238 / Poster Number: 021.16P

Deciphering Host-Microbe Interactions in the Drosophila melanogaster Gut Ecosystem

Presenting author: Dr. Aryan Rahimi-Midani, Max Planck Institute for Infection Biology, BERLIN, Germany

Co-authors: *Igor Iatsenko*

The gut microbiota of Drosophila melanogaster plays a crucial role in the host physiology, yet the viral components, particularly phages, remain largely unexplored. This study aims to investigate the modulation of the Drosophila gut bacterial communities by the phages, focusing on a dominant gut commensal Lactiplantibacillus plantarum and its physiological impact on the host. We collected 950 wild-type fruit flies from nature using standard trapping methods(1). Through culture-dependent techniques and genome sequencing, we analyzed the isolated bacterial species from the Drosophila gut. Consequently, we isolated wild-type L. plantarum from fruit flies collected across various locations in Germany. Our results confirmed that the Drosophila gut is predominantly inhabited by Lactobacillaceae. However, we also identified other bacterial species, such as Leuconostoc mesenteroides. We investigated the dynamics of the bacterial community by examining the stable colonization of L. plantarum strains in the Drosophila gut. Gnotobiotic flies were generated and colonized with defined L. plantarum strains(2) to observe changes in bacterial populations. We characterized specific species capable of proliferating in the gut and forming stable, mutually beneficial associations with the host. Furthermore, we hypothesize that phages have a regulatory effect on the gut microbiota. To explore this, we aim to apply bacteriophages that infect gut colonizers to understand their role in shaping the microbial community. This research highlights the complex interplay between the Drosophila gut microbiota and host physiology, emphasizing the importance of bacterial community dynamics in shaping host responses. Understanding these interactions provides valuable insights into host-microbiota relationships.

Abstract ID: 2208 / Poster Number: 065.16P

Deciphering Metabolic Microbiome-Host Crosstalk in Colorectal Cancer

Presenting author: Ms. Catarina Correia Tavares Bernardino, University of Luxembourg, Esch-Belval, Esch-sur-Alzette, Luxembourg

Recently, it became abundantly clear that microbial metabolites play a role in colorectal cancer (CRC). To date, most studies have investigated the effect of single bacteria on the host. However, since bacteria and their metabolites do not occur in isolation, there is an urgent need to understand metabolic microbiome-host crosstalk in more complex microbial communities.

To study this, we selected a cluster of seven co-occurring CRC-associated bacteria named CROC (CRC onco-cluster), identified through analysis of our own in-house CRC cohort, various publicly-available datasets and an extensive literature search. We will utilize the CROC to illustrate microbial metabolic crosstalk in CRC by studying ecological and metabolic interactions between CROC members in planktonic and biofilm settings. We will also assess the effect of CROC metabolites on primary CRC tumor growth in *in vivo* mouse models. Moreover, we will be involved in creating a spatial map of the tumor microbiome and its secreted metabolites during CRC development, using MetaFISH which combines fluorescence *in situ* hybridization (FISH) with MALDI-mass spectrometry imagining. Preliminary FISH experiments with the CROC member *Fusobacterium nucleatum* show that upon oral gavage, *F. nucleatum* can be found forming biofilms, infiltrating mouse CRC adenomas, and even invading colonic crypts.

By studying the effect of a microbial consortium in CRC development, instead of single bacterial species, we will be able to understand the role of microbial metabolites in CRC in a setup that is more relevant to disease settings. Ultimately, these findings can contribute to new personalized microbiome-based therapy options for CRC patients.

Abstract ID: 3065 / Poster Number: 342.15S

Deciphering organic matter turnover across the euphotic tropical North Atlantic using microbial community transcriptomics

Presenting author: Benjamin Pontiller, PhD, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

Co-authors: *Wanja Feodor Böhme, Helmke Hepach, Daniel Lundin, Anja Engel*

The ocean microbiome hosts an exceptionally high degree of taxonomic and functional diversity, playing a pivotal role in driving global element cycles. While environmental conditions and biogeochemical factors are known to determine microbial community composition and function over spatiotemporal scales, deciphering the molecular mechanisms that regulate organic matter turnover across biogeographic gradients remains a significant challenge. To address this, we investigated microbial processes and activities driving marine dissolved organic matter (DOM) turnover during cruise SO287 within the framework of the CONNECT project. The study spanned a 4347 km longitudinal transect in the euphotic tropical North Atlantic (23°W to 65.5°W, between 17°N and 30.5°N). Using 16S rRNA gene sequencing and metatranscriptomics, we analyzed the taxonomy and function of the free-living microbiome (0.22–3.0 µm fraction) alongside detailed assessments of DOM composition, quality, and quantity, including dissolved hydrolysable carbohydrates and amino acids. Our findings reveal substantial changes in chemical and microbiological parameters along the longitudinal transect from east to west. Phytoplankton biomass, primary production, and bacterial production decreased in near-surface waters, alongside dissolved organic carbon (DOC) concentrations. Interestingly, the contribution of carbohydrates to the DOC pool increased from the Canary upwelling system to the oligotrophic North Atlantic Gyre, while amino acid contributions remained stable, indicating an accumulation of semi-labile sugars westward. Microbiome composition and function differed significantly between surface and deep chlorophyll-*a* layers, with strong longitudinal patterns in near-surface samples driven by nutrient availability and DOM composition. Further analyses of DOM-relevant genes (e.g., KEGG orthologs for nitrogen and phosphorus metabolism as well as carbohydrate-active enzymes) revealed that sugars and amino acids (mol%) explained approximately 20% of the transcriptional variance. This was supported by changes in the relative proportion of transcribed genes along the transect, linked to nitrogen (e.g., ammonium utilization in the nutrient-rich eastern region) and phosphorus metabolism (e.g., phosphate and phosphonate utilization in the oligotrophic west), coinciding with

declining concentrations of both inorganic and organic nutrients. Our results highlight the pivotal role of nutrient availability and physical conditions in shaping the structure and function of the ocean microbiome, thereby, the fate of organic matter components such as semi-labile sugars with important consequences for carbon sequestration in the tropical North Atlantic.

Abstract ID: 3072 / Poster Number: 166.17P

Deciphering the role of the ancillary residues in maintaining the activity and substrate specificity of OXA-232 beta-lactamase

Presenting author: Tejavath Ajith, PhD, Indian Institute of Technology Kharagpur, Kharagpur, India

Co-authors: *Anindya Sundar Ghosh*

Background: OXA-232, a variant showing five amino acid substitutions from OXA-181, demonstrates unique hydrolytic properties that warrant detailed investigation. While active site residues of these enzymes are well-characterized, the contribution of non-catalytic residues to enzyme function remains poorly understood.

Objectives: To elucidate the role of the residues S118, V120, L158, and D159 in structure and functioning of $bla_{OXA-232}$.

Methods: $bla_{OXA-232}$ gene was cloned in pBAD-18cm vector for *in-vivo* studies. Alanine substitutions were created using site directed mutagenesis. Minimum inhibitory concentrations were determined to analyze the effect of the mutation on the susceptibility pattern of *E .coli* against beta-lactams. Further, the truncated genes devoid of nucleotides coding for signal peptide proteins were cloned in pET28a and expressed in *E. coli* BL21. Proteins were purified using affinity chromatography and characterized through circular dichroism spectroscopy and kinetic analyses.

Results: *E. coli* cell expressing $bla_{OXA-232}$ exhibited enhanced resistance towards tested bet-lactams. S118A substitution in OXA-232 resulted in >62-fold increased susceptibility to penicillins and cephalosporins, while V120A substitution led to >16-fold increased susceptibility to cephalosporins. Noteworthily, D159A substitution led to complete loss of resistance while L159A substitution led to a drastic increase in the susceptibility of the cells towards all the antibiotics. Circular dichroism spectroscopy revealed substantial alterations in protein secondary structure in the purified OXA-232_S118A, while L158A substition affected the thermal stability. Overall, it can be inferred that S118, V120, L158, and D159 are crucial for the activity and stability of OXA-232.

Conclusion: OXA-232 beta-lactamase mutations of S118, V120, L158, D159 revealed critical roles in enzyme function, structure and antibiotic resistance.

Abstract ID: 4153

Decision-making tools for optimizing sanitation practices

Presenting author: Valentina Trinetta, Kansas State University, Manhattan, KS, United States of America

Effective control of *Listeria monocytogenes* biofilms in food processing environments requires more than routine sanitation—it demands strategic decision-making grounded in scientific evidence. This presentation introduces decision-support tools designed to optimize cleaning and sanitizing practices by integrating microbial risk assessment, environmental monitoring data, and surface hygiene evaluation. Dr. Trinetta will explore how to tailor sanitation protocols to specific facility conditions, accounting for surface type, organic load, and microbial ecology. Emphasis will be placed on proper sequencing of cleaning and disinfection steps, selection of active compounds, and validation of sanitation efficacy. Real-world case studies will illustrate how these tools have improved food safety outcomes by reducing biofilm-associated contamination risks.

Abstract ID: 3972 / Poster Number: 097.16P

Decoding bacterial interactions of an in vitro reconstructed Human Gut Microbiota the presence of a prebiotic Maitake extract

Presenting author: Dr. Margherita Finazzi, University of Milano-Bicocca, Milan, Italy

Co-authors: *Vanessa Piva, Patrizia Di Gennaro*

Introduction: Microorganisms inhabit body surfaces, particularly the gastrointestinal tract, forming the human gut microbiota (HGM), which performs essential functions through interactions among bacterial populations.

Objective: This work evaluates the interaction network in a reconstructed in vitro HGM during the fermentation of a β-glucan-enriched prebiotic Maitake extract.

Methods: The HGM in vitro model included a minimal core of *Bacteroides cellulosilyticus*, *Clostridium symbiosum*, *Flavonifractor plautii*, and *Escherichia coli*, along with probiotics (*Lactobacillus* and *Bifidobacterium* sp.). Growth experiments with Maitake and β-glucans were conducted in a batch bioreactor under anaerobic conditions, and bacterial strain modulation was monitored using RT-qPCR. Bacterial metabolites were extracted and analyzed via GC-MS.

Findings: In the HGM model, *L. plantarum*, *B. lactis*, and *E. coli* are the main strains that grow with Maitake extract. *L. acidophilus* is inhibited by *B. lactis*, which more efficiently utilizes the carbon source. Moreover, the probiotics inhibit the growth of *C. symbiosum* and *F. plautii*. In addition, *B. cellulosilyticus* competes with *B. lactis* and *E. coli*. *E. coli* does not grow in the presence of β-glucans, indicating it cannot use this molecule as a carbon source. *L. plantarum* and *B. lactis* are the dominant strains. *B. lactis* and *B. cellulosilyticus* still appear to have a competitive relationship. The results indicate that *E. coli* growth on Maitake extract isn't linked to β-glucans, which are primarily utilized by *B. lactis* and *B. cellulosilyticus*.

Conclusion: These results helped us understand the complex dynamics in the model, highlighting the competitive and cooperative interactions between the strains.

Abstract ID: 3802 / Poster Number: 024.16P

Decoding Gut Microbial Imbalances in Ulcerative Colitis: Insights from Metagenomics and Culturomics in Lebanon

Presenting author: Mr. Hassan Abbas, American University of Beirut, Beirut, Lebanon

Co-authors: *Aya Hanna, Fayez Yassine, Jinane Samaha, Alia El Bush, Melhem Bilen*

Ulcerative colitis (UC) is a chronic inflammatory bowel disease marked by relapsing inflammation in the colon and rectum, driven by genetic, environmental, and immune factors. Dysbiosis, or gut microbial imbalance, is increasingly recognized as central to UC pathogenesis. This study investigates the gut microbiota of UC patients in Lebanon using metagenomics and culturomics to delineate microbial profiles and identify potential therapeutic targets.

Culturomics, which isolates previously undetectable microorganisms, complements metagenomics by broadening the spectrum of microbial diversity captured. By comparing UC patients and healthy controls, this research identifies microbial taxa linked to disease severity and symptoms, contextualized within Lebanon's unique dietary and genetic landscape. Comparative analyses with global datasets further elucidate region-specific patterns.

The findings highlight microbial taxa associated with UC in Lebanon and underscore culturomics' role in advancing microbiome research. This work lays the foundation for personalized bacteriotherapy and microbiota-based interventions, contributing to the understanding of UC pathogenesis and the development of innovative, region-specific treatments.

Abstract ID: 2390

Decoding Marine Fungi: mapping their diversity, distribution and ecological insight

Presenting author: Raquel Ledo Doval, PhD, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

Marine fungi play essential roles in ocean ecosystems, influencing microbial diversity, ecological interactions, and molecular processes. Despite recent advances in documenting their phylogeny, physiology, and ultrastructure, our understanding of the diversity, distribution, and ecological roles of marine fungi remains incomplete. The ocean offers a largely underexplored reservoir of fungal communities with potential ecological and biotechnological significance.

The primary aim of this study was to assess the diversity and distribution of marine-derived fungal species collected during the NICO expedition (2018). Particular focus was placed on their distribution across various substrates and environmental conditions. Additionally, substrate utilization preferences and growth patterns were analysed to better understand their ecological niches.

Biological samples were collected from multiple marine stations across the North Sea, Caribbean waters and Atlantic Ocean, targeting diverse water column depths, latitudes and collection substrates. Fungi were isolated using selective culturing techniques and identified through molecular sequencing and phylogenetic analyses. Growth profiling was employed to evaluate substrate preferences and growth behaviours of the identified species.

From 240 fungal isolates recovered and identified, most were classified to the species level, representing a wide range of fungal taxa and expanding the known diversity of marine fungi. Growth profiling revealed ecological versatility, with environmental conditions such as salinity and temperature significantly influencing growth patterns. The discovery of previously undocumented fungal species contributed to a deeper understanding of fungal biogeography (Figure 1).

This study advances the knowledge of marine fungi, emphasizing their diversity, distribution, and ecological roles. Mapping fungal species to substrates and environmental conditions provides insights into their contributions to marine ecosystem functioning and their potential for biotechnological applications. These findings pave the way for deeper investigations into the roles of marine fungi within the vast and complex oceanic microbial communities.



Figure 1. Infographic of the related study illustrating the workflow, from sample collection during the NICO expedition to fungal identification and substrate mapping, along with key findings on marine fungal diversity and distribution.

Abstract ID: 1223 / Poster Number: 134.15P

Decoding the Regulatory and Spatial Dynamics of m-Xylene Biodegradation by Pseudomonas putida mt-2

Presenting author: Mr. Francisco Moreno-Garzón, CNB-CSIC, National Center for Biotechnolgy, Madrid, Spain

Co-authors: *Peter Graumann, Víctor De Lorenzo, Tomás Aparicio*

The biodegradation of m-xylene by *Pseudomonas putida* mt-2 is governed by a regulatory system encoded in the TOL plasmid. This metabolic pathway comprises two operons: the upper pathway, which converts m-xylene into toluates, and the lower pathway, which transforms these intermediates into central metabolic compounds.

The objective of this study was to investigate the roles of the xylS promoters PS1 (inducible) and PS2 (constitutive) in regulating metabolic efficiency and redox balance, as well as to characterize the cellular localization and dynamics of key enzymes involved in the pathway.

We employed growth assays to evaluate the impact of promoter deletions on cellular behavior and used high-resolution STED microscopy and single-molecule tracking (SMT) to visualize enzyme localization and dynamics. Enzymes were fluorescently tagged with mNeonGreen (XylC, upper pathway) and mKate2 (XylE, lower pathway).

Our findings revealed that the absence of PS2 caused delayed growth and increased ROS activity, emphasizing its role in directing intermediates efficiently into the lower pathway and maintaining redox balance. In contrast, PS1 deletion had no significant impact. STED microscopy suggested substrate channeling during specific steps, while SMT demonstrated that most enzymes diffuse freely, indicating a balance between localized and dynamic activity.

These results provide new insights into the regulatory and spatial mechanisms underlying m-xylene biodegradation, enhancing our understanding of *P. putida* mt-2's metabolic efficiency and robustness.

Abstract ID: 1058 / Poster Number: 081.17P

Decoding the synergistic interaction between spectinomycin and tetracycline in Escherichia coli

Presenting author: Dione L. Sánchez-Hevia, PhD, Uppsala University, Uppsala, Sweden

Co-authors: *Eunate Valdaliso-Diez, Sanne Westhoff, Dan Andersson*

Antibiotic combination therapies are important and effective strategies for treating infections with antibiotic resistant pathogens. When antibiotics are present in combinations their efficacy can increase (synergy) or decrease (antagonism) from the predicted combinatory effect (additivity). Previous work has shown that the spectinomycin and tetracycline combination is synergistic in *Escherichia coli* MG1665; however, the mechanism underpinning this interaction remains unknown.

We aimed to study the mechanism underlying this antibiotic interaction by selecting and characterizing mutants that had lost the synergistic interaction. To this end, *E. coli* was exposed to inhibitory concentrations of spectinomycin and tetracycline. The resulting spontaneous mutants were analysed, excluding those had become resistant.

The fitness cost and minimal inhibitory concentration values were compared with those from the parental strain; and the spectinomycin-tetracycline interaction outcome was determined using CombiANT. Genome sequencing revealed diverse DNA modifications, including, SNPs, amplifications and deletions. Measurements of intracellular antibiotic concentrations showed, overall, no-changes in the spectinomycin intracellular concentration, but a reduction for tetracycline. Even though a low convergence among the DNA modifications, several mutants seemed to share an increase in the AcrAB-TolC multidrug efflux pump expression. Preliminary data shows an increase in expression of the *acrA* and *acrB* genes in the selected mutants, and a change when spectinomycin is present.

Our data shows that selection and analysis of mutants with altered interactions is a useful approach to study the molecular mechanisms of antibiotic interactions.

Abstract ID: 2667 / Poster Number: 136.17P

DEDGRAM – Targeted detection of antimicrobial resistance genes

Presenting author: Helena Torrell Galceran, PhD, Eurecat, Reus, Spain

Co-authors: *Mirko Paparella, Lucia Tadeo Orellana, Adrià Cereto Massague, Lorena Garcia Alcalde, Nuria Canela Canela*

Antimicrobial resistance (AMR) poses a significant global health threat, complicating the treatment of infections in both clinical and environmental contexts. Traditional detection methods, such as bacterial cultures and second-generation sequencing, are slow, expensive, and require specialized infrastructure, delaying critical decision-making in life-threatening conditions like sepsis. To overcome these challenges, a rapid and cost-effective system has been developed to detect AMR genes within 8-12 hours using Oxford Nanopore Technologies' third-generation sequencing platform.

This system includes a capture panel designed to target all 8,278 known AMR genes, with periodic updates to incorporate newly discovered resistance markers. The methodology emphasizes simplicity, enabling its implementation in molecular biology laboratories, including hospital diagnostic facilities. To enhance usability, a cloud-based bioinformatics platform has been developed, providing automated data analysis and generating intuitive visual reports that can be easily interpreted without specialized expertise.

Preliminary evaluations demonstrate that targeted gene capture combined with nanopore sequencing can effectively identify a wide range of AMR genes in diverse biological samples. The system's rapid processing time, coupled with its affordability and scalability, makes it particularly suitable for clinical diagnostics, environmental surveillance, and real-time outbreak management.

This innovative approach not only accelerates diagnostic timelines but also reduces operational costs, offering a practical solution to a critical global challenge. By equipping clinicians and researchers with timely, actionable insights, the system has the potential to transform AMR management, contributing to improved patient outcomes and public health strategies.

Abstract ID: 2572 / Poster Number: 076.15P

Degradation and recovery of microbial ecosystem functions in rivers exposed to multiple stressors

Presenting author: Dr. Verena Brauer, University of Duisburg-Essen, Essen, Germany

Microorganisms in sediments are key players for the functioning of rivers, yet, we do not understand how they respond to and recover from multiple anthropogenic stressors, which are increasingly threatening river ecosystems worldwide. We therefore investigated the combined effects of the stressors temperature increase and salinization on two microbe-driven ecosystem processes, which are degradation of dissolved organic carbon (DOC) and degradation of bacterial dead biomass (necromass). Using controlled lab experiments with isolated sediment microbial communities, we found that temperature increase had clear positive and salinization clear negative effects on the degradation of DOC and necromass, even at rather low stressor levels. Using outdoor mesocosm experiments where sediment microbial communities are integrated into the riverine food web, we found that the same low stressor levels had no effect on DOC degradation but on necromass degradation, whereas stressor release increased DOC degradation but not necromass degradation. The same differential effects occurred for microbial community composition. Our results indicate that the effects of multiple stressor increase and release on sediment microbial processes are controlled by the concentration and the bioavailability of the organic carbon source, and that changes in energy availability may mask multiple stressor effects.

Abstract ID: 4046 / Poster Number: 384.16P

Degradation of plasma pre-treated LDPE films by a fungal consortium

Presenting author: Ms. Marie Joie, University of Mons (UMons), Mons, Belgium

Co-authors: *Alice DELACUVELLERIE, Ruddy Wattiez*

Plastic production reaches around 400Mt nowadays. A majority become wastes and mostly end-up in the ocean or landfill, causing environmental and health issues because of micro- and nano-plastics formation and release of toxic molecules.

Taking inspiration from Nature could help us find a way to solve the plastic pollution problem. Fungi degrade complex natural polymers, and studies found that they can also degrade plastics, but at a rate far from sufficient.

The main objective is to combine different strategies that have been shown to improve degradation: (i) the use of three fungi, that have demonstrated an ability to degrade plastics, in a consortium, (ii) plastic treatment to improve adhesion and growth of fungi on the surface, and (iii) addition of black liquor to increase biodegradation rates. The end-goal of this thesis is to use the fungal biomass obtained to make a mycelium-based material.

We treated LDPE films with plasma at different powers and analysed the roughness, wettability and chemical composition of the surface. We are currently studying the growth and enzymatic production and activities of our fungi (in mono and tri-culture) in minimal media with glucose or diluted black liquor, with and without LDPE films. We found that plasma treatment as a real impact on the measured parameters. Fungal growth also varies depending on the cultures conditions that we tried.

We still need to go further in the analysis and experiments, to measure biodegradation rates in these conditions.

Abstract ID: 4149

Demystifying bacterial dark matter: functional genomics of uncharacterized genes in *Enterococcus faecalis*

Presenting author: Julia Willett, University of Minnesota Medical School, Minneapolis, Minnesota, United States of America

Uncharacterized genes, or genes with no known function, are prevalent in bacteria. Nearly 100 million unique bacterial proteins are annotated as hypothetical or uncharacterized, and many more have predicted functions that do not align with the known physiology of a given bacterium. These poorly characterized genes are often differentially expressed or important for fitness in biologically relevant conditions, including during biofilm growth, after antibiotic treatment, *in vivo*, and in the environment. Discovering organism-specific functions for uncharacterized loci is a major challenge for modern microbiology. *Enterococcus faecalis* is a Gram-positive bacterial pathobiont that is a commensal in the gastrointestinal tract and also a prevalent nosocomial pathogen. Like many bacteria, ~25-30% of the core genome of *E. faecalis* comprises uncharacterized genes. We sought to identify uncharacterized genes important for *E. faecalis* establishment and persistence in the gastrointestinal tract using transposon sequencing (TnSeq) in a germ-free mouse model. To guide prioritization of candidate genes, we compared our *in vivo* TnSeq results to previous *in vitro* screens for *E. faecalis* genes involved in biofilm formation or resistance to cholic acid. This approach identified ~90 uncharacterized genes important for both *in vivo* and *in vitro* growth. We generated predicted functions for more than half of these genes using bioinformatics. We demonstrated that a cluster of poorly characterized genes is important for tolerance to compounds that target the cell envelope, including antibiotics and detergents. Together, this work highlights the importance of studying uncharacterized genes and demonstrates how genetic screens can be used to prioritize functional characterization of understudied loci in bacteria.

Abstract ID: 1097 / Poster Number: 048.16P

Depletion of host DNA enables deeper sequencing of microbial gene content from tissue samples

Presenting author: Mr. Dominic O'Neil, QIAGEN GmbH, Hilden, Germany

Co-authors: *Janina Leinberger, Nicola Scholle, Markus Sprenger-Haussels*

Tissue microbiomes are an important aspect of animal biology and recent evidence highlights their impact on host health, behavior and development. Recent studies have demonstrated that animal tissue microbiomes undergo significant changes during disturbances in the host environment and that presence or absence of certain taxa correlates with factors such as temperature or anthropogenic antibiotic discharge.

Major challenges in tissue microbiome studies arise from low residual bacterial biomass and a therefore high host-to-bacterial cell ratio. To overcome these particular issues, we have developed a specialized workflow designed to ensure reliable host DNA removal from mucosal, soft or fibrous tissue, while yielding high quality bacterial DNA in quantities that allow for subsequent metagenome sequencing.

To ensure conservation of the microbial community structure and to rule out discrimination for individual taxa during our extraction procedure, we sequenced mock microbial community standards prepared with the new protocol, as well as commercially avalaible kits. Multiple sample types, including swabs, intestine, and internal organs were analyzed by whole genome sequencing after extraction with the new protocol, revealing much deeper diversity of microbial genes, including antibiotic resistance genes, which were not visible when these samples were extracted without host depletion. The new protocol allows additional insights to be derived from tissue samples, potentially illuminating host-microbial interactions.

Abstract ID: 3323 / Poster Number: 138.16S

Design of a phage cocktail targeting extensively drug-resistant Acinetobacter baumannii

Presenting author: Ms. Caterina Ferretti, University of Pisa, Pisa, Italy

Co-authors: *Andrea Bonacorsi, Novella Cesta, Laura Rindi, Mariagrazia Di Luca*

Introduction: The spread of difficult-to-treat infections, for example those caused by *Acinetobacter baumannii*, represents a challenge in medical practice[1]. Phage therapy (PT) is an encouraging strategy to treat infections caused by multidrug-resistant bacteria[2]. However, bacteria can develop resistance to bacteriophages, and this can be overcome by combining phages with antibiotics or by designing phage cocktails[2].

Objectives: This work aimed to select and combine therapeutic bacteriophages targeting extensively drug-resistant (XDR) *A. baumannii*, isolated from a patient with prosthetic joint infection (PJI), candidate for PT. The resulting phage cocktail should prevent the selection of phage-resistant *A. baumannii* clones.

Method: *A. baumannii* bacteriophages from a proprietary collection were tested by spot assay against the clinical isolate to identify the matching of phages. Phage L1 was selected. Sequencing and annotation of L1 genome was performed, ensuring the therapeutic application of the active phage. Efficiency of plating and growth kinetics were evaluated for phage efficacy determination. Synograms and interaction plot were created to investigate synergism between L1 and cefiderocol against the strain in planktonic form. Synergy testing was performed to evaluate the effect of phage-cefiderocol combination against *A. baumannii* biofilm. Phage-bacteria co-incubation was used to select six phage-resistant mutants, which resistance was confirmed by plaque assay and growth curves. Finally, a new bacteriophage (ABKATE) targeting phage-resistant clones was isolated and tested in combination with phage L1, by monitoring the growth kinetic of *A. baumannii* up to 48h.

Findings: Out of seven phages stored in our bank screened, only L1 phage was active against the strain. Genotypic analysis of L1 revealed its suitability for PT, since it is a lytic phage, lacking toxins, antibiotic resistance and virulence determinants. Growth kinetics showed that phages (MOI 0.1, 1 and 100) controlled bacterial growth for 48 hours, without totally inhibit the bacterial load (Figure 1A). Synograms were created to investigate synergism between L1 and cefiderocol, following by interaction plots, which showed additive effect at 24 and 48 hours. L1 (10^5 PFU/mL) in combination with cefiderocol at

sub-biofilm-bactericidal concentration (0.5 µg/mL) showed a synergistic bactericidal effect against the biofilm, which was not achieved with higher phage titres, maybe due to lysis from without. Finally, a new phage, named ABKATE, active against the six phage-resistant mutants was isolated. Growth kinetic of the clinical isolate treated with the phage cocktail, that include L1 and ABKATE, revealed a completely inhibition of the bacterial growth for 48 hours (Figure 1B). Molecular characterisation of phage-resistant bacteria and phage ABKATE is ongoing.

Conclusion: Results obtained show the possibility of using phage L1 in therapy in combination with cefiderocol to treat XDR *A. baumannii* PJI. Additionally, the design of a phage cocktail composed of an additional phage active against phage-resistant mutants, might improve the efficacy of phage treatment.

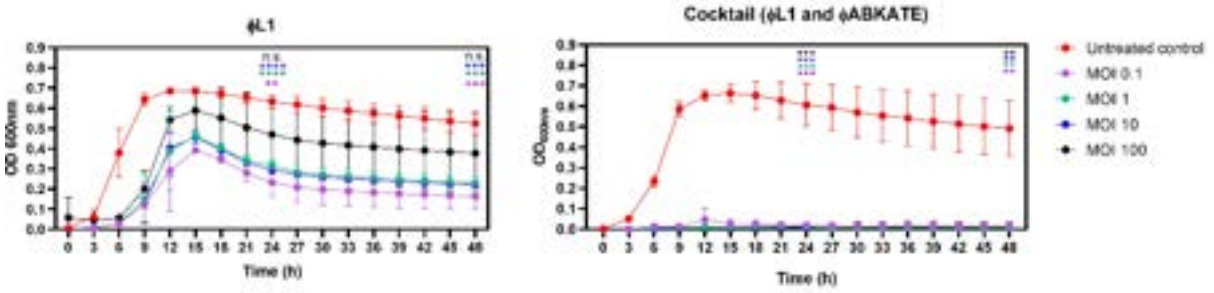


Figure 1: Growth kinetics of a XDR A. baumannii treated with*: (A) phage L1. (B) phage cocktail composed of L1 and ABKATE phages. Multiple t-test between untreated control and each condition. p-value>0.05 n.s., p-value≤0.01 **; p-value≤0.001 ***; p-value≤0.0001 ****.*

Abstract ID: 3688 / Poster Number: 292.16F

Designing a synthetic alkaliphile to increase bioreactor efficiency for more affordable biomanufacturing

Presenting author: Dr. Tiffany Halvorsen, Lawrence Livermore National Laboratory, Livermore, United States

Co-authors: *Christina Kang-Yun*

Introduction: Biomanufacturing promises to displace single use petrochemical-derived products with bio-derived alternatives to create a closed loop economy. However, high operational costs still prevent the widespread adoption of biomanufacturing. To improve bioreactor cost efficiency, costly steam sterilization, expensive feedstocks, and loss of productivity due to fluctuating culture conditions (e.g., pH, temperature) must be addressed. All these issues could be solved by replacing mesophilic biomanufacturing strains with organisms evolved for growth in extreme conditions, which would enable the use of modified aseptic media (e.g., adjusted to pH 10) and/or free waste streams. However, using extremophiles for synthetic biology is hindered by a lack of foundational knowledge, metabolic models, and genetic engineering tools. Because these tools exist for bioproduction strains already in use, we aim to develop a general approach for converting mesophiles into synthetic extremophiles.

Objectives: We provide a framework for converting an established bioproduction strain into a synthetic extremophile for use in naturally aseptic bioproduction processes. Importantly, our approach requires a moderate extremophile and a closely related mesophile to 1) link genotype to phenotype using a pan-genome, 2) identify recent evolutionary adaptations more likely to be encoded in portable genes, and 3) ensure genetic compatibility.

Methods: We chose a moderate alkaliphile isolated from seawater (*Pseudomonas alcaliphila*) and a close relative with bioproduction capability isolated from soil (*Pseudomonas putida*) to establish a comparative genomics pipeline for identification of portable pH tolerance genes. We built a pangenome of 80 pH-tolerant species related to *P. alcaliphila* and 60 *P. putida* isolates, used OrthoFinder [1] to identify orthologous genes unique to each clade, then identified Orthogroups capable of determining clade membership (neutrophile or alkaliphile) with a decision tree. Finally, using RB-TnSeq [2], we verify our computational approach by experimentally determining which genes are required for growth at pH 9.5 and pH 10 in *P. alcaliphila*.

Findings: Using our *Pseudomonas* pangenome (Fig. 1a), we identified 773 Orthogroups exclusive to the alkaliphiles (Fig. 1b). By classifying Orthogroups by enrichment and gene copy number, we discovered a unique compatible solute transporter present in every member of the alkaliphile group (Fig. 1c). Furthermore, by training a decision tree on Orthogroup presence/absence, we identify 70 Orthogroups capable of determining clade membership with high confidence. Finally, using RB-TnSeq, we find consistencies between our computationally determined tolerance genes and those necessary for survival of *P. alcaliphila* in alkaline growth media.

Conclusion: This study provides methodology for identifying tolerance genes in moderate extremophiles that can be transported into mesophiles for more robust, cost competitive biomanufacturing. Our results demonstrate the feasibility of designing polyextremophiles for broad use within the bioeconomy, for bioremediation and, eventually, to support human spaceflight and interplanetary terraformation.

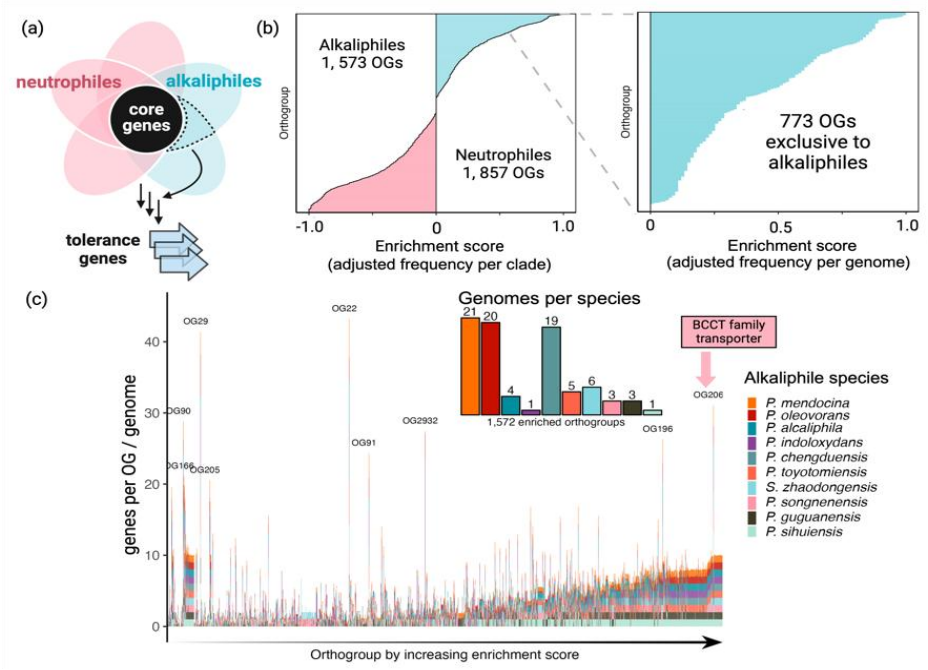


Figure 1: OrthoFinder analysis of pangenome. ***(a) Schematic depicting pH tolerance gene identification strategy. (b) OrthoFinder analysis of Pseuomonads, filtered for P<0.05 and plotted by enrichment per clade (left) and per genome (right). (c) Gene copy number for Orthogroups enriched by any amount in the alkaliphile clade.***

Abstract ID: 1493 / Poster Number: 424.15P

Designing E. coli bacteria to co-deliver ART toxins into PD-L1+ tumor cells in mice

Presenting author: Eva Pico Sánchez, PhD, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain

Co-authors: *Alejandro Asensio-Calavia, Luis Ángel Fernández Herrero*

Bacteria have been investigated as therapeutic agents against cancer because their natural capacity to colonize solid tumors and their intrinsic immunogenicity. Based on this and through synthetic biology, we engineer non-pathogenic *Escherichia coli* with functional genetic modules of interest for anti-cancer therapies. For that, we developed Synthetic Injector *E. coli* strains (SIEC) carrying a delivery module based on Type III Secretion System (T3SS) from enteropathogenic *E. coli*; a payload module for injection of the catalytic domains of ADP-ribosyl transferase (ART) toxins; a three-repressor regulatory module to control T3SS and cargo expression; an adhesion module based on synthetic adhesins (SAs) enabling to program the adhesion of bacteria toward an antigen expressed by the surface of tumor cells. SIEC strains binding to human Epidermal Growth Factor Receptor (EGFR) and carrying one ART-toxin domain (PE25 or TccC3hvr) were shown to trigger cell death of HCT116 human tumor cells expressing EGFR and to reduce the growth of HCT116 tumors in immunodeficient mice.

We aim to develop SIEC strains targeting mouse tumors that could be tested in immunocompetent mice. For that, we have developed SIEC strains with SAs binding Programmed Death-Ligand 1 (PD-L1) antigen expressed in different types of mouse tumors: bladder, melanoma, colon. In addition, we have developed a bicistronic cargo module coinjecting the two ART-toxin domains by the same bacterium. We will show recent *in vitro* data demonstrating on various mouse tumor cells the efficacy of these novel SIEC strains for adhesion to PD-L1+ cells and coinjection of PE25 and TccC3hvr ART-toxins.

Abstract ID: 65 / Poster Number: 297.16S

Detection and Molecular Characterization of Aflatoxin and Ochratoxin Produce Aspergillus Species in Capsicum Spices in Saudi Arabia

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Capsicum is a widely used spice known for enhancing food flavor and color, but it is particularly vulnerable to mycotoxin contamination, which poses serious health risks. Mycotoxins, natural toxins produced by certain fungi, especially Aspergillus species, can contaminate food products. This study aimed to assess the prevalence of Aspergillus species producing aflatoxins and ochratoxins in powdered Capsicum sourced from markets in Riyadh, Saudi Arabia.

A total of 130 samples were randomly collected from various vendors in Riyadh. The detection of fungal contamination was carried out using the ISO 21527-2:2008 method, designed for samples with low water activity. Both morphological and molecular techniques were employed to identify toxigenic Aspergillus species accurately.

The results revealed alarming contamination levels: approximately 84.61% of samples exceeded the acceptable limit set by the Gulf Cooperation Council (GCC) Standardization Organization (GSO1016:2015), indicating a fungal count above 102 CFU/g. Contaminated samples were primarily labeled as originating from Saudi Arabia (23.07%), with others imported from Turkey (17.69%), India (16.15%), and Syria (14.6%). Only 15.38% of the samples fell within the acceptable limit (≤102 CFU/g).

Interestingly, sample packaging did not significantly reduce fungal contamination, as fungal counts were similar between packaged (78.57%) and unpackaged (91.66%) samples. This finding raises concerns about the effectiveness of packaging in preventing mycotoxin contamination.

In terms of fungal diversity, Aspergillus species made up about 51.13% of the identified isolates, with Aspergillus flavus (38.8%) and Aspergillus niger (37.7%) being the most prevalent. Other species like Aspergillus ochraceous and Aspergillus fumigatus were present but in lower percentages.

Molecular characterization of Aspergillus isolates was conducted to identify key aflatoxin biosynthesis genes (apa-2, omtA, nor1, ver1) and ochratoxin gene (pks1). Of the 90 isolates tested, 13 (14.4%) were positive for all aflatoxin-targeted genes. The presence of these genes was distributed as follows: nor1 in 62.2% of isolates, omtA in 35.5%, apa-2 in 32.2%, and ver1 in 25.5%. The ochratoxin gene pks1 was found in only two A. niger isolates.

Mycotoxin analysis using liquid chromatography-mass spectrometry (LC-MS) was performed on 34.6% of samples. Aflatoxin B1 (AFB1) was detected in 28.8% of samples, with concentrations ranging from 0.2 to 13.8 µg/kg. Ochratoxin A (OTA) was found in 35.5% of samples, with levels ranging from 6.87 to 59.00 µg/kg.

These findings highlight the urgent need for stringent regulations regarding the storage, shipping, and packaging of spices in Saudi Arabia to mitigate the prevalence of toxigenic fungi and mycotoxins. This study is the first of its kind in Saudi Arabia focusing on Aspergillus contamination in Capsicum products, providing critical insights for future food safety initiatives. As global demand for Capsicum increases, ensuring its safety and quality is essential for protecting consumer health.

Abstract ID: 3231 / Poster Number: 230.17F

Detection of antibiotic resistance genes in the Onyar River, Spain, using metagenomics

Presenting author: Dr. Jose Luis Balcazar, Catalan Institute for Water Research (ICRA-CERCA), Girona, Spain

The increasing prevalence of antibiotic resistance presents a major challenge to antibiotic therapy, compromising the effectiveness of prescribed antibiotics and posing a critical public health threat. Thus, efforts to mitigate its impact should include interventions at the interface of human, animal, and environmental health. Importantly, aquatic ecosystems are not exempt from the effects of antibiotic resistance. Urban rivers, in particular, are constantly exposed to several chemical and biological contaminants, including antibiotic residues, heavy metals, and antibiotic-resistant bacteria from anthropogenic sources, creating ideal conditions for the exchange of genetic material among bacterial communities. This study aimed, therefore, to investigate the presence and abundance of antibiotic resistance genes (ARGs) in the genomic DNA of sediment samples collected from the Onyar River, both upstream and downstream of its passage through the urban area of Girona (northeast Spain). A combination of metagenomics and bioinformatics techniques was used to conduct the study. The results showed a higher abundance of ARGs in samples collected downstream, particularly genes conferring resistance to aminoglycosides, beta-lactams, macrolides, polypeptides, sulfonamides, and tetracyclines. In conclusion, our study highlights the impact of urban areas on the abundance of ARGs in the environment.

Abstract ID: 3738 / Poster Number: 068.15P

Detection of Yersinia enterocolitica ystB gene in milk samples from dairy cows and tank milk in Poland

Presenting author: Ms. Zuzanna Polak, University of Warmia and Mazury, Olsztyn, Poland

Co-authors: *Agata Bancerz-Kisiel, Karolina Lipczyńska-Ilczuk*

Introduction: Yersiniosis can be a serious problem both in human and animals. People can be infected due to consumption of products contaminated with *Yersinia enterocolitica*, a zoonotic bacteria.

Objective: The aim of the study was to determine if *Y. enterocolitica* is present in milk samples collected directly from cows and from tank.

Method: Samples were obtained from one herd in Poland, that has around 200 lactating dairy cows (145 samples including 142 samples from individual animals and 3 samples from tank milk from 3 different days). Samples were transported to laboratory directly after collection. Bacterial isolation and biochemical identification of isolated colonies were performed. Colonies that showed characteristic biochemical features for *Yersinia*, were transferred to American agar solid media in tubes and stored in a fridge. Then, DNA isolation and multiplex PCR (looking for ail, ystA and ystB genes) were performed and DNA bands in agarose gel were evaluated. Obtained amplicons were purified using clean-up kit and sequenced to confirm specificity.

Findings: Multiplex PCR revealed that 3 samples included ystB gene. None of this samples came from tank milk. Sequencing confirmed that 2 amplicons have a sequence typical for ystB.

Conclusion: This data allows to assume that some cows can be carriers of *Y.enterocolitica,* what did not result in occurrence of this bacteria in tank milk. Further examination will evaluate greater number of cattle, which can show if isolation of this bacteria from milk from udder will not affect presence of this bacteria on a tank level in different herds.

Abstract ID: 1662 / Poster Number: 428.15P

Determining the ability of E. coli strains to attach to specialised materials

Presenting author: Ms. Emily Horsburgh, University of Glasgow, Glasgow, United Kingdom

Co-authors: *Andrew Roe*

Introduction: Many Gram-negative bacteria contain the ability to produce pili, the most extensively studied pili are the Type 1 pili – more commonly referred to as Type 1 fimbriae (T1F). They are the most prevalent type of pili in *E. coli* UTI infections; however, most *E. coli* strains encode the T1F. Expression is determined by the orientation of an invertible 314bp region (*fimS*) which contains the promoter of the *fim* operon. Therefore, the expression of T1F is phase-variable; permanent expression of T1F has been shown when a mutation occurs in the left invertible repeat (LIR). The use of T1F is well studied to allow them to adhere and invade cells in *E. coli* infections, however, their role in adhering to specialised materials is not.

Objective: To determine the ability of different *E. coli* strains to adhere to these materials when expressing Type 1 fimbriae. To determine if permanent expression allows for higher adherence to specialised materials.

Method: Using fluorescent microscopy, mesocopy, and single-cell force spectroscopy to determine the adhesion level and force.

Findings: We identify that different strains expressing the same *fim* operon have varying levels of adhesion. Although, this adherence is seen in inconsistent levels across the surface of the material. Permanent expression of T1F allows for higher levels of adhesion to the material in certain strains.

Conclusion: These results could lead to the use of T1F being utilised to develop microbial systems for whole-cell living attachment to specialised industrial materials.

Abstract ID: 522 / Poster Number: 174.16P

Determining the antibacterial efficacy of spices against Escherichia coli and Staphylococcus aureus

Presenting author: Ms. Anu Ghimire, ST.XAVIER'S COLLEGE, KATHMANDU, Nepal

The rising concerns of foodborne pathogens and the increasing concern of antibiotic resistance has generated an urgent need for alternative natural antibacterial agents. Spices, renowned for their rich phytochemical profiles, have been utilized in traditional medicine for their antimicrobial and antioxidant properties.

This study aims to evaluate the antibacterial efficacy of ethanolic extracts from 10 common spices against foodborne pathogens Escherichia coli and Staphylococcus aureus and standard cultures Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 by agar well diffusion method. The spice extracts were obtained through Soxhlet extraction. Phytochemical analysis was performed in order to evaluate the presence of secondary metabolites. The antioxidant properties of the spice extracts were assessed using DPPH radical scavenging activity and IC50 values were determined to gauge their antioxidant potential.

The results demonstrated that Trigonella foenum exhibited highest yield of 17.6 %, while Piper nigrum yielded the lowest at 0.49 %. Phytochemical analysis revealed the presence of flavonoid, alkaloid, tannins and terpenoids. Syzygium aromaticum exhibited the most potential antibacterial activity, with maximum zone of inhibition (ZOI) of 15mm and 22mm against E.coli and S.aureus respectively at the concentration of 300mg/ml. Against the ATCC strains, Syzygium aromaticum demonstrated the highest ZOI, measuring 19mm against E.coli ATCC 25922 and 21mm against S.aureus ATCC 25923. In terms of antioxidant activity, Zanthoxylum armatum displayed the highest DPPH radical scavenging activity at 91.5 %, while Piper nigrum exhibited the lowest at 71.7 %. Zingiber officinale exhibiting an IC50 value of 6.59µg/ml, showed strong antioxidant potential.

The findings of this study suggests antibacterial and antioxidant activities of common spice extracts, suggesting their potential application in food preservation and therapeutic treatment against bacterial infections and oxidative stress –related disease.

Keywords: antibacterial efficacy, ethanolic extracts, phytochemicals, therapeutic treatment, spices, antioxidant

Abstract ID: 2740 / Poster Number: 344.17P

Development and Application of Lactobacillus with Anti-photoaging and Anti-melanogenesis Properties

Presenting author: Ms. Hsiu-Ping Yu, Soochow University, Taipei, Taiwan

With population aging and lifestyle changes, skin health has become a key concern, especially issues like photoaging and melanogenesis caused by UV exposure, which negatively impact both aesthetics and skin health. Lactobacillus, a widely used probiotic, has garnered attention for its potential in regulating the immune system and promoting skin health. However, the mechanisms and applications of Lactobacillus in anti-photoaging and melanogenesis inhibition remain underexplored.

This study aims to develop Lactobacillus strains with both anti-photoaging and anti-melanogenesis properties. Through strain screening, supernatants and precipitates were tested for tyrosinase and elastase inhibition to identify promising candidates. Cellular models using HaCaT keratinocytes, melanocytes, and fibroblasts were employed to assess effects on skin health and safety, validated via fluorescence imaging (PI/SYTO9). Genomic analysis (PCR sequencing) and principal component analysis identified key active components in strain supernatants.

Further evaluations using CCK-8, MTT assays, flow cytometry, and Western blotting revealed the impact on cell viability and expression of skin-related proteins such as collagen degradation markers and tyrosinase. Notably, Leuconostoc mesenteroides demonstrated significant tyrosinase inhibition, providing valuable insights for future applications of this strain in functional health products. This study offers a foundation for innovative solutions to skin health challenges and promotes Lactobacillus applications in cosmetics and medicine.

Abstract ID: 3784 / Poster Number: 244.16P

Development and Characterization of Biocomposites Functionalized with Lactobacilli for Topical Application

Presenting author: Vesna Lazic, University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

Co-authors: *Tanja Krunic, Andrea Osmokrovic*

There is an urgent need for innovative strategies and therapeutic options to treat various types of wounds, including diabetic wounds. One promising approach is the development of bioactive materials for topical applications. Hyperglycaemia is known to delay wound healing, while reducing local glucose levels can improve the wound microenvironment and promote healing. The aim of this study was to develop biocomposites by immobilizing four *Lactobacilli* strains (*L. acidophilus, L. plantarum, L. Gasseri,* and *L. rhamnosus*) onto activated charcoal fabric (ACF) and characterizing them after freeze-drying. The viability and growth kinetics of the immobilized strains were evaluated over 24 hours in simulated wound fluid under normo- and hyperglycaemic conditions. Additionally, the biocomposites were further analysed using field-emission scanning electron microscopy and characterized for their textural properties. Results demonstrated that ACF maintained approximately 85% bacterial viability after freeze-drying, confirming its suitability as a carrier. Also, simulated wound fluid under hyperglycaemic conditions supported the growth of the selected *Lactobacilli*. Further experiments will assess the ability of these biocomposites to inhibit the growth of the most relevant pathogens affecting chronic wounds in the conditions mimicking hyperglycaemic wound environment. In conclusion, these biocomposites show significant potential as bioactive wound dressings, providing a novel approach to diabetic wound management.

Abstract ID: 94 / Poster Number: 246.17P

Development and characterization of microencapsulated Pichia kluyveri CCMA 0615 with probiotic properties and its application in fermented beverages

Presenting author: Assoc. Prof. Rosane Schwan, Federal University of Lavras, Lavras, Brazil

Co-authors: *Iara Ferreira, Dirceu Melo, Pâmela Martins, Marciane Magnani, Disney Dias*

This study aimed to develop innovative microencapsulated formulations of Pichia kluyveri CCMA 0615 and Saccharomyces cerevisiae CCMA 0732, strains known for their probiotic potential. The yeasts (8 log CFU/mL) were microencapsulated using the spray drying technique with whey powder (WP at 15%, 20%, and 30%) and sodium alginate (ALG at 1%). Scanning electron microscopy was initially performed to confirm microcapsule integrity and provide ultrastructural characterization. The viability of the cells was evaluated immediately after drying and over two months of storage at 4 ºC and 25 ºC. Selected formulations were subsequently applied in functional beverage fermentation, and their viability and survival under simulated gastrointestinal tract (GIT) conditions were assessed. The viability of spray-dried microencapsulated yeasts was shown to be dependent on both the strain and encapsulating matrix, ranging from 84% to 99%. Notably, P. kluyveri microcapsules required refrigeration for optimal stability. Microencapsulated yeasts maintained consistent fermentative profiles during functional beverage fermentation, demonstrating carbohydrate consumption, lactic acid production (0.30–1.10 g/L), alcohol production (0.2–1.61 g/L), and high viability during storage. Furthermore, microencapsulation of P. kluyveri with 15% WP and 1% ALG maintained high viability under GIT conditions, with survival rates exceeding 84% when isolated and over 94% when incorporated into a food matrix. This study demonstrates that innovative microencapsulation techniques can enhance the viability of probiotic yeasts, thereby improving their biotechnological application and facilitating the efficient delivery of probiotics to the host.

Abstract ID: 3096 / Poster Number: 375.16P

Development and Validation of Safety Assessment Guidelines for Heat-Killed Bacteria and Metabolites Derived from Agro-Bio Microbiome Resources

Presenting author: Ms. Jiseon Hwang, Department of Agricultural Biotechnology, Seoul National University, Seoul, South Korea; Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, South Korea ; Center for Food and Bioconvergence, Seoul National University, Seoul, South Korea

Co-authors: *Sungwoo Choi, Jihye Yang, Liha Kim, Yunjeong Kim, Ju-hoon Lee*

Introduction: Agro-bio microbiome technology is crucial for developing safe, functional food ingredients to improve global health. Heat-killed bacteria and metabolites offer significant potential in promoting health and ensuring food safety. However, standardized global safety guidelines are lacking. This study establishes and validates safety guidelines, focusing on affordable and accessible solutions for public health.

Objective: To develop harmonized safety guidelines by analyzing global standards and validate their applicability through experimental evaluation of commercially available heat-killed bacteria and metabolites.

Method: Safety criteria were collected from Korea, Europe, the United States, Canada, Brazil, India, Japan, Thailand, and Australia/New Zealand. Parameters for heat-killed bacteria and metabolites were compared to form a draft guideline. This was validated using commercially available, safe products through experimental evaluations.

Findings: Analysis identified key safety parameters across countries, forming the draft guideline. Validation showed the guidelines effectively assessed the safety of commercial products, aligning with international standards.

Conclusion: This study delivers validated safety guidelines for heat-killed bacteria and metabolites, supporting their use as safe food ingredients. By providing affordable solutions, the guidelines address public health challenges and advance sustainable food biotechnology.

Abstract ID: 1296 / Poster Number: 350.16P

Development of a flow-controlled biofilm model to facilitate the testing of debridement treatments of dental implant surfaces

Presenting author: Mr. Jan-Ole Reese, Universitetet i Oslo, Oslo, Norway

Co-authors: *Ingrid Maria Castro Lund, Athanasios Saragliadis, Ståle Petter Lyngstadaas, Håvard Jostein Haugen, Dirk Linke*

Introduction: Microbial biofilms are the primary cause of peri-implantitis infections, affecting 40% of all dental implants[1]. An increasing prevalence of these infections necessitates the development of new therapeutic approaches while avoiding reliance on antibiotics. The extensive testing and validation process for these comes at the expense of financial and ethical constraints when depending on traditional animal studies. A solution to this dilemma is presented by sophisticated *in vitro* biofilm models, adapting the specific environmental conditions in the oral cavity.

Objectives: We aimed to develop a dynamic multi-species biofilm model specifically suited for evaluating debridement treatment efficacy against oral biofilms. While flow is crucial in modern *in vitro* systems, the influence of varying flow and shear forces on biofilm development remains largely unknown. The intent of our model was to simulate key aspects of biomaterial-derived oral infections while allowing for the identification of optimal flow conditions to enable debridement testing.

Methods: Biofilms are grown on implant surfaces within a modified Robbins device flow chamber coupled to a bioreactor for anaerobic bacterial cultivation. Two independent cycles enable separate adjustment of biofilm flow exposure and bioreactor residence time. Biofilms are analysed through biomass quantification, scanning electron microscopy, as well as species-specific bacterial quantification by qPCR and fluorescence *in situ* hybridization.

Findings and Conclusion: Varying flow exposure induces alterations in biofilm composition and quantity. Reduced flow results in higher biomass but weaker attachment. Higher flow exposures were found to be more suitable for facilitating debridement testing by allowing differentiation between treatments and controls.

Abstract ID: 3821 / Poster Number: 361.17P

Development of a High-Sensitivity Electrochemical Aptasensor for Pseudomonas aeruginosa Detection

Presenting author: Somayeh Maghsoomi, Center for Water and Environmental Sensors, Department for Integrated Sensor Systems, University for Continuing Education Krems; Institute of Specific Prophylaxis and Tropical medicine, Medical University of Vienna, Vienna, Austria

Co-authors: *Mai Lan Pham, Martin Brandl, Julia Walochnik*

Introduction: *Pseudomonas aeruginosa* is a high-priority pathogen identified by the WHO due to its clinical significance and environmental prevalence. It is a gram-negative bacterium responsible for acute and chronic infections, especially in immunocompromised individuals. Rapid and accurate detection of this pathogen is critical for environmental monitoring and infection control.

Objective: To develop a highly sensitive and selective electrochemical aptasensor employing the F23 aptamer for *P. aeruginosa* detection, emphasizing its application as a point-of-care diagnostic tool.

Method: An advanced aptasensor was constructed using gold screen-printed electrodes functionalized with the F23 aptamer as the biorecognition probe. Methylene blue was incorporated as a redox indicator to enhance signal sensitivity. Electrochemical characterization was performed using cyclic voltammetry and square wave voltammetry to assess the aptasensor's performance.

Findings: The aptasensor demonstrated a detection limit of 8 CFU/ml and exhibited high specificity for *P. aeruginosa* compared to other bacterial species. Methylene blue significantly amplified the sensor's signal response, improving detection sensitivity. The aptasensor effectively detected *P. aeruginosa* in tap water samples, showcasing its real-world applicability.

Conclusion: The developed aptasensor offers a reliable and efficient solution for rapid detection of *P. aeruginosa*, highlighting its potential for use in environmental monitoring and clinical diagnostics.

Abstract ID: 2424 / Poster Number: 279.16F

Development of a High-throughput Method Harnessing CRISPR-Cas Selection for the Mutagenesis and Delivery of Genetic Cargo to Phage Genomes

Presenting author: Ms. Natalie Kyte, University of Otago, Dunedin, New Zealand; Genetics Otago, Dunedin, New Zealand

Co-authors: *Leah Smith, Peter Fineran*

Bacteriophages, or phages, are viruses that infect bacteria. With an estimated 10^{31} phages on Earth, their vast number and diversity represent a significant portion of biological "dark matter" that remains poorly understood. We aim to unravel the complexities of phage genomes and develop tools to facilitate this understanding. We have created a method to generate random gene disruptions in lytic phage genomes, including a New Zealand isolated nucleus forming jumbo phage. This method employs CRISPR-Cas and anti-CRISPRs for selection and is adaptable to a range of phages and hosts requiring minimal knowledge of the target phage. Our method enables the exploration of roles of non-essential phage genes and the delivery of genetic cargo into phage genomes. Our approach will fast-track our understanding of phage gene function and the optimisation of phages for multiple applications, including their use in the battle against antibiotic-resistant bacterial infections.

Abstract ID: 2367 / Poster Number: 253.16P

Development of a non-infectious assay for rapid screening of antiviral entry/fusion inhibitors

Presenting author: Dr. Lorenzo Lupi, University of Padova, Padova, Italy

Co-authors: *Adriana Vitiello, Chiara Sandro, Eric A. Legenzov, Arianna Calistri, Maria Cristina Parolin, Mohammad M. Sajadi, Joseph P.Y. Kao, Alfredo Garzino Demo*

The increased risk of viral spillover due to globalization and global warming poses a significant risk to human health, as exemplified by the SARS-CoV-2 pandemic and the increasing frequency of H5N1 influenza virus emergence. Preventive and therapeutic approaches are urgently needed against old and new viruses. Viral entry is, in theory, a desirable target to halt viral spread and disease in their early stages. To identify and develop viral entry inhibitors, a high-throughput screening system is needed. Ideally, a screening system should combine ease of use with high reproducibility, without the need for higher biosafety containment facilities and trained personnel. To meet this need, we developed a fusion assay, optimized for SARS-CoV-2. This assay comprises two cell lines: one stably expresses hACE2 and the GFP11 motif, while the other stably expresses the SARS-CoV-2 Spike protein and the GFP1-10 domain. When co-cultured, the cells fuse, allowing the split GFP to reconstitute to generate a fluorescent signal. Within hours after mixing, the two cell types fused, as demonstrated by fluorescence microscopy and flow cytometry. The assay was optimized in a 96-well plate format, and the fluorescence signal was measured by a plate reader representing a convenient platform for rapidly evaluating multiple drugs candidates. The assay was validated by testing three neutralizing antibodies targeting SARS-CoV-2 Spike: the results mirrored data obtained in pseudotype assays. Our novel split GFP fusion assay is suitable for screening candidate antiviral compounds, including antibodies, that inhibit SARS-CoV-2 spike-mediated membrane fusion. More importantly, our assay offers high reproducibility and ease of use, in a BSL-2 laboratory. The assay is suitable for high-throughput screening in a convenient 96-well format, providing useful dose-effect data. We envision adapting the assay to various emerging viruses, thus allowing identification of new antiviral molecules for use as a rapid response to novel pandemic events.

Abstract ID: 3531 / Poster Number: 445.16P

Development of a Personalized Web Platform for Biochemistry Students at the University of León

Presenting author: Dr. Ana Ibáñez, Universidad de León. Área de Bioquímica y Biología Molecular, León, Spain

Co-authors: *Elías R. Olivera, Carlos Barreiro*

In response to the growing need for **innovative educational tools in higher education**, we have developed a **customized web platform** tailored to the academic and practical needs of undergraduate students in Proteomics and Protein Engineering at the University of León. This platform combines two essential functionalities to enhance both **theoretical understanding** and **practical skills**.

The first feature integrates **virtual laboratory modules** designed to support students in mastering the experimental techniques and molecular concepts central to their coursework. These modules simulate real-life lab scenarios, offering an immersive learning environment where students can explore and practice protocols, analyze data, and reinforce practical knowledge without the constraints of physical lab resources.

The second component of the platform is a **private web application** dedicated to fostering student interaction and self-assessment. This space allows students to **share questions** and clarify doubts with their peers in a collaborative setting. Additionally, it provides access to a question bank for **practicing exam scenarios**. This feature aims to reinforce theoretical concepts and help students strategically prepare for assessments by focusing their efforts on key topics. Additionally, the platform includes tools for instructors to monitor student usage and identify aspects of the course that may require further clarification.

This personalized platform is designed to improve **student engagement**, support **active learning**, and enhance academic performance by **bridging the gap between theoretical knowledge and practical application**. The implementation of this web-based tool marks a significant step forward in adapting educational methodologies to the digital era while promoting collaborative and self-directed learning.

Abstract ID: 3266 / Poster Number: 215.16P

Development of a rapid diagnostic lateral flow strip coupled with recombinase polymerase amplification (RPA) for detecting clinical isolates of Streptococcus suis

Presenting author: Dr. Mantana Jamklang, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

Co-authors: *Ekkasit Kanklang, Nanthanat Pansuksan, Wissarut Srisakvarangkool*

Streptococcus suis is a zoonotic bacterium that represents a significant threat to both public health and the pig farming industry. In Thailand, human infections are prevalent, predominantly affecting males, and typically present as sepsis or meningitis, with hearing loss being a common complication. Diagnosing *S. suis* is challenging due to the absence of simple biochemical tests. While MALDI-TOF MS offers rapid and reliable identification, it is expensive and accessible only in large hospitals. Other methods, such as PCR or 16S rDNA sequencing, are either costly or time-consuming. Consequently, this study aims to develop a faster, simpler, and more cost-effective detection method for *S. suis* using a lateral flow strip coupled with recombinase polymerase amplification (LF-RPA). Two sets of primers, labelled with FITC and Biotin, were designed for the RPA. The assay's detection limit was assessed using serial dilutions of *S. suis* suspension, and specificity was tested against other streptococcal pathogens. The detection time and temperature were optimized for highest performance. Our results show that the RPA assay detected *S. suis* DNA in 20 minutes, with 100% specificity. The system performed well across a broad temperature range, with optimal results at 40°C, and could detect bacterial cells as few as 10^6 cells. The RPA assay will be coupled with lateral flow DNA dip-strip chromatography for visual detection. In conclusion, our LF-RPA system for detecting *S. suis* is a sensitive, rapid, and accurate method, making it ideal for preliminary clinical screening, particularly in blood samples.

Abstract ID: 1794 / Poster Number: 303.17P

Development of an efficient method for identifying and typing genetically closely related microorganisms

Presenting author: Mr. Satoshi Shimotsu, Asahi Breweries, Ltd., Moriya, Japan

Co-authors: *Masayuki Omote, Kazumaru Iijima*

Bacterial species identification and strain typing technologies are effective methods for sources tracking of microbial contamination. However, in many cases, it is difficult to identify genetically related microorganisms using DNA sequencers or MALDI-TOF MS, and further analysis is required, which takes time and effort. Therefore, we aimed to establish a method for efficiently identifying and strain typing bacteria using the recently developed DNA-GSTK method.

Genomic DNA was extracted from the Bacillus cereus group strains and beer spoilage lactic acid bacteria strains cultured on agar medium. Then, after performing random amplified polymorphic DNA (RAPD) method, DNA sequence data in FAST5 format was obtained for each sample using the MinION nanopore sequencer, and approximately 1000 reads of data were obtained per sample. We then performed genomic analysis on the acquired data using GSTK (Genome Search Tool Kit) and genome databases.

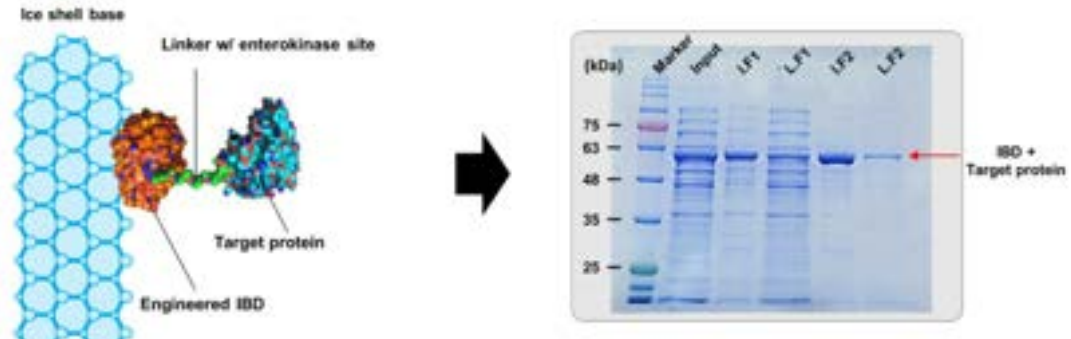
As a result, the species names identified by the GSTK method were the same whether RAPD products or genomic DNA were used. In addition, when the genomic DNA of each type strain of the B. cereus group was subjected to the GSTK method, it was correctly identified. Therefore, it is suggested that efficient investigation of the source of microbial contamination can be achieved by performing species identification with the GSTK method after performing strain typing with the RAPD method. The combined use of the DNA-GSTK method and the RAPD method is considered to be one of the effective means of quality control in the food industry.

Abstract ID: 975 / Poster Number: 416.17P

Development of an Ice Affinity Purification System for Recombinant Proteins Using Engineered Ice-Binding Domains

Presenting author: Prof. Hackwon Do, Korea Polar Research Institute, Incheon, South Korea

Protein purification is essential for isolating specific proteins from complex mixtures for therapeutic and industrial applications. We developed a novel purification method using the ice-affinity properties of ice-binding proteins (IBPs), named the Ice Affinity Purification (IAP) system. Three new DUF3494 ice-binding domains (IBDs) (*Co*IBD1, *Co*IBD2, *Co*IBD3) were identified in *Candidatus Cryosericum odellii* SMC5. These IBDs exhibited hyperthermal hysteresis, ice recrystallization inhibition, and binding to multiple ice planes, suggesting they can bind to ice with maximum density per unit area. Furthermore, we generated the engendered IBD (e*Co*IBD) by analyzing high-resolution X-ray structures and sequences. The e*Co*IBD exhibited increased thermal and chemical stability without losing ice-binding characteristics. An IAP system with the e*Co*IBD-coded plasmid was subsequently established, and its purification efficiency was estimated using GFP. The IAP system successfully purified e*Co*IBD-GFP fusion proteins, with 87% purity observed after two rounds. This yielded approximately 29% of the initial protein from the crude extract. Moreover, additives such as dithiothreitol, PMSF, and glycerol did not affect the purification efficiency of the IAP system. This system is cost-effective, scalable for large-scale purification, and environmentally friendly. Moreover, it has potential applications in the bioremediation of heavy metal pollution and isolation of rare earth elements.



Ice Affinity Purification (IAP) system

Figure: Schematic representation of the Ice Affinity Purification (IAP) system. The IAP system utilizes engineered ice-binding domains (eCoIBD1) to facilitate the selective purification of recombinant proteins.

Abstract ID: 1215 / Poster Number: 342.16P

Development of DNA aptamers as therapeutic tools against Pseudomonas aeruginosa

Presenting author: Dr. Federico Bosetto, University of Cambridge, Cambridge, United Kingdom

Co-authors: *Tongyuan Wei, Maria Zacharopoulou, Ioanna Mela*

Antibiotic resistance is posing a serious threat to human health. Therefore, innovative therapeutic tools are needed to efficiently treat resistant bacteria. Aptamers are small synthetic oligonucleotides which show high specificity and affinity for a designated target[2,3]. They evolve from random DNA oligonucleotide pools by an in vitro selection process called Systemic Evolution of Ligands by EXponential enrichment (SELEX)[4]. Aptamers have multiple applications in the biotechnological field and can be considered as biological drugs to target resistant microorganisms. In this work we selected DNA aptamers to specifically target the hemophore of the haem assimilation system (HasA) from *Pseudomonas aeruginosa*. Starting from a DNA library with a random region of 40 nucleotides, we used SELEX to select DNA aptamers with HasA as target. Twelve rounds of SELEX were performed, and the selection was monitored by qPCR and melting curve analysis. From round 1 to 12, the main fluorescence signal peak at 70°C, typical of the library, gradually shifted to 84°C. Selected putative aptamers were sequenced by Next Generation Sequencing and, the most abundant and relevant candidates are currently being tested for their binding affinity and specificity. At the same time, our group is also working on the aptamer selection towards the receptor of the haem assimilation system (HasR). Since iron is an essential micronutrient for *P. aeruginosa*, as for many bacteria. Targeting and potentially blocking elements of the bacterium's iron acquisition mechanisms, such as HasR and HasA[1], can put the bacterium in significant metabolic disadvantage.

Abstract ID: 2256 / Poster Number: 309.17P

Development of Escherhichia coli to guide stem cell differentiation

Presenting author: Dr. David Mark, University of Glasgow, Glasgow, United Kingdom

Co-authors: *Massimo Vassalli, Andrew Roe*

Introduction: The PRISM-LT consortium seeks to develop an engineered living material platform where naïve stem cells are 3D bioprinted alongside "helper" microorganisms, which produce genetically encoded growth factors to guide differentiation. The challenges facing this are two fold: control of the growth of microorganisms so that they don't outgrow and outcompete the stem cells, and controlled growth factor expression. Minicells, anucleoid cells produced by bacteria whose cell division is disrupted, present a potential solution to these problems, as they are incapable of growth

Objective: Engineer a minicell producing bacterial strain that can sense mammalian metabolites and secrete growth factors in turn

Methods: *E. coli* MG1655 was engineered using λ-red recombineering to delete *minCD* – facilitating minicell production – and knock-in mBeRFP under a constitutive promoter. Minicells were separated by centrifugation. Purity of these preparations was assessed by microscopy. Detection of recombinant growth factors was performed by spot-blotting and Western blotting. A library of GFP-transcriptional reporters was screened against stem cell conditioned media to identify candidates for control of growth factor expression.

Findings: We found that the minicells produced by our strains accumulate mBeRFP to levels detectably by microscopy. The promoter library screen identified candidate promoters, specific to adipogenic and osteogenic lineages

Conclusion: This work highlights the challenges facing the development of cross-kingdon engineered living materials, and how known biology can be exploited to solve those problems. Minicells, owing through their inability to grow and ability to carry genetic cargo, offer an exciting avenue for development of these.

Abstract ID: 3766 / Poster Number: 256.16P

Development of fluorogenic antibiotics to study antimicrobial permeability, activity, and resistance

Presenting author: Prof. Ming Hammond, University of Utah, Salt Lake City, United States

Co-authors: *Sepehr Sebghati*

Introduction: Some antimicrobial compounds are active against Gram positive bacteria but show poor or no activity in Gram negative bacteria due to differences in membrane permeability and cytoplasmic accumulation. Furthermore, while there are PCR-based methods to identify resistant genotypes, for some bacterial pathogens there is not a perfect correlation between genotype and sensitive/resistant phenotypes due to unknown or novel mechanisms of antimicrobial resistance.

Objective: To develop novel fluorogenic antibiotics to study antimicrobial permeability, activity, and resistance.

Methods: We synthesized a tagged version of the clinically prescribed antibiotic ciprofloxacin that fluoresces under two conditions: either when bound to a specific RNA aptamer or when bound to its enzyme target. By expressing this RNA aptamer in bacterial cells, for example, it is possible to assess how well an antibiotic permeates the membrane and accumulates in the cytoplasm. Furthermore, cells without the aptamer will show fluorescent labelling of the enzyme target by the fluorogenic antibiotic. The cellular fluorescence profiles of *B. subtilis* as a model Gram positive and *E. coli* as a model Gram negative were analyzed by flow cytometry and fluorescence microscopy. MIC values were determined by broth dilution method and IC50 values were determined by *in vitro* enzymatic assays.

Findings: We showed that it was possible to 'watch' real-time cytoplasmic accumulation of different antibiotics in both *E. coli* and *B. subtilis*. Marked differences were observed for drug permeability between these Gram positive and Gram negative species, and our results were consistent with prior quantitation by mass spectrometry. We even could observe the effect of an efflux pump on antibiotic accumulation.

In addition, we showed that fluorogenic ciprofloxacin co-localized with its enzyme targets, DNA gyrase and topoisomerase IV, in live cells. The level of fluorescence signal is highest at the exponential growth phase, which should have highest replication activity. These tagged antibiotics retain antimicrobial activity, and most excitingly, show a difference in fluorescence between sensitive and resistant strains.

Conclusion: Together these results present the concept of fluorogenic antibiotics, as exemplified by fluorogenic ciprofloxacin, as novel chemical probes to monitor two critical steps for a drug to have antimicrobial activity: cell permeability and inhibition to the enzyme target. Ongoing work seeks to advance these fluorogenic probes to study and potentially diagnose antimicrobial resistance.

Abstract ID: 1177 / Poster Number: 356.17P

Development of Functional Cosmetic Materials Using Sargassum horneri Exosomes Based on Upcycling

Presenting author: Mr. Sijung Kim, Jeonbuk National University, Jeonju, South Korea

Co-authors: *Jiho Min, Yang-Hoon Kim*

Introduction: Sargassum horneri, a brown algae from the Sargassaceae family, accumulates along Jeju Island's coast, disrupting ecosystems and creating disposal challenges. Despite these issues, S. horneri contains bioactive compounds, offering opportunities for sustainable applications. By upcycling S. horneri into valuable materials, this study aligns with global efforts to reduce waste and promote circular economies. Additionally, exosomes derived from yeast separated from beer lees were used as a comparative model to evaluate the properties of S. horneri-derived exosomes.

Objective: This study aims to extract exosomes from S. horneri and evaluate their potential as functional cosmetic materials. The research specifically focuses on their ability to promote skin health through three mechanisms: whitening, wound healing, and antioxidant activity. By repurposing this invasive species, the study addresses environmental concerns while creating high-value products.

Method: Exosomes were isolated from both S. horneri and yeast derived from beer lees and subjected to various cellular assays. Wound healing efficacy was evaluated in keratinocytes, antioxidant effects were assessed in fibroblasts, and whitening potential was analyzed in melanocytes. These experiments provided insights into the mechanisms of exosome activity and their functional roles in cosmetic applications.

Findings: S. horneri-derived exosomes demonstrated multifunctional properties, including wound healing, antioxidant activity, and pigmentation reduction. Compared to yeast-derived exosomes, S. horneri exosomes exhibited unique and potent bioactivity.

Conclusion: These findings highlight the potential of S. horneri as a sustainable and innovative source for cosmetic ingredients, offering a solution to ecological challenges while enabling the development of high-value, multifunctional products.

Abstract ID: 3235 / Poster Number: 360.16P

Development of innovative and sustainable miso using Portuguese legumes: the impact of fermentation in the protein legume digestibility

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Miso is a traditional fermented food made with soybean, koji (*Aspergillus oryzae* inoculum) and salt. Koji and indigenous microorganisms – yeast and lactic acid bacteria (LAB) – play a crucial role in the fermentation, producing enzymes capable of hydrolyse proteins, polysaccharides and lipids, enhancing miso nutritional properties and providing a unique taste – *umami flavour*.

This work aimed to develop innovative and sustainable miso using traditional Portuguese legumes instead of soybean and specific yeast and LAB.

Chickpea, lupin, and cowpea miso were produced with 12% salt and fermented for 18 months. The total protein, peptide and free amino acid content were analysed before and after the fermentation.

After 18 months, the protein content remained stable; only cowpea miso showed a slight decrease (64.97 to 53.82 mg/g fresh biomass). A significant increase was observed in peptide content, rising 114% for chickpea miso (5.57 to 11.91 mg tyrosine/g fresh biomass), followed by lupin (85%) and cowpea miso (79%). A similar behaviour was observed for the free amino acids content: chickpea miso increased by 22%, lupin by 57% (0.18 to 0.28 mg tyrosine/g fresh biomass) and cowpea miso by 15%. Among the three final miso, chickpea and lupin miso stood out from the cowpea miso: higher protein, peptides (chickpea miso) and free amino acids content (lupin miso).

The results highlight the crucial role of microbial fermentation in legume digestibility and reveal the possibility of producing a locally sourced miso using Portuguese legumes, offering a sustainable alternative to soybean-based miso.

Abstract ID: 3704 / Poster Number: 409.16P

Development of innovative antimicrobial packaging activated with natural compounds for preserving fresh Italian favette strawberries

Presenting author: Valeria Poscente, PhD, ENEA, Rome, Italy

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(Regulation (EU) 10/2011 and Directive 94/62/EC), and carvacrol migration using inductively coupled plasma mass spectrometry (ICP-MS) and Fourier-transform infrared spectroscopy (FTIR).

The results demonstrated the enhanced antimicrobial activity of antimicrobial PADs functionalised with the combination of carvacrol, nisin, and chitosan compared to the single treatments. This innovative solution offers a sustainable approach to reducing the environmental impact of the distribution chain while enhancing the value of Italian agricultural products by improving their quality and safety.

Abstract ID: 499 / Poster Number: 182.16P

Development of Species-specific Assays Based on rpoB Gene using Droplet Digital PCR for Quantification Probiotic Bacteria

Presenting author: Seil Kim, Korea Research Institute of Standards and Science, Daejeon, South Korea ; Korea National University of Science and Technology, D; ejeon, South Korea

Co-authors: *Jinyoung Park,*

Accurate quantification of individual species in multi-species probiotic products is essential for quality control. Traditional colony counting methods and Next-Generation Sequencing (NGS) have limitations in accurate quantification due to technical difficulties and platform-dependent bias, respectively. This study aimed to develop and validate a species-specific quantification method for 19 probiotic species designated by the Korean Ministry of Food and Drug Safety. We developed droplet digital PCR (ddPCR) methods targeting both 16S ribosomal RNA (rRNA) and RNA polymerase β-subunit (rpoB) genes. While both assays targeting 16S rRNA and rpoB genes were initially evaluated, the 16S rRNA-based assays showed cross-reactivity in mixed samples due to sequence similarity among probiotic species. The rpoB gene-targeted method demonstrated species-specificity with no cross-reactions in mixed genomic DNA samples from all 19 species. Five commercial probiotic products were analyzed with rpoB gene-targeted ddPCR and NGS sequencing methods (GridION and MiSeq). Bias Index (BI) was calculated for the relative abundance obtained from each method. BI of MiSeq showed lower values (0.15-0.36), indicating that the ddPCR method can be a reliable tool for assessing sequencing accuracy. The ddPCR method using the specific assays developed in this study offers a precise and efficient complementary approach for evaluating NGS for microbial quantification in probiotic products. Its absolute quantification capability without external reference demonstrates potential for establishing reference materials of probiotic bacteria and enhancing quality control processes in health-functional foods and other probiotic-utilizing industries.

Abstract ID: 1426 / Poster Number: 018.16P

Development of the genetic toolkit identifies capsular polysaccharides as a competitive colonization factor in Ruminococcus gnavus

Presenting author: Dr. Nozomu Obana, University of Tsukuba, Tsukuba, Japan

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Ruminococcus gnavus (Mediterraneibacter gnavus) is a commensal human gut bacterium associated with various diseases, and its abundance often increases in the intestines of active inflammatory bowel disease (IBD) patients. However, the genetic factors establishing gut colonization and virulence are still elusive due to the lack of a system for genetic modifications. This study develops several genetic tools, including a shuttle vector, an inducible promoter, fluorescent proteins, and gene disruption or deletion systems in R. gnavus to identify the genes involved in R. gnavus colonization. Using these genetic tools, we find the sortase enzymes necessary for cell wall localization of the superantigens and capsular polysaccharide (CPS) production, respectively, in R. ganvus ATCC 29149. We identify the putative new gene cluster responsible for CPS production in the adjacent region of the sortase gene. We confirm that CPS production is crucial for competitive fitness during the colonization into germ-free mouse intestines and that CPS production is inversely correlated to the inflammatory activity of R. gnavus. Furthermore, CPS cluster-possessing strains tend to be distributed in healthy human isolates compared to Crohn's disease patient isolates. This implies that CPS production involves their inflammatory activities and pathogenesis for intestinal diseases, such as IBD. We highlight that precise gene modification systems promise to identify the genetic factors facilitating intestinal colonization in gut bacteria.

Abstract ID: 3040 / Poster Number: 301.16S

Diazotrophic Purple Phototrophic Bacteria in the fast lane: A growth rate advantage revealed

Presenting author: Dr. Luis Diaz Allegre, University of Antwerp, Antwerp, Belgium

Co-authors: *Sara Olyslaegers, Siegfried Vlaeminck*

Introduction: Nitrogen fixation is a vital process for ecosystem sustainability and nutrient cycling. While the energetic demands of diazotrophy are well-studied, its growth-related effects on purple phototrophic bacteria (PPB) are less understood[1]. PPB, as anoxygenic phototrophs, grow primarily photoheterotrophically by suing light as an energy source and organic compounds as carbon and electron sources, reaching substrate to biomass efficiency of up to 100%[2]. Moreover, diazotrophic growth may impose selection pressures that reduce contamination risks, positioning PPB as a valuable tool for resource recovery and high-value product generation.

Objective We hypothesize that diazotrophic PPB can sustain comparable growth rates, while simultaneously undergoing changes in protein composition and nutritional profile.

Method Three PPB species (*Rp. palustries, Rb. capsulatus and Rp. Rubrum)* were cultivated under photoheterotrophic conditions in 250 mL flasks at 28 ± 2 °C, with acetic acid (2 gCOD L^{-1}) as the sole organic carbon source. Continuous illumination was provided by three halogen lamps at a minimum intensity of 8 W L^{-1}. To ensure anaerobic conditions, the flasks were sealed and flushed with either nitrogen gas (diazotrophic condition) or argon gas (NH_4^+ controls). Macromolecular composition was assessed using standard analytical methods.

Findings Among the three species studied, *Rp. palustris* and *Rb. capsulatus* exhibited 38% and 8% higher growth rates, respectively, under diazotrophic conditions compared to growth with reactive nitrogen, while *Rp. rubrum* maintained similar growth rates (Figure 1, left). These results demonstrate that PPB can effectively use nitrogen gas as a nitrogen source to grow on high-organic-carbon waste streams, achieving comparable or superior rates and yields with minimal competition. Nutritionally, diazotrophic PPB demonstrated similar protein content to their NH_4^+-grown counterparts, with *R. capsulatus* showing higher levels (Figure 1, right). PHA content was reduced under diazotrophic conditions, likely because PHA serves as an electron sink, competing with nitrogen fixation for electrons[3]. Conversely, pigment production was enhanced, representing the most valuable biomolecules in PPB biomass. This increase is likely driven by the higher ATP

demand of diazotrophic growth (16 ATP and 8 electrons per mole of N_2 fixed). Amino acid analysis revealed an improved essential-to-non-essential amino acid (EEAA/NEAA) ratio in diazotrophic PPB, reaching 52–54%. Leucine and methionine exhibited the most significant increases, further enhancing the nutritional profile.

Conclusion PPB can grow diazotrophically at equal or higher rates than when using reactive nitrogen, with biomass of similar or enhanced nutritional value. This highlights a promising avenue for nitrogen cycling research, leveraging diazotrophic PPB for resource recovery from waste streams and applications as microbial protein in feed and food production.

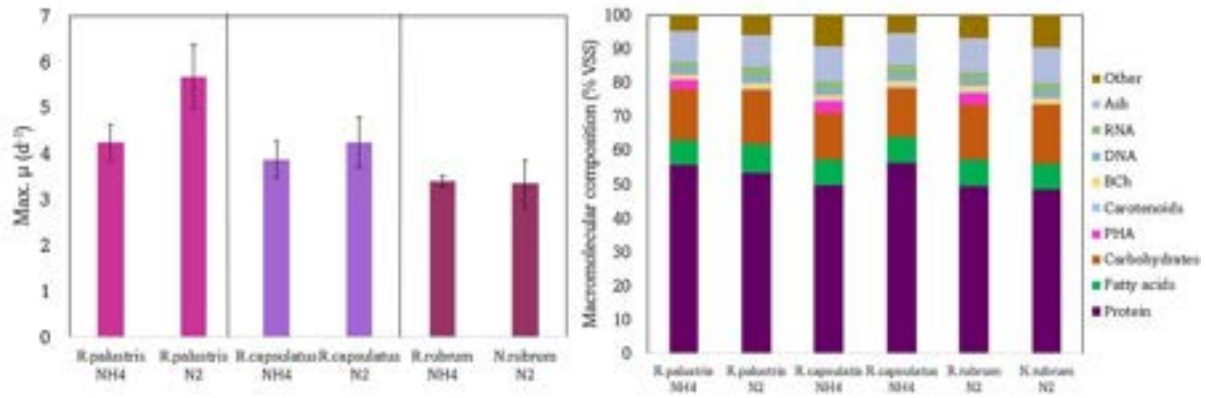


Figure 1: Growth rates (left) and macromolecular composition (right) of three PPB strains (Rp.s palustris, Rb. capsulatus, and Rp. rubrum) under photoheterotrophic conditions using NH_4^+ (reactive nitrogen) or N_2 (diazotrophic). Error bars represent the standard error.

Abstract ID: 2895 / Poster Number: 023.16P

DictDb5: an updated 16S rRNA gene reference database for improved taxonomic classification of arthropod gut microbiota

Presenting author: Ms. Joana Maria Kästle Silva, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

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Information on the microbial diversity in the intestinal tracts of arthropods has dramatically increased with the high-throughput sequencing technologies developed in the past decade. Short-read and long-read amplicon libraries of cockroaches and termites have expanded the available datasets on bacteria and archaea in the gut of termites, cockroaches, and other arthropods. Coupled with metagenomic studies, these efforts allow a taxonomic revision of the bacterial and archaeal groups involved in the symbiotic digestion of lignocellulose, methanogenesis, and associations with intestinal protists. Here, we introduce DictDb5, a comprehensive database that integrates robust genome-based phylogenies with the broader diversity captured in 16S rRNA-based studies, providing both a highly curated reference alignment and the taxonomy files required by automated sequence classification software. DictDb5 is based on the latest releases of Silva (r138.2) and the taxonomy of GTDB (r220), and has been expanded with 16S rRNA gene sequences from the gut metagenomes of 34 termite species and more than 1,800 metagenome-assembled genomes (MAGs) obtained from these samples. In addition, it includes representatives of all lineages of bacteria and archaea from newly obtained long-read amplicon libraries of the gut microbiota of 28 termite, 19 cockroach, 3 millipede, and 4 scarab beetle species. When tested with amplicon data from previous studies, DictDb5 yielded a substantially increased classification success at genus and family level, providing a detailed picture of microbial community structure and the organisms that play key roles in the complex intestinal ecosystems in the guts of Dictyoptera (termites and cockroaches) and other arthropods.

Abstract ID: 2207 / Poster Number: 013.16P

Diet Impacts Microbiome Based Therapies Against Antibiotic-Resistant Pathogens In Mice

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Antibiotic-resistant pathogens (ARPs) are a major health concern. Infections caused by ARPs, such as vancomycin-resistant enterococci (VRE) and carbapenem-resistant Enterobacteriaceae (CRE), often begin with intestinal colonization, a critical step that is normally prevented by the gut microbiota under homeostatic conditions. Antibiotic-induced disruptions of the microbiota allow ARPs to colonize and persist in the gut. Consequently, microbiota restoration therapies (e.g., fecal microbiota transplantation – FMT) are being implemented to reduce colonization by ARPs. This promising treatment exhibits significant variability in ARP eradication rates and most importantly, the factors influencing the efficacy of FMT remain unknown. In this study, using a mouse model, we demonstrated that the donor's diet, through its impact on microbiome, is key for the effectiveness of FMT as a therapy against ARPs. To simulate a clinical scenario, mice were treated with vancomycin, which facilitates colonization by VRE or CRE. To restore the microbiota and eliminate the pathogen, FMT were performed using donors fed either a western-diet rich in fats and simple sugars (WD-FMT) or a control diet low in fat and simple sugars (CD-FMT). The donor's diet influenced microbiota reconstitution, leading to significant differences in intestinal decolonization of ARPs, with CD-FMT proving significantly more effective.

Ex vivo experiments, along with metatranscriptomic and metabolomic analyses, suggest that direct mechanisms by which the microbiome inhibit ARPs (i.e. nutrient competition) are involved in the effect of donor’s diet on fecal transplantation efficacy. Our study indicates that diet microbiome modulation can impact the efficacy of microbiome-based therapies against ARPs.

Abstract ID: 2379 / Poster Number: 073.15P

Dietary Rhythms and Gut Microbiota: Unveiling Metagenomic Patterns in dry Fasting

Presenting author: Mrs. Yomna Khaled, Misr university for science and technology, Giza, Egypt

Co-authors: *Marwa Amer, Ingy Badawy*

Dry fasting, a distinctive type of intermittent fasting that involves abstaining from food and water for 12 to 18 hours daily, is attracting attention for its potential effects on health, including gut microbiome dynamics and systemic biomarkers. Despite its increasing popularity, research exploring the impacts of dry fasting on gut health is still limited, highlighting the need for further investigation. This study aimed to evaluate the effects of dry fasting on gut microbial composition and biochemical markers by monitoring eight participants over a 30-day fasting period. Fecal samples were collected at three time points—before, during, and after the fasting period—and analyzed using DNA extraction and 16S rRNA sequencing. The data were processed with QIIME2, a bioinformatics platform capable of analyzing microbial diversity, taxonomic composition, and phylogenetic relationships. Blood samples were also collected to examine correlations between biochemical changes and bacterial shifts. The results indicated no significant differences in gut microbial composition across the three-time points, the dominant bacterial groups in the gut microbiome were classified within the *Bacteroidota* and *Firmicutes*, These bacterial groups are known for their roles in carbohydrate fermentation, short-chain fatty acid production, and maintaining gut homeostasis., with only slight variations in bacterial abundance such as *Veillonellaceae, Prevotellaceae* and *Rikenellaceae* and phylogenetic relationships seen during fasting. Similarly, blood biomarkers exhibited minor changes such as urea and the alanine and aspartate aminotransferases (ALT and AST, respectively) that were weakly correlated with microbial variations. Notably, both gut microbiota and biochemical markers reverted to baseline post-fasting, suggesting that dry fasting induces minimal and transient effects. These findings emphasize the resilience of the gut microbiome and indicate that dry fasting, as practiced in this study, is unlikely to impact gut microbial composition or overall health negatively.

Abstract ID: 680

Diet-induced sex-dependant metabolomic and metagenomic changes in the small intestine: Insights from a pig model

Presenting author: Axel Ranson, PhD, INSERM, Paris, France

Co-authors: *Marta Vazquez-gomez, Eugeni Belda, Karim Chikh, Tiphaine Le Roy, Karine Clément*

The intestinal microbiota is recognized as a key player in obesity pathophysiology[1]. While the small intestine is a critical site for nutrient sensing and absorption, its specific alterations in obesity are still barely explored[2,3]. Pioneering studies in mice suggest that the small intestine microbiota can significantly influence intestine functions, and its composition is altered in people with obesity[2]. However, it remains unclear whether changes in the microbiome, particularly in the duodenojejunal area, influence obesity phenotypes or act as mediators of dietary effects, thereby contributing to metabolic alterations and shaping obesity-related outcomes[4]. We therefore sought to investigate, in a sex-dependent manner, the specific role of diet in metabolomic and metagenomic changes along the digestive tract using a non-coprophage model, the pig.

40 Iberian pigs were fed either a control diet (LFD) or a medium fat diet (MFD), not obesogenic[5,6]. After 12 weeks, the pigs were sacrificed, and bile and duodenojejunal fluid metabolomic analyses were conducted. Illumina sequencing was performed on luminal content from three segments of the small intestine and two of the large intestine. The Metaphlan4 pipeline was used to profile microbial communities. Multivariate and multidimensional models were performed to identify significant differences between diets and correlations between morphometric, biological, and “omics” data.

Our results reveal sex-dependent diet-induced shifts in bile and duodenojejunal fluid metabolite levels. These metabolites include p-cresol, stachydrine, or indole derivatives. These metabolic changes were strongly correlated with diet-induced changes in microbiota composition, both in microbial diversity within groups (α-diversity) or between groups (β-diversity) ($p \leq 0.001$). We notably observed a decrease in *Lactobacillus johnsonii* and an increase in *Streptococcus alactolyticus* in the 5 segments studied in pigs fed the MFD, compared to LFD. These microbial modifications were associated for significant changes in metabolic pathways, such as an increase in succinate synthesis or lactose degradation pathways in MFD pigs in the small intestine, which has an effect on bacterial development in the lower segments, such as an increase of *Clostridium* in the large intestine of MFD pigs ($p \leq 0.005$).

Diet significantly impacts the upper intestine, influencing both microbial communities and produced metabolites, previously shown to be linked to metabolic diseases in humans[7-10]. Our findings underscore the interplay between dietary fat, sex-specific metabolomic responses, and intestinal microbiota composition. These findings offer promising insights into the role of nutrition in gut microbiota modification and in metabolic disease development. This highlights the need for further analyses to explore the potential mediating role of diet in shaping male and female pig phenotypes through metagenomic changes, via changes in small intestinal microbiota impacting large intestinal microbiota[11,12].

Abstract ID: 3894 / Poster Number: 095.16P

Difference in the urinary tract microbiome of males with spinal cord injury and those without injury when they have asymptomatic bacteriuria

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Co-authors: *Xin Zhou, Jörg Krebs, Jürgen Pannek, Jivko Stoyanov*

Introduction: Urine is not sterile. When bacteria are present in the urine without symptoms it is classified as asymptomatic bacteriuria and is a risk factor in developing urinary tract infections (UTI). UTI is common among individuals with spinal cord injury (SCI). In this study, we investigated whether there is a difference in the urinary tract microbiome of asymptomatic males with SCI vs without SCI.

Methods: In total, we collected 87 urine pellets (32 without injury; 55 with SCI). The DNA was extracted and full-length 16S rRNA were sequenced using Oxford Nanopore technology. Internal controls were used to estimate microbial load. Positive and negative controls were included for each batch of sequencing. The amplicons were classified using emu.

Findings: All samples had microbial load and ranges 10^2 to 10^{10} bacteria/mL urine (upper range for without injury was 10^7bacteria/mL). There was significant differences in the alpha indices (Observed Species, Shannon and Simpson) and the beta-diversity (PERMANOVA 0.024, r^2 0.023, PERMDISP 0.22). Microbial diversity was lower in the urinary tract of those with SCI compared to those without injury. Differential abundance analysis showed that members of *Enterobacteriaceae* were significantly more abundant while members of *Staphylococcaceae* were significantly less abundant in the urinary tract of males with SCI compared to those without injury.

Conclusion: There are significant differences in the urinary tract microbiome of males with SCI vs without injury. The loss of diversity and the more abundant presence of *Enterobacteriaceae* explains the higher risk of UTI in the SCI population.

Abstract ID: 4052 / Poster Number: 100.16P

Differences in Oral Microbial Communities Between Smokers and Non-Smokers

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Co-authors: *Razmik Sargsyan, Nelli Vardazaryan, Lilit Nersisyan*

Smoking is a well-known risk factor leading to various health issues and has been linked to changes in the oral microbiome, a form of dysbiosis that may contribute to adverse effects on health.

This study explores the notable differences in the oral microbiota of smokers and non-smokers, aiming to identify the driver species contributing to these alterations and assess the potential of the oral microbiome as a predictive indicator of smoking status. To achieve this, shotgun metagenome sequencing data from the salivary microbiota of smokers and non-smokers in Hungary were analysed.

Taxonomic labels were assigned, and bacterial abundances were estimated using Kraken and Bracken, respectively. PCoA and alpha diversity analyses were applied to assess differences in microbial composition and community richness/evenness. Differential abundance analysis using ANCOM-BC compared microbial communities between smokers and non-smokers. Subsequently, Receiver-Operating Characteristic curve (ROC) analysis was used to evaluate the capacity of microbial data for distinguishing between smokers and non-smokers.

Shannon Index indicated significantly lower alpha diversity in smokers compared to non-smokers. PCoA analysis revealed distinct differences in microbiota composition between the two groups. Nine species, including *Veillonella dispar* and *Prevotella multiformis*, showed significantly different abundances between smokers and non-smokers. ROC analysis demonstrated effective classification, with AUC values of 0.818 for the test set and 0.710 for the validation set.

The study identifies key microbiome differences between smokers and non-smokers, highlighting potential microbial biomarkers for smoking status. ROC results represent a promising first step toward developing a microbial-based tool for smoking status prediction.

Abstract ID: 2789 / Poster Number: 110.15F

Differential gene expression in Shewanella putrefaciens T2.3D-1.1 during lactate oxidation with and without electron acceptor

Presenting author: Guillermo Mateos, PhD, Universidad Autónoma de Madrid, Madrid, Spain; Centro de Biología Molecular Severo Ochoa, Madrid, Spain

Co-authors: *Karel Olavarria Gámez, Irene Sánchez Andrea, Ricardo Amils Pibernat*

Deep subsurface isolate, *Shewanella putrefaciens* T2.3D-1.1, response to electron acceptor availability was studied using RNA-seq when grown on lactate with fumarate compared to lactate with nitrate as electron acceptors. Sampling occurred during the first hydrogen peak detected via gas chromatography, marking fumarate depletion, while the nitrate culture remained in exponential growth. Lactate metabolism by *Shewanella* involves its oxidation to acetate and acetyl-CoA, entering the TCA cycle, or conversion to formate, hydrogen, and CO_2. Mapped reads were converted to TPM for expression comparisons, and differential expression analysis (log2FC > 2.5, padj < 0.01) was combined with ORA analysis using GO terms.

Out of 4179 mapped genes, 202 were overexpressed, and 135 were repressed. Among 80 genes of interest, no clear expression patterns emerged. Although hydrogenase genes themselves were not overexpressed, four hydrogenase maturation genes showed significant fold changes (>9.28). Periplasmic fumarate reductase exhibited a remarkable fold change (15.76), while non-periplasmic fumarate reductase had four genes with fold changes above 6.58. Genes related to flagella assembly, biofilm dispersal, vitamin B_{12} transport, and iron uptake were upregulated. Conversely lactate oxidation, pyruvate oxidation, and acetate production genes were repressed. Repression was also observed for genes encoding formate dehydrogenase and some TCA cycle genes (e.g., 2-oxoglutarate dehydrogenase and succinate dehydrogenase).

These findings highlight the complexity of *S. putrefaciens* T2.3D-1.1's metabolic adaptation to electron acceptor availability. Despite fumarate depletion, non-periplasmic fumarate reductase overexpression suggests an alternative electron disposal route alongside hydrogen production. Further research is needed to elucidate the regulatory mechanisms underlying these metabolic shifts.

Abstract ID: 2564 / Poster Number: 228.17F

Differential Transcriptomic Profiles of E. coli under Different Sub-inhibitory Concentrations of Penicillin

Presenting author: Dr. Ping Lung Chan, Hong Kong Metropolitan University, Hong Kong, Hong Kong

Co-authors: *Sophie, Ling Shi, Kwok Tai Lam*

Introduction: Although it is well-known that antibiotics at sub-inhibitory concentrations would induce antimicrobial resistance by adaptation through adaptive mutations, its underlying biochemical and molecular mechanisms are not well characterised. Therefore, this study aims to utilise a transcriptomic approach to depict the transcriptomic changes of *E. coli* for identifying genes whose expression level was significantly affected by various subinhibitory concentrations of penicillin.

Objective: To characterise the transcriptomic profile of *E. coli* under various subinhibitory concentrations of penicillin.

Method: *E. coli* (ATCC 25922) was cultured in LB broth to OD 0.5 and was sub-cultured into a new flask of LB broth supplemented with 0.1, 0.01, and 0.001X MIC of penicillin for 12 hours. *E. coli* was harvested and subjected to transcriptomic analysis.

Findings: Our data indicated that subinhibitory concentrations of penicillin induced the expression changes of about 2,000 – 3000 genes in *E. coli*. Interestingly, *E. coli* challenged with 0.001X MIC of penicillin exhibited a transcriptomic profile distinct from that with 0.1X and 0.01X MIC of penicillin and the negative control. In addition, enrichment analysis indicated that expression level genes related to the amino acid metabolism and the two-component system were increased and decreased, respectively, and these were not observed in the other two penicillin concentrations.

Conclusion: Our data suggested that different metabolic pathways in *E. coli* may be affected when challenged by different subinhibitory concentrations of penicillin. It might differentially affect its cellular functions, thus the potential of inducing AMR.

Abstract ID: 3777 / Poster Number: 221.16P

Differentially Methylated Nucleotides in Heat-Treated vs. Untreated Bacteria: insights from Nanopore PCR-Free Sequencing

Presenting author: Mr. Amir reza Varzandi, University of Turin, Turin, Italy

Co-authors: *Ramón Christian Gortazar Schmidt , Lucas Domínguez, Ezio Ferroglio*

Environmental DNA/RNA (eNA)-based methods, which provide researchers with information on species presence without the need for capture or direct observation, are increasingly utilized for detecting and investigating pathogens. However, eNA methods require further advancement to distinguish stages (in parasites), living from dead organisms, or resistant strains (in bacteria). Nanopore sequencing offers a promising solution as it can detect epigenetic modifications, such as methylated versus non-methylated nucleotides, in addition to sequence variations. To address this need, nanopore PCR-free sequencing of DNA was applied to differentiate methylation patterns between distinct states of an organism.

In this pilot study, DNA was extracted from two *Mycobacterium bovis* cultures: one untreated before extraction and the other subjected to gradual heat increment for 50 minutes. DNA from each sample type was sequenced in several replicates using the ONT-Native Barcoding kit, enabling native DNA sequencing. Base- and methylation-calling (5mC, 5hmC, and 6mA) were performed with the super-high accuracy configuration in Dorado. The MicrobeMod package was employed to identify methylation patterns, motifs, and differentially methylated nucleotides.

Our results revealed similar methylation sequence motifs and restriction-modification systems between the two sample types. Importantly, we identified hundreds of differentially methylated nucleotides and motifs that could be informative biomarkers of an organism's state or life-cycle stage.

Abstract ID: 2629

Dimethyl sulfoniopropionate (DMSP) metabolism in green tide causing seaweed

Presenting author: Ms. Catherine Philip, Plymouth Marine Laboratory, Plymouth, United Kingdom; University of East Anglia, Norwich, United Kingdom

Introduction: *Ulva* seaweeds, notorious for causing green tides, are prolific producers of dimethyl sulfoniopropionate (DMSP), one of Earth's most abundant organosulfur molecules. They can cleave DMSP to release the climate-cooling gas dimethylsulfide (DMS). The precise role of DMSP in *Ulva* is unclear, but could relate to anti-stress mechanisms, or to the signaling and recruitment of beneficial bacteria which use DMSP as a nutrient and facilitate further DMS production.

Objectives:

1. Elucidate the bacteria that metabolise DMSP produced by *Ulva*.
2. Determine the DMS production rates by *Ulva* associated bacteria.

Methodology: The current study investigates the bacterial communities associated with *Ulva* using 16S rRNA sequencing, DNA-stable isotope probing and metagenomics, targeting both culture-dependent and independent communities. DNA stable-isotope probing provides a means to identify the microorganisms that incorporate DMSP by substituting it with C-13 labeled DMSP without the prerequisite of cultivation. This is followed by separation and molecular analysis of C-13 labeled DNA which reveals phylogenetic and functional information about the microorganisms responsible for the metabolizing DMSP in *Ulva*. This technique helps to identify both culture dependent and independent microorganisms responsible for DMSP cycling in *Ulva* seaweeds.

Findings: To date, nine bacterial species have been isolated and identified through 16S rRNA gene sequencing belonging to the *Flavobacteriaceae, Nocardiaceae, Micrococcaceae, Vibrionaceae, Psychromonadaceae, Alteromonadaceae,* and *Pseudoalteromonadaceae* families. Four of these isolates used DMSP as a sole carbon source, indicating their active role in DMSP metabolism on *Ulva*. Furthermore, the isolated bacterial species do not indicate any DMSP production, but has noticeably higher DMS production rates, indicated in figure 1. It was also found that *Ulva* microbiome rapidly metabolizes DMSP and releases DMS. Additionally, data collected through DNA stable isotope probing is expected to provide further insights on bacterial metabolisers of DMSP in *Ulva* seaweed.

Conclusion: This study underscores the roles of DMSP in *Ulva* and its surrounding microbial communities. By elucidating the microbial dynamics and DMSP metabolism, these findings help us understand the microbial and environmental drivers of sulphur cycling in marine ecosystems.

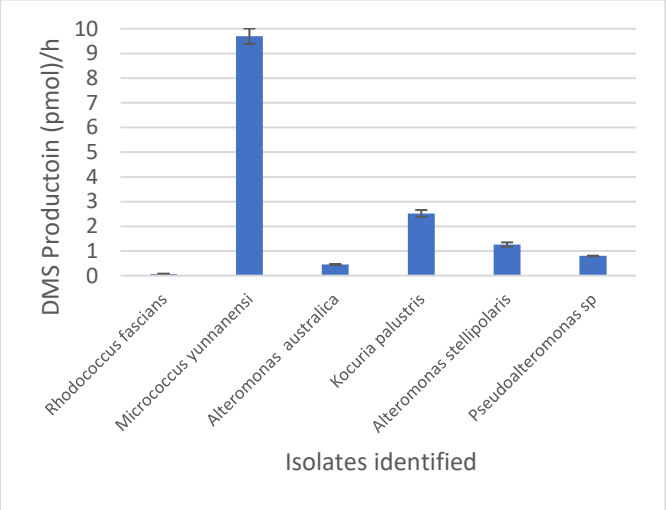


Figure 1: DMS production by bacteria isolated from Ulva

Abstract ID: 2143

Direct CO conversion into medium-chain carboxylates with a novel thermophilic microbiome

Presenting author: Dr. Rodolfo Palomo-Briones, University of Valladolid, Valladolid, Spain

Co-authors: *Raul Muñoz, Diana Sousa*

Introduction: Conversion of CO-containing gas streams (*e.g.*, waste-derived syngas containing CO, CO_2, and H_2, among other gases) into organic chemicals has become a promising strategy for establishing and strengthening a circular bioeconomy. Anaerobic gas fermentation is a biotechnology that utilizes anaerobic microbes to convert syngas components into acetate, ethanol, and a few other short-chain carboxylates *via* the Wood-Ljungdahl metabolic pathway (WLP). Other processes, such as anaerobic chain elongation, can be coupled to obtain longer products that can be easily separated and further upgraded to sustainable fuels and high-value chemicals. In the anaerobic chain elongation *via* reverse b-oxidation, short-chain carboxylates produced in the WLP are elongated two carbons at a time to obtain medium-chain carboxylates (between 5 and 10 C-atoms). Combining the two processes has been hypothesized as a clear route for upgrading syngas. However, the experimental evaluation of single-stage syngas to medium-chain carboxylates has been scarcely studied. Here, we report for the first time a thermophilic microbiome that transforms syngas and pure CO into n-caproate, opening a whole new path for further biotech development.

Objective: Select, demonstrate, and evaluate the potential of novel thermophilic anaerobic microbiomes for converting syngas into n-hexanoate in a single step.

Method: A composite inoculum (pond sediment + anaerobic sludge + activated sludge) was grown at 60 °C in an MES-buffered mineral salt medium with either a) 100 mM acetate, 100 mM acetate + 200 mM ethanol, or c) 100 mM acetate + 200 mM lactate. Microbiomes were set with 1.5 bar H_2:CO:CO_2 (16:16:68 (v/v)) at increasing CO concentrations. Gas conversion and *n*-hexanoate production were monitored *via* gas and liquid chromatography, respectively. The microbiome composition was investigated through 16S-rRNA gene sequencing (MinION, Oxford Nanopore).

Findings: We found that our novel selection strategy promoted the production of *n*-hexanoate from the first transfer using syngas as carbon and energy sources. After eight transfers, we observed that the microbiome selected with acetate at pH 7 was able to use CO as the sole carbon and energy source, resulting mainly in 46.6 mM-C acetate (57

%) and 32.8 mM-C *n*-hexanoate (41 %). Other selected microbiomes were also found to grow efficiently on CO, but their *n*-hexanoate was lower or nonexistent. Future analysis will resolve the microbial community composition and relate such composition to their product profiles.

Conclusion: Here, we report the direct CO conversion into *n*-hexanoate using thermophilic anaerobic microbiomes using a novel selection strategy. The relevance of this report relies on the potential use of the selected microbiomes for producing sustainable fuels and high-value chemicals from CO-containing gas streams in a single bioreactor.

Abstract ID: 2988 / Poster Number: 265.15P

Discovering Ecological Principles Through Neural Network Concept Extraction in Microbial Communities

Presenting author: Mr. Matteo Peluso, The University of Zurich, Zurich, Switzerland

Co-authors: *Janko Tackmann*

Modern deep learning techniques and advances in mechanistic-interpretability can distill abstract and complex microbial relationships into a human-intelligible format. Models trained on masked prediction (masked language modelling) or generative de-noising (diffusion) objectives must learn implicit rules guiding ecological niche formation in order to accurately predict missing OTUs or generate plausible OTU samples. The internal states of these models can then be stratified by a sparse autoencoder trained on a reconstruction objective. This helps to extract 'concepts' from the underlying model.

By combining large scale microbial abundance data with context-defining metadata - per-sample (SRA, ENA) and per-taxon (BacDive, Progenomes3) - it is possible to gain insight into the 'concepts' recovered by the interpreter models. Concepts can be validated through enrichment analysis using independent phenotypic or environmental data, strengthening confidence in their biological relevance.

To explore this space, we are currently developing several masked prediction and diffusion-based models, initially based on Human Microbiome Project presence/absence data and BacDive phenotype information. We found these early models to already predict missing OTUs with F1 score nearing 75%, recapture co-occurrence patterns of the original data to a high degree and, upon internal state visualization, recover clear body site, with notable body subsite and primer segmentation. This suggests they capture fundamental and possibly nuanced ecosystem properties. Supporting this, 'concepts' derived from these models are enriched for phenotypic features such as oxygen-tolerance level and pathogenicity.

The promise of these methods is the self-supervised discovery of deep ecological characteristics, allowing for hypothesis generation from large scale data.

Abstract ID: 1475 / Poster Number: 154.16P

Discovering the species-specific dynamics within a model biofilm system for subgingival infections

Presenting author: Ms. Ingrid Maria Castro Lund, University of Oslo, Oslo, Norway

Co-authors: *Jan-Ole Reese, Dirk Linke, Athanasios Saragliadis, Ståle Petter Lyngstadaas, Håvard Jostein Haugen*

Background: Subgingival infections pose a significant global health issue [1,2]. People with dental implants are at risk for chronic infections and inflammation that ultimately leads to bone degradation [3]. This occurs due to the rugged surface of implants, that while necessary for bone attachment, also promotes bacterial growth. The bacteria responsible for the infection form a biofilm, making the infection harder to treat due to increased antimicrobial resistance.

Objectives: Our aim was to develop a multispecies biofilm model that accurately replicates infection in patients. This model was then used to determine the species-specific structure of the biofilm by identifying the quantity and location of each species. Further the model will be used for treatment testing.

Methods: Biofilms were grown in a Robbins device. The biofilm model included six species known to be present in subgingival infections around titanium implants [4]. Various methods for DNA extraction were tested and the best was selected for downstream analysis.

Quantification of bacterial species was done by qPCR. Fluorescence in situ hybridisation (FISH) was used to evaluate the spacial distribution of species within the biofilm.

Results: Supported by qPCR data, we will showcase the relative amounts of each species in the biofilm. Additionally, FISH images will shed light on species localisation within the biofilms formed on the implants. This study will help our understanding of the dynamics involved in these infections which may aid future therapies. Furthermore, it will demonstrate the suitability of our multispecies biofilm model for testing of diverse treatments.

Abstract ID: 3666 / Poster Number: 347.17P

Discovery and exploitation of psychrotolerant bacteria and novel cold-adapted enzymes in regenerative agriculture

Presenting author: Dr. Anna-Karina Kaczorowska, University of Gdansk, Gdansk, Poland

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Regenerative agriculture is a modern farming approach that blends sustainable innovation with tradition. It focuses on restoring and improving the health of the soil, ecosystem, and overall farm environment by implementing innovative technologies. Some common regenerative practices include cover cropping, crop rotation, reduced tillage, integrating livestock into farming systems, and biocomposting. One of the advantageous approaches in regenerative agriculture could be the introduction of cold-active bacteria and their enzymes which efficiently accelerate (bio)chemical processes of choice at comparatively low temperatures, *e.g.* enhance biocomposting of postharvest or remaining plant residues on fields. These psychrotolerant bacteria and their enzymes function at lower temperatures, making them valuable for agricultural applications in diverse climates. Amongst cold-active (but also generally) enzymes, those processing polysaccharides, *i.e.* cellulases, pectinases, amylases, and glycosidases, play a crucial role in regenerative agriculture by facilitating the breakdown of complex plant organic macromolecules, improving nutrient availability, and promoting overall soil health. In the RegAgro project, we utilise the microbiological resources of Arctic and Antarctic soils to identify novel psychrotolerant bacteria and novel cold-active enzymes that may be exploited in regenerative agriculture.

Abstract ID: 1454 / Poster Number: 039.15P

Discovery of a functional tandem repeat sequence that controls gene expression in bacteria

Presenting author: Dr. Hikaru Suenaga, National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan

Co-authors: *Tomohiko Matsuzawa, Takehiko Sahara*

The ability to degrade exogenous compounds is acquired by adaptive processes of microorganisms when they are exposed to compounds that are foreign to their existing enzyme systems. Previously, we reported that simultaneous point mutations and mobile genetic elements cause the evolution and optimization of the degradation systems for aromatic compounds (Suenaga *et al.*, ISME J. 2009(3): 1335-1348.). In the present study, we propose another element with this role—tandem repeats. The novel metagenomic tandem repeat (MTR) sequence T(G/A)ACATG(A/C)T was identified in the 5'-untranslated regions of catechol 2,3-dioxygenase (C23O)-encoding genes by metagenomic analysis. Recombinant *Escherichia coli* carrying a C23O gene with various numbers of MTRs exhibited increased C23O protein expression and enzyme activity compared with cells expressing the C23O gene without MTRs. Thus, MTRs would affect the translation efficiency of the gene expression system. MTRs may change the expression levels of their downstream genes for adaptation to a fluctuating environment. MTRs also have the potential to be a simple and effective tool in bioproduction and synthetic biology.

Abstract ID: 1213 / Poster Number: 183.16P

Discovery of aminoglycoside potentiators for re-sensitizing multi-drug resistant bacterial pathogens

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Introduction: The rise of multi drug-resistant infections, particularly within Gram-negative ESKAPE pathogens, has emerged as a threat in clinical settings globally. Aminoglycosides (AM), vital for treating severe bacterial infections, are increasingly rendered ineffective due to acquisition of aminoglycoside modifying enzyme (AMEs). Re-sensitization of resistant pathogens by inhibiting resistance functions through AM potentiators offers promising avenue.

Objective: Development of a High-Throughput Screening (HTS) assay using reporter strains to Identify AM potentiators against resistant pathogens.

Methods: Prevalent AMEs encoding genes were cloned into an integrative expression vector, and irreversibly inserted into the chromosome of sensitive *Vibrio cholerae* N16961 and *Escherichia coli* ATCC 25922 strains. Screening of natural and synthetic compounds (n= 4417) to identify hit molecules was completed with AM antibiotic. The optical density, viability, and resazurin assay of reporter strains were monitored to identify hit molecules.

Findings: The established HTS assay using sensitive reporter strains facilitated rapid and efficient identification of 326 compounds that demonstrates antibacterial, potentiating, or synergistic effects. Screening of with AM antibiotic identified eight compounds displaying potentiating or synergistic effect. The compound BMM_1567 at low concentration showed potentiating effect (100%) with multiple AM antibiotics against multidrug-resistant Gram-negative ESKAPE pathogens. In silico analysis indicates that BMM_1567 has strong binding affinity with AMEs and competes with antibiotics to form a complex.

Conclusion: The development of AM potentiators undoubtedly represents a significant pace toward advancing our capacity to combat antimicrobial resistance.

Abstract ID: 1130 / Poster Number: 152.15P

Discovery of species-specific non-coding RNAs from methanogens as novel targets for methane mitigation from ruminants

Presenting author: Dr. Mi Zhou, The University of British Columbia, Vancouver, Canada

Co-authors: *Sinead Leahy, Tim McAllister, Leluo Guan*

Introduction: Reducing ruminant enteric methane is a priority for mitigation of greenhouse gases (GHG) in agriculture.

Objective: The current study aimed to identify and verify non-coding RNAs (ncRNAs) from rumen methanogens, which can serve as the novel targets for methanogen manipulation.

Method: Genome-wide search of ncRNAs from four key strains representative of rumen methanogens (Methanobrevibacter ruminantium M1, Mbb. smithii DSM861, Mbb. boviskoreani JH1, and Methanosphaera stadtmanae DSM3091) was performed. Expressions of ncRNAs were measured with digital droplet PCR.

Findings: Three major ncRNAs identified with sequencing methods at high abundance were further examined in three populations of cattle that varied in CH4 emissions and/or feed efficiency classification (an indirect indicator for CH4 emissions of the tested herds) using digital droplet PCR. Expressed methanogen ncRNAs differed between pure culture and rumen content samples. In beef steers and beef bulls, ncRNA expressions did not differ between high and low CH4 emitters or between H- and L-RFI groups. In dairy cows, M1 RNase_P and M1 SRP expression was associated with a decrease in CH4 emissions adjusted for gross energy intake, suggesting a regulatory potential of these two ncRNAs for CH4 mitigation in dairy cows.

Conclusion: The identified CH4-associated ruminal ncRNAs may provide a new opportunity to develop ncRNA-based strategies to manipulate ruminal methanogens.

Abstract ID: 2807 / Poster Number: 147.17P

Disinfectant efficacy and biocide resistance determinants in MDR pathogens from hospital environments

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Co-authors: *Luminița Măruțescu, Carmen Mariana Chifiriuc, Marcela Popa, Ilda Barbu*

Background: Hospital-acquired infections (HAIs) caused by multidrug-resistant (MDR) pathogens remain a global health challenge. Surface disinfectants are essential in mitigating pathogen transmission and antimicrobial resistance in healthcare settings. However, the increasing prevalence of biocide resistance demands a deeper understanding of its genetic basis and an evaluation of disinfectant efficacy.

Objectives: This study aimed to: i) explore the genetic basis of bacterial resistance to disinfectants; ii) evaluate the antimicrobial and antibiofilm properties of commercial disinfectants against ESKAPE pathogens; iii) assess their impact on bacterial virulence.

Methods: Nine disinfectants (alcohols, quaternary ammonium compounds, hydrogen peroxide, glutaraldehyde, and sodium hypochlorite, were tested against four reference strains and 40 MDR strains (e.g., *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*). Antimicrobial efficacy and biofilm inhibition were evaluated via microdilution and crystal violet assays. Whole-genome sequencing identified biocide resistance genes, while enzymatic activity tests assessed virulence.

Results: The tested disinfectants showed significant antimicrobial and antibiofilm activity at the concentrations recommended for use. At inhibitory and higher concentrations, disinfectants significantly reduced hemolysis and moderately inhibited enzymatic activities, though bacteria remained viable under stress. Key resistance genes were identified, including *qacEΔ1, msr(C), tet,* and *catA*, and were linked to efflux pumps and membrane modifications.

Conclusions: This study underscores the importance of tailored disinfectant use in preventing biofilm formation and infections caused by virulent, resistant strains. Elucidating the genetic basis of biocide resistance can guide the selection of effective disinfectants to combat MDR pathogens in healthcare settings.

Abstract ID: 1617 / Poster Number: 265.16P

Dispersin-Based Latex Agglutination for Rapid and Accurate Detection of Enteroaggregative Escherichia coli (EAEC): A Breakthrough in EAEC Diagnostics

Presenting author: Prof. Doc. Roxane Piazza, Instituto Butantan, São Paulo, Brazil

Co-authors: *Caroline Florêncio, Miriam Silva, Claudia Moraes, Juliana Moutinho, Waldir Elias*

Enteroaggregative *Escherichia coli* (EAEC) is one of the major global causes of both acute and persistent diarrhea, which is particularly associated with growth and cognitive impairments in children living in low-income settings. The standard method for characterizing EAEC is the detection of the aggregative adherence adherence (AA) on cultured epithelial cells. However, this method is limited to reference laboratories equipped with cell culture facilities. Molecular diagnostic techniques have been proposed as alternatives, but also require specialized infrastructure. Whole-genome sequencing has identified the *aap* gene, which encodes dispersin, as a highly specific marker for EAEC. This study aimed to develop and standardize a latex agglutination assay targeting dispersin for the rapid diagnosis of EAEC. Recombinant dispersin was produced and a specific polyclonal antibody was generated. After purification, the antibody was conjugated to latex particles. The assay's performance was optimized using Tryptic Soy Broth (TSB) and MacConkey agar bacterial cultures. The best results were obtained after TSB growth, since 83% of *aap*-positive EAEC isolates were detected. The latex agglutination assay itself exhibited 90% sensitivity, 100% specificity, and 95% efficiency, with positive and negative predictive values of 100% and 92%, respectively. The latex agglutination test developed is a highly sensitive, specific, and easy-to-use diagnostic tool, particularly suitable for clinical laboratories with limited infrastructure. Additionally, it is a cost-effective solution, especially for resource-limited settings.

Abstract ID: 3041

Dissecting collateral sensitivity mediated by beta-lactamases

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Major antibiotic groups are losing effectiveness due to the uncontrolled spread of antimicrobial resistance (AMR) genes. In Enterobacterales, the most common resistance mechanism is β-lactam resistance genes -encoding β-lactamases- due to their frequent association with mobile genetic elements, such as plasmids. The dissemination of AMR genes encoded in plasmids and the critical shortage of new antibiotics underscores the urgent need for innovative therapeutic strategies that counter mobile AMR. One promising approach that capitalises on the resistance acquisition event is to exploit collateral sensitivity (CS), which occurs when the acquisition of resistance to one antibiotic increases the susceptibility to a second antibiotic. However, most CS networks described so far emerge as a consequence of chromosomal mutations and cannot be leveraged to tackle mobile AMR[1].

We dissected the CS response elicited by the acquisition of a prevalent antibiotic resistance plasmid, revealing that the expression of the β-lactamase bla_{OXA-48} induces CS to colistin (COL) and azithromycin (AZI; Fig 1a). We next showed that other clinically relevant mobile β-lactamases produce similar CS responses (fig 1b) in multiple, phylogenetically unrelated *E. coli* strains. Furthermore, by combining experiments with surveillance data comprising thousands of antibiotic susceptibility tests, we show that β-lactamase-induced CS is pervasive within Enterobacterales (Fig. 1c)[2].

Given that it is a widespread effect in enterobacteria and its potential applicability in the design of new therapeutic strategies, we focused on deciphering the underlying molecular mechanism. To achieve this, we performed transcriptomic analyses, and our results suggest that β-lactamase accumulation in the periplasm destabilizes the bacterial envelope, thus increasing AZI permeability and enhancing COL's action. Furthermore, low AZI concentrations kill plasmid-carrying strains at significantly higher rates than plasmid-free strains.

These findings highlight that the physiological side effects of β-lactamases can be leveraged therapeutically, paving the way for the rational design of specific therapies to block plasmid-mediated AMR or at least counteract their effects.

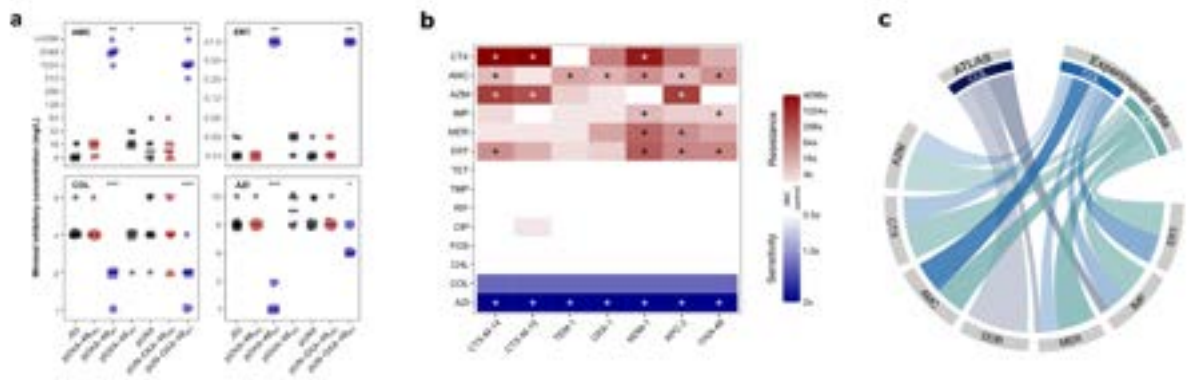


Figure 1: β-lactamase expression induces collateral sensitivity in Escherichia coli. **a.** MIC determination in four antibiotics (AMC, ERT, AZI, COL) for *E. coli* J43 plasmid-free and carrying the pOXA-48 plasmid variants. **b**. Heat map representing collateral responses to antibiotics associated with β-lactamase acquisition. **c.** Antibiotic resistance surveillance data show patterns consistent with β-lactamase-induced CS.

Abstract ID: 1500

Dissecting N2O Isotopic Signatures: Insights into Denitrification Pathways and Enzymatic Contributions

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Co-authors: *Paul Magyar, Jakob Zopfi, Lukas Emmenegger, Moritz F Lehmann, Joachim Mohn*

Nitrous oxide (N_2O), a potent greenhouse gas, is largely driven by anthropogenic activities, especially agriculture. Microbial denitrification is a major source of N_2O, where nitrate (NO_3^-) or nitrite (NO_2^-) is reduced by nitrate reductases (Nar, Nap), nitrite reductases (NirS, NirK), and nitric oxide reductase (NorB). Flavohemoglobin (Fhp), a detoxifying NO reductase, also produces N_2O under anoxic conditions. These enzymes shape N_2O's isotopic composition, including $\delta^{15}N$, $\delta^{18}O$, and site preference (SP), which reflects the intramolecular distribution of ^{15}N. Notably, N_2O produced by NorB shows an SP of ~0 ‰, while Fhp imparts a distinct SP of ~+10 ‰[1], underscoring the importance of enzyme-specific contributions to isotopic signatures.

This study investigates two aspects of N_2O production during denitrification using NO_3^- as substrate. First, we examine how nitrite reductase types (NirK vs. NirS) influence $\delta^{15}N$ and $\delta^{18}O$[2]. Second, we explore SP variations, specifically by replicating conditions that produce higher SP values and using genetic sequencing to identify active genes for NorB and Fhp. NO_3^- reduction experiments were conducted with denitrifying strains *Pseudomonas chlororaphis subsp. aureofaciens* (NirK-type) and *Pseudomonas chlororaphis subsp. chlororaphis* (NirS-type) under batch- and continuous-culture conditions. Quantum-Cascade Laser Absorption Spectroscopy (QCLAS) provided continuous high time-resolution data to track isotopic dynamics ($\delta^{15}N$, $\delta^{18}O$, SP) for all experiments.

Distinct isotopic behaviours between the strains reflected differences in nitrite reductase types. $\delta^{18}O$ variations revealed distinct oxygen isotope exchange dynamics between N_2O precursors and water, shaped by nitrite reductase types (Figure 1, which plots $\delta^{15}N$, $\delta^{18}O$, and SP of N_2O against the fraction of substrate remaining, f). Early-stage SP variability suggests differential NorB activity and possible contributions from Fhp. These findings

highlight the role of enzyme diversity in isotopic dynamics, advancing our understanding of microbial N_2O emissions.

By linking enzyme diversity to isotopic signatures, this study provides new insights into microbial processes underlying N_2O production. Continuous QCLAS measurements enable a nuanced understanding of isotopic dynamics, advancing source attribution strategies for N_2O emission mitigation.

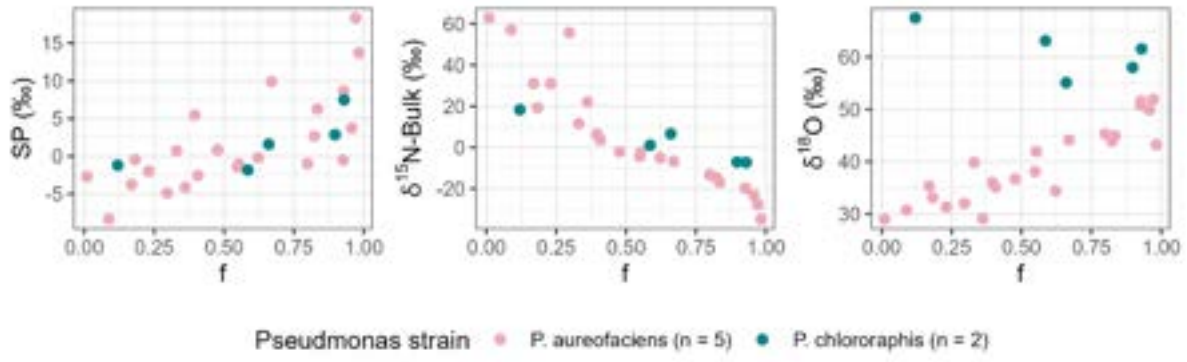


Figure 1: *Isotopic composition ($\delta^{15}N$, $\delta^{18}O$) and site preference (SP) of N_2O produced during denitrification by Pseudomonas chlororaphis subsp. aureofaciens (*P. *aureofaciens) and Pseudomonas chlororaphis subsp. chlororaphis (*P. *chlororaphis), plotted against f, the fraction of substrate remaining. Lower f values represent earlier stages of the reaction.*

Abstract ID: 474 / Poster Number: 157.15P

Distinct adaptive responses of clinical Acinetobacter baumannii to indole stress

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Co-authors: *Woojun Park*

Indole, a bacterial signaling molecule, is critical in regulating various physiological processes, although elevated concentrations can harm bacterial survival. At high concentrations (2.5 mM), indole induced phenotypic changes in both a clinical strain (CS), NCCP 16011, and a wild-type strain (WT), ATCC 17978. These changes were characterized by altered cell morphology and increased outer membrane vesicles (OMVs) production. Short-term (2 hours) killing curve assays demonstrated that CS exhibited significantly greater resistance to indole than WT, maintaining a stable coccobacillus morphology, as observed through scanning electron microscopy (SEM). In contrast, WT displayed an elongated cell morphology under the same conditions. Interestingly, indole-derived pigments, such as indigo and indirubin, were exclusively produced by CS, as confirmed via spectrophotometric analysis. This indicates active indole detoxification pathways that likely enhance the survival of CS. Supporting this, hydroxylamine-based indole assays revealed a marked reduction in indole levels in the supernatant of CS cultures, providing strong evidence for ongoing indole detoxification. Additionally, quantitative analysis of OMV production using the lipophilic dye FM4-64, combined with SEM imaging, showed significantly increased OMV production in CS compared to WT. These findings highlight the need for further genomic comparisons to uncover the mechanisms underlying the observed phenotypic differences and the enhanced indole tolerance in CS.

Abstract ID: 2539 / Poster Number: 129.17P

Distribution of antibiotic resistance genes and antibiotic residues in drinking water production facilities: Links to bacterial community

Presenting author: Dr. Karabo Tsholo, North-West University, Potchefstroom, South Africa

Co-authors: *Lesego Molale-Tom, Suranie Horn, Carlos Bezuidenhout*

There is a rapid spread of antibiotic resistance in the environment. However, the impact of antibiotic resistance in drinking water is relatively underexplored. Thus, this study aimed to quantify antibiotic resistance genes (ARGs) and antibiotic residues in two drinking water production facilities (NW-E and NW-C) in North West Province, South Africa and link these parameters to bacterial communities. Physicochemical and ARG levels were determined using standard procedures. Residues (antibiotics and fluconazole) and ARGs were quantified using ultra-high performance liquid chromatography chemical analysis and real-time PCR, respectively. Bacterial community compositions were determined by high-throughput 16S rRNA sequencing. Data was analysed using pairwise correlation. Although some physicochemical levels were higher in treated than in raw water, drinking water in NW-E and NW-C was safe for human consumption using the South African Water Quality Guideline (SAWQG). ARGs were detected in raw and treated water. In NW-E, the concentrations of ARGs (*sul1*, *intl1*, EBC, FOX, ACC and DHA) were higher in treated water than in raw water. Regarding antimicrobial agents, antibiotic and fluconazole concentrations were higher in raw than in treated water. However, in NW-C, trimethoprim concentrations were slightly higher in treated than in raw water. Pairwise correlation showed significant differences (p-value <0.05) between some bacterial communities and environmental factors. The presence of ARGs and antibiotic residues indicated that antibiotic resistance is not only a clinical-associated phenomenon but also an environmental one. Thus, regulating and monitoring ARGs and antibiotic residues in drinking water production facilities should be regarded as paramount.

Abstract ID: 3396 / Poster Number: 328.16F

Distribution of fibrinolytic and anticoagulant proteases across the phylogenetic tree of some Aspergillus species

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Co-authors: *Anastasia Koval, Anna Shestakova, Sofia Syrtseva, Veronika Liamina*

Anticoagulants and fibrinolytics are two main groups of drugs used for therapy of thrombosis which mortality rate is almost 25 million people worldwide. Currently, several fibrinolytic proteases of microbial origin are used to treat thrombosis; however, despite the abundance of microorganisms producing fibrinolytic proteases, few sources of enzymes with anticoagulant activity have been described. At the same time, a combination of anticoagulant and fibrinolytic agent is crucial to effectively fight excessive thrombi formation. Thus, finding a single producer that secretes proteases with both anticoagulant and fibrinolytic effects would greatly reduce the overall cost of antithrombotic therapy and make it more accessible to different segments of the population.

In present work abundance of fibrinolytic activity and its joint appearance with anticoagulant activity was studied among secreted enzymes of several *Aspergillus* species including representatives of 4 subgenera, 8 sections and 13 series. Both proteolytic activities were assesed spectrophotometrically by the rate of different protein/peptide substrates hydrolysis, fibrinolytic activity was also measured with fibrin plates method. Coexistence of two required activities is characteristic mainly of studied representatives of the series *Versicolores* and *Speluncei* of the section *Nidulantes* which form one clade on the phylogenetic tree of *Aspergillus* species. Discovered correlation between proteolytic activity and phylogeny of one of the most widely used enzyme producers for biotechnology will significantly narrow the spectrum of potentially applicable in antithrombotic therapy species.

Abstract ID: 664 / Poster Number: 035.16P

Distribution of staphylococcal species on the skin of healthy dogs and horses detected by MALDI-TOF spectrometry

Presenting author: Lucia Štempelová, PhD, Centre of Biosciences of the Slovak Academy of Sciences, Košice, Slovakia

Co-authors: *Viola Strompfová, Filip Koľvek, Jana Farbáková*

The skin is the body's largest and most exposed organ system which is colonized by a diverse microbial population. This population comprises especially staphylococci, which play an important role in epidemiology of animals and humans.

The aim of this study was bacterial species identification of strains isolated from healthy skin of horses and dogs with a focus on the genus Staphylococcus. The skin swabs were collected from 50 healthy horses (5 body areas) and 30 healthy dogs (8 body areas). The swabs were inoculated on Mannitol Salt agar and incubated at 37°C for 24–48 h. One or two morphologically different colonies from each sample were selected and identified based on MALDI-TOF mass spectrometry after previous sample preparation.

The most common bacterial species on healthy skin of horses were coagulase-negative staphylococci. Staphylococcus succinus was the most frequently isolated bacterial species detected in 74% of horses (dominated on back, abdomen and muzzle). S. xylosus, S. warneri and S. vitulinus were also abundant species. Interestingly, coagulase-positive and opportunustic pathogen S.aureus was identified only in 6% of healthy horses. On the contrary, the skin of healthy dogs was dominated by the coagulase-positive species Staphylococcus pseudintermedius (80% of dogs). It was predominantly found on the nasal area, chin, inner pinna, interdigital area and perianal area. Coagulase-negative staphylococci (S. hominis, S. warneri, S. capitis) were also found, but less frequently. These results confirm the fact that the staphylococcal composition at the species level can differ in various animal species defined by the skin microenvironment conditions.

Abstract ID: 1370 / Poster Number: 091.17P

Divergent Mechanisms of Signal Transduction in Two-Component Systems: Insights into the ATP-Binding Properties of GraS

Presenting author: Prof. Dasantila Golemi-Kotra, York University, Toronto, Canada

Antimicrobial resistance (AMR) in bacterial pathogens poses a global health challenge, with resistance mechanisms in *Staphylococcus aureus* particularly alarming due to their impact on treatment efficacy. Two-component systems (TCSs) play a central role in bacterial adaptability to environmental stressors, including antibiotic pressure. The GraSR TCS, associated with resistance to cationic antimicrobial peptides (CAMPs), relies on auxiliary factors such as the VraFG ABC transporter and GraX to facilitate signal transduction. GraS, an intramembrane histidine kinase (IM-HK) lacking a traditional extracellular sensor domain, exhibits non-canonical mechanisms of activation and interaction with ATP.

This study investigates the intrinsic ATP-binding properties of GraS using isothermal titration calorimetry (ITC) and compares it to other IM-HKs, including VraS and BceS. While VraS demonstrated robust ATP-binding affinity, GraS exhibited a markedly weak, likely biologically irrelevant, binding affinity despite hosting the conserved histidine kinase motifs. Structural variations between GraS and BceS may underlie this ATP-binding difference. Our data support a model wherein GraS relies on auxiliary components for ATP binding and signal transduction, distinguishing it from canonical autophosphorylating histidine kinases.

These findings highlight the diversity of mechanisms in TCS-mediated signal transduction, emphasizing the importance of accessory proteins in the mechanisms of signal transduction mediated by two-component systems. Understanding these variations may inform the development of antimicrobial strategies to combat TCS-driven resistance in *S. aureus* and other pathogens.

Abstract ID: 3340 / Poster Number: 345.15S

Diverse defense and antidefense systems shape the cloudwater microbiome

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Co-authors: *Rebecca Büschel, Julia Micheel, Manuela Van Pinxteren, Janina Rahlff*

Introduction: Ongoing arms races between bacteria and their viruses (phages) across diverse ecological niches drive the former to evolve intricate and highly specialized defense mechanisms. A considerable knowledge gap remains in our understanding of the comprehensive repertoire of bacterial defence mechanisms, particularly in unique environments like clouds. These niches host diverse microbes (Delort *et al.,* 2017; Péguilhan *et al.,* 2023) and present resource-limited conditions where bacterial-phage interactions influence microbial adaptation. However, the impact of the anti-viral systems of bacteria in the cloud environment and their interactions with viruses are not yet fully understood.

Objectives: Metagenomic profiling of the cloud water microbiome collected from Mount Verde in the tropical Atlantic Ocean was followed by the identification and characterization of defense and antidefense systems in isolated bacterial strains, metagenome-assembled genomes (MAGs), and mobile genetic elements (MGEs). We further studied the distribution patterns of defense systems in the world's oceans.

Methods: In this study, six compact Caltech active strand collectors (Demoz *et al.,* 1996) were used to collect cloud water on Mount Verde (1330 m above sea level) in the tropical Atlantic Ocean (van Pinxteren *et al.,* 2020). DNA was short-read sequenced from iron-flocculated and filtered samples for metagenomics, and additionally, 24 bacterial strains were isolated and whole genomes were sequenced. DefenseFinder (Tesson *et al.,* 2022) was used to identify defense and antidefense systems in 16 genomes of bacterial isolates, 22 dereplicated MAGs, 458 viral operational taxonomic units (vOTUs), and 7,712 plasmid sequences. Additionally, the distribution of homologs of cloudwater-derived defense systems in the major oceans was examined using the Ocean Gene Atlas v2.0 (Vernette *et al.,* 2022).

Findings: We observed a diverse set of antiviral defense systems in the cloudwater bacterial isolates, averaging five per strain, with highest prevalence of restriction-modification systems. Notably, a *Sphingomonas* isolate encoded up to 12 distinct defense systems, underscoring its potential ecological importance in this unique environment. A strong positive correlation between defense system density and the presence of vOTUs indicated active bacterial-viral interactions. These were further supported by CRISPR spacer hits from *Sphingomonas* sp. and *Deinococcus* sp. isolates against cloudwater vOTUs and plasmids. Negative correlations were observed between defense system density and genome size, as well as between defense system density and coding density. This indicates a potential trade-off in genome organization of the host, where larger genomes and higher coding efficiency may limit the relative allocation of space to defense systems. Antidefense systems were also found in the vOTUs, indicating an ongoing molecular arms race between bacteria and phages. Additionally, plasmids within the metagenome encoded both defense and antidefense genes, highlighting the role of MGEs in shaping microbial defense landscapes. The widespread distribution of homologs for these defense systems across the world's oceans further emphasizes their broad environmental significance.

Conclusion: Our study highlights the ecological and evolutionary dynamics between phages and their host in the cloudwater microbiome. The presence of diverse defense and antidefense systems indicates an ongoing adaptation likely driven by environmental pressures. Our data provide important insights into rarely studied viral-bacterial interactions in atmospheric ecosystems.

Abstract ID: 3382 / Poster Number: 027.16P

Diversification of cropping systems for the One Health of soils, plants and humans (DCropS4OneHealth)

Presenting author: Dr. Lena Simon, Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany

Co-authors: *Annette Prochnow*

Introduction: Diversification of cropping systems has the potential to increase biodiversity and support human health.

Objective: The DCropS4OneHealth project pursues a OneHealth approach and aims to test the following hypotheses along a causal chain in the area of agrobiodiversity: (i) Diversification in cropping systems increases farm biodiversity in terms of soil and plant microbiome, vegetation and invertebrate fauna. (ii) The higher diversity of cultivated crops, soil and plant microbiome, vegetation and invertebrate fauna positively affects health-relevant characteristics of the field produce in terms of the produce microbiome and health-promoting plant secondary metabolites. (iii) These improved characteristics foster human health. Thus, relations between biodiversity and human health are mediated and can be analysed via the interlinked microbiomes and the plant secondary metabolites.

Method: The consortium brings together scientists from different disciplines and practitioners. A large-scale on-farm experiment will be established in a commercial farm to test the hypotheses. For the analyses, OMICS technologies will be used, plants and invertebrates will be recorded, and methods of chemo- and bioinformatics and artificial intelligence will be applied.

Expected findings and Conclusion: In exchange with relevant stakeholders, recommendations for favourable diversification measures and for unlocking the benefits for biodiversity and human health will be derived and options for their implementation will be identified.

Abstract ID: 3735 / Poster Number: 301.15P

Diversity and Ecological Roles of Deep-Sea Sediment Microbiomes in the Gulf of Mexico

Presenting author: Dr. Asuncion Lago-Lestón, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico

Co-authors: *Mónica Torres-Beltrán, Mario Hernández-Guzman, Valentín Pérez-Hernández*

The Gulf of Mexico (GoM) is a deep oceanic basin with depths reaching up to 4,000 m. Sediments at these depths harbor highly diverse microbial communities adapted to extreme conditions such as high pressure, low temperature, and limited nutrients. Despite their ecological importance, these communities remain underexplored.

To elucidate the diversity and function of this microbiota, we collected sediment samples at depths from 550 to 4,000 meters, and along of the sediment column, during several oceanographic campaigns. After, they were sequenced using high-throughput technologies (Illumina) and analyzed.

Analyses of the 16S rRNA gene and metagenomes revealed a high microbial diversity, with Archaea comprising nearly 40% in the deepest areas. Depth clearly influenced the microbial diversity and abundance both below sea level and the seabed. We also observed functional redundancy in amino acid and energy production metabolisms in taxa such as alpha and deltaproteobacteria, highlighting the importance of the composition and structure of this microbiota in ensuring environmental resilience. Metagenome-assembled genomes (MAGs) identified 27 bacterial and 3 archaeal genomes, with key metabolic pathways such as sulfate reduction, methanosulfonate degradation, methanogenesis, and nitrate reduction. Notably, four bacterial MAGs carried genes for nearly complete nitrification pathway. Additionally, our work contributes to a better understanding of genes involved in hydrocarbon degradation and alternative metabolic pathways, offering insights into the efficient utilization of hydrocarbons in deep marine sediments.

These findings enhance our understanding of the microbiota's potential for biogeochemical cycling and hydrocarbon utilization in deep-sea environments, establishing a baseline for future research.

Abstract ID: 1090 / Poster Number: 104.16P

Diversity and function of bacterial vesicles of the human gut microbiome

Presenting author: Mr. Andre Geisler, University Clinic of Schleswig-Holstein, Kiel, Germany

Co-authors: *Ana Paula Schaan, Hannah Jebens, Marcus Lettau, Franziska Theilig, Mathilde Poyet, Mathieu Groussin*

The interaction between the human host and its intestinal microbiome is shaped by a multitude of microbial mediators, including bacterial extracellular vesicles (BEVs). BEVs can diffuse over long distances due to their small size, and encapsulate a variety of potent factors, including proteins, metabolites and sometimes virulence factors that shape host-microbiome interplay. Yet, our knowledge on the diversity and function of BEVs produced by gut commensals is very limited. Here, we profiled the BEV molecular diversity of multiple key gut bacterial species and whole microbiomes associated with health and disease. For this, we leveraged the Global Microbiome Conservancy biobank, which contains microbiome resources from a diverse range of human lifestyles from industrialized and non-industrialized countries worldwide. Our preliminary proteomic and genomic investigations revealed that BEVs are produced by a large diversity of gut bacteria in the microbiome *in vivo*, that their composition reflects bacterial phylogeny, and that they are likely shaping mucosal immunoglobulin responses. Further experiments will give additional insight about their mechanistic role in shaping bacteria-bacteria interactions and in contributing to, or protecting against, inflammatory responses. Our project will thus make important contributions to the understanding of BEVs in the context of host-microbiota interaction.

Abstract ID: 3255 / Poster Number: 358.15F

Diversity and metabolic potential of cave microorganisms

Presenting author: Mr. ETTORE LOPO, University of Bologna, Bologna, Italy

Co-authors: *Bernardo Barosa, Francesco Sauro, Jo De Waele, Martina Cappelletti*

Bacteria inhabiting subterranean environments have evolved metabolic strategies to cope with the absence of sunlight and the scarcity of organic compounds. Despite caves can be considered extreme environments for life, they have been found to be hotspots of microbial diversity attracting research interests focusing on the discovery of novel bacteria and metabolic pathways also in relation with the microbial interaction with the rock substrates and the use of alternative metabolic pathways to survive. Microorganisms are also bioindicators of the cave health being some bacterial groups potential proxies of human impact and environmental contamination. The study of the diversity and metabolic potential of microorganisms colonizing caves can therefore provide information on the evolution and protection of subterranean environments and might result in the discovery of novel microbes and metabolites with biotechnological interest. The aim of this work is to (in the framework of the PNRR-DM118/2023 project) explore and compare the microbiology of different gypsum, limestone and quartzite caves to determine the correlation between microbial taxa/functions and environmental parameters and to dig into the microbial roles in different types of subterranean environments. For this purpose, we collected and analysed samples from different caves and applied sequencing technologies, culture isolation and metabolic assays.

The results indicated the presence of microorganisms associated with different caves presenting functions correlated with biogeochemical cycles, the interaction with the rock substrate and the establishment of cooperative and competitive relationships.

In conclusion, our study investigates the biodiversity and metabolic potential of cave microorganisms, highlighting their ecological and evolutionary significance.

Abstract ID: 225 / Poster Number: 179.15P

Diversity in Acclimatory Strategies to Polar Night across Antarctic Phytoplankton: An Integrated Study

Presenting author: Ms. Devon Popson, Miami University, Oxford, United States

Co-authors: *Zev Cariani, Conall McGinness, Cristina Takacs-Vesbach, Peter Doran, John Priscu, Rachael Morgan-Kiss*

In high latitude environments, polar night is a period of continuous darkness during wintertime. Historically, study of the polar night has been minimal as it is logistically challenging for field science and has been regarded as a period of quiescnece. However, recent advancements in autonomous sensors/sampling have revealed dynamic seasonal shifts in microbial community structure. Phytoplankton, the primary producers of photic polar aquatic habitats, are expected to be particularly sensitive to the lack of light. Previous research has proposed a few mechanisms underlying winter survival, but there remains a gap connecting lab-controlled experiments and responses of native algal communities. Here we utilized an integrative approach between field- and lab-based studies to evaluate how key phytoplankton species acclimate to polar night within Lake Bonney (McMurdo Dry Valleys, Antarctica). Seasonal dynamics of dominant algal groups were monitored by year-round autonomous profiling using a spectral Chlorophyll-a fluorometer. Additionally, four Lake Bonney isolates, Chlamydomonas priscui, Chlamydomonas sp. ICE-MDV, Isochrysis sp. MDV, and Geminigera cryophila possessing variable trophic abilities were subjected to mimicked Antarctic seasonal light cycle. Here we found green algae abundance declined during the polar night, whereas mixotrophic haptophytes and cryptophytes increased in abundance during early winter in native Lake Bonney communities. Additionally, chlorophytes species functionally downregulated photosynthesis but retained their photosystems, while the mixotrophic species retained photosynthetic capacity during mimicked polar night and showed increased capacity for complex carbon breakdown. These differing responses to mimicked polar night conditions represent two distinct strategies for surviving the long darkness of polar night.

Abstract ID: 1027 / Poster Number: 313.15P

Diversity of amoebae and bacteria in the environment of cattle herds in bovine tuberculosis outbreak zones

Presenting author: Prof. Yann Héchard, University of Poitiers, Poitiers, France

Free-living amoebae (FLA) are phagotrophic protists that feed on bacteria. However, they are described as environmental reservoirs for some bacteria able to resist their phagocytosis. In the environment, the survival of *Mycobacterium bovis (Mbo)* and *Mycobacterium avium* subsp. *paratuberculosis (Map)*, responsible for bovine tuberculosis and paratuberculosis respectively, remains poorly understood and is considered potentially problematic in the eradication and control of these diseases. Our objective was to test whether FLA may play a role in persistence of *Mbo* and *Map* in the environment.

In this study, 90 samples, water and soils, were collected from herd affected by one or both diseases. FLA were cultured on plate and their DNA extracted. To investigate the diversity of amoeba and their associated bacteria, 16S and 18S rRNA gene amplicon sequencing was conducted by Nanopore. *Mbo* and *Map* DNA was also detected via ddPCR.

FLA were found in all samples and some were isolated. The sequencing analysis revealed that *Acanthamoeba*, *Copromyxa*, *Naegleria* and *Vermamoeba* were the most represented FLA genera. Regarding the bacteria, Pseudomonadota sequences were the most commonly found and some have been already described in previous studies as associated to FLA. *Mycobacterium* were not found in the sequences but traces of *Mbo* and *Map* DNA were detected by ddPCR.

In conclusion, we described a wide diversity of FLA and associated bacteria in this environment. The traces of *Map* or *Mbo* DNA detected by ddPCR, suggests that these bacteria might be present in association with FLA, but in very low amounts in our samples. This may be due to a poor association in the environment or a sampling bias, as we are searching for a needle in a haystack. Further experiments are needed to better understand if FLA could participate to *Mbo* or Map survival and transmission in the environment.

Abstract ID: 3951 / Poster Number: 304.15P

Diversity of Chaetothyriales in Freshwater Sediments of the Spanish Mediterranean Basin

Presenting author: Enrique Monzón, PhD, Universitat Rovira i Virgili (URV), Reus, Spain

Co-authors: *Josepa Gené, José Francisco Cano, Dania García*

Black yeasts, classified within the order Chaetothyriales (Ascomycota), exhibit remarkable morphological diversity and ecological adaptability. These fungi occupy diverse habitats, acting as phytopathogens, human and animal pathogens, or environmental saprobes. While their presence in freshwater sediments has been noted, their diversity and ecological roles remain poorly understood.

To investigate Chaetothyriales diversity in freshwater sediments, samples were collected from rivers and streams in the Spanish Mediterranean Basin during four surveys carried out between 2023-2024. A total of 55 strains were isolated using Potato Dextrose Agar supplemented with Actidione (PDA+A) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC). Preliminary morphological characterization was followed by multi-locus phylogenetic analysis, targeting the ITS and LSU regions, with additional sequencing of tub2 and tef1-α when necessary.

The strains obtained were representatives of 10 species in the family Herpotrichiellaceae, including Capronia pulcherrima, Cladophialophora denticulata, Cladophialophora emmonsii, Cladophialophora irregularis, Exophiala equina, Exophiala oligosperma, Exophiala tremulae, Phialophora americana, Phialophora cf. verrucosa, and Phialophora submersa, and Knufia tsunedae in the family Trichomeriaceae. Phylogenetic and morphological analyses also revealed 12 potential novel taxa across Capronia, Cladophialophora, Exophiala, Rhinocladiella, and Knufia.

These findings underscore freshwater sediments as an underexplored habitat for black yeasts, highlighting the need for further research to clarify their phylogenetic relationships and ecological significance. This study expands our understanding of Chaetothyriales diversity and distribution in freshwater ecosystems.

Abstract ID: 530 / Poster Number: 003.15P

Diversity of Saccharomyces cerevisiae strains isolated from Borassus akeassii palm wines from Burkina Faso in comparison to other African beverages

Presenting author: Dr. Tapsoba François, University Joseph K-ZERBO, Ouagadougou, Burkina Faso; French National Institute for Agriculture, Food, and Environ; ent (INRAE) |, Montpellier, France

Co-authors: *LEGRAS Jean-Luc, DEQUIN Sylvie, SAVADOGO Aly*

In South-West of Burkina Faso, palm wine is produced by spontaneous fermentation of the sap from a specific palm tree Borassus akeassii and plays an important role in people's lives. Saccharomyces cerevisiae is the main agent of this alcoholic fermentation but little is known about the diversity of the isolates from palm. In this work, 39 Saccharomyces cerevisiae strains were isolated from palm wine samples collected from 14 sites in Burkina Faso, as well as 7 isolates obtained from sorghum beer (Dolo) from 3 distant sites. Their diversity was analyzed at 12 microsatellite loci, and compared to the genotypes obtained for other African yeast populations isolated from Cocoa hulks from Ghana, sorghum beer from Ivory Coast, palm wine from Djibouti Republic, and to our database of strains from miscellaneous origins (bread, beer, wine, sake, oaks...). The ploidy of these strains has been assessed as well by flow cytometry. Our results show that B. akeassii palm wine contains a specific yeast population of diploid strains, different from Dolo produced in the same area and from other palm wine strains from Ivory Coast, Nigeria, or Djibouti Republic. In contrast, Dolo strains appeared as a group of related and mainly tetraploid strains despite being isolated from different countries.

Keywords: Palm wine Borassus akeassii Diversity Saccharomyces cerevisiae Microsatellite typing Flow cytometry

Abstract ID: 316

DNA damage response and cell cycle regulation in radioresistant bacterium *Deinococcus radiodurans* that lacks LexA/RecA canonical pathway of it as known in other bacteria

Presenting author: Prof. Hari Misra, NMIMS (Deemed to be University), Mumbai, India

Introduction: *Deinococcus radiodurans* is characterized for its extreme resistance to DNA damaging agents including radiations and desiccation. Although the bacterium adjusts its proteome and transcriptome in response to DNA damage, it lacks LexA/RecA mediated canonical pathway of DNA damage response (DDR) and cell cycle (CC) regulation that is well characterized in other bacteria and is known as a paradigm. Then, our findings on how it responds to DNA damage would be worth presenting during this the most appropriate conference.

Objective(s): To understand the molecular mechanisms underlying the change in genome functions in response to gamma radiation exposure? If it does not obey the paradigm that others follow, then what is the new in this bacterium?

Method: Identified an oxidative stress responsive antioxidant and radioprotector from this bacterium using metabolomics approach. Subsequently, gene knockout studies showed its involvement in metabolism of DSB repair. Identified a couple of proteins having interaction motifs for this molecule and knockouts were created. The gamma radiation response and molecular biology approaches finally pinpointed a protein responsible for responding to both oxidative stress and gamma radiation mediated damages. This was further characterized with potential substrates using mass spectrometric, biochemistry, molecular biology approaches.

Findings: A radiation responsive Ser/Thr quinoprotein kinase (RqkA) has been characterized for its roles in DSB repair and extraordinary radiation resistance. The effects of RqkA phosphorylation on a number of proteins involved in both DNA metabolism and cell division have been characterized. Interestingly, RqKA phosphorylation to DNA metabolic proteins has either stimulated their activities *per se* or has altered the macromolecular interactions required for their better functions. On contrary, RqkA phosphorylation showed negative impact on proteins required for cell cycle in this bacterium (Figure 1).

Conclusion: *Deinococcus radiodurans* deploys a different type of DDR and CC regulation that seems to be an alternate to the LexA/RecA mediated canonical pathways of DDR and CC regulation known in other bacteria. Functional analogies indicated its closeness to eukaryotic type DDR and CC in this bacterium.

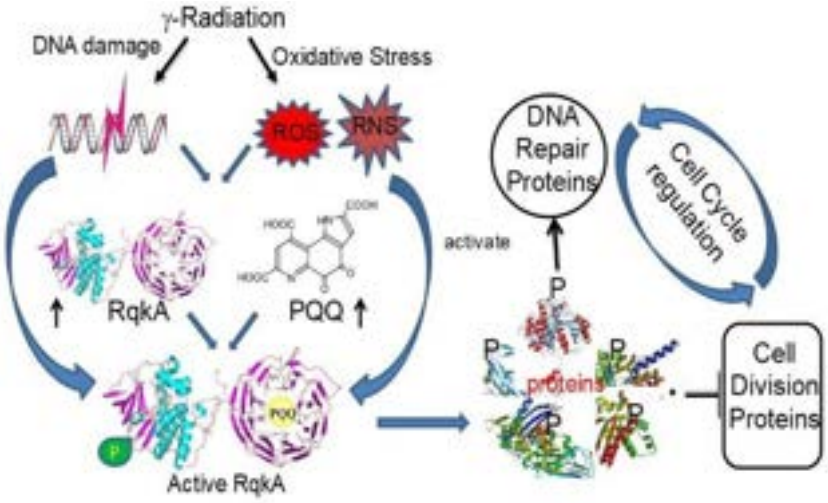


Figure 1: Schematic representation of potential mechanism underlying DNA damage response and cell cycle regulation in *Deinococcus radiodurans*. An antioxidant signalling molecule pyrroloquinoline quinone (PQQ) and transcript encoding RqkA were co-induced in response to gamma radiation. PQQ interacts with RqkA and stimulated the autokinase activity of RqkA leading to the phosphorylation of both DNA metabolism and cell division proteins. While activity of DNA metabolic proteins is favourably enhanced, the activity of cell division proteins is attenuated upon RqkA phosphorylation. This might present an example of a new type of DDR and CC regulation in this bacterium and opened up for further investigation in other bacteria.

Abstract ID: 3757 / Poster Number: 238.16P

DNA polymerase IV, a key player in the phenotypic evolution of the human pathogen Pseudomonas aeruginosa

Presenting author: Dr. Mariela Monti, CIQUIBIC, Córdoba, Argentina

Co-authors: *Consuelo Fernandez*

Pseudomonas aeruginosa (PA) is an acute virulent pathogen that transitions to chronic lung infections. This adaptive process is driven by mutations that inactivate virulence factors (pigments, motility) and enhance traits like biofilm growth, and responds to selective forces including the highly oxidative environment. Identifying factors involved in this adaptation may aid in designing better antimicrobial treatments. One such factor could be the DNA polymerase (Pol) IV, which erroneously incorporates oxidized nucleotides leading to mutagenesis. In the present work, we evaluated the involvement of Pol IV in the phenotypic and genomic evolution of PA under oxidative damage. With this aim, we analyzed a *mutT* deficient strain (T), which accumulates high levels of oxidized nucleotides, and a *mutT mutS*$^{\beta}$ (Tβ) strain, where the repair factor MutS does not avoid Pol IV mutagenesis. We also included the Pol IV-deficient strains *mutT dinB* (TD) and *mutT mutS*$^{\beta}$*dinB* (TβD). In order to study the role of Pol IV in the mutagenesis of the entire PA genome, we performed mutation accumulation experiments in which de novo spontaneous mutations accumulate across the genome randomly. Tβ lines showed increased mutagenesis in cellular pathways involved in the adaptation of PA to chronic infections. An important proportion of Tβ lines displayed lower production of pyocyanin and pyoverdine pigments and proteases, decreased flagellar mobility and increased biofilm growth. Conversely, lines from T, TD and TDβ did not display major phenotypic changes. In conclusion, our work reveals that Pol IV might play an important role in the acute-chronic switch of PA.

Abstract ID: 3566 / Poster Number: 411.15S

Do effects observed in laboratory plasmid transfer experiments translate to ARG levels in natural microbial communities? – A case study on carbamazepine

Presenting author: Ms. Eda Deniz Erdem, Technische Universität Dresden, Dresden, Germany

Co-authors: *Uli Klümper, Thomas U. Berendonk*

Carbamazepine (CBZ) is an emergent chemical pollutant that has been increasingly found in water bodies, mainly due to its widespread use as an anticonvulsant and ability to resist biodegradation. In previous studies, CBZ has been shown to facilitate the intra- and inter-species spread of synthetic plasmids among bacterial communities. However, it is still unclear whether this effect could lead to an increased rate of horizontal gene transfer of plasmids that harbour antibiotic resistance genes (ARGs) in natural microbial communities with low plasmid loads.

In this work, we explored the potential of CBZ to act as a promoter of mobile genetic element (MGE)-mediated ARG horizontal transfer through an experimental evolution approach. For this, a complex community derived from wastewater effluent was exposed to five concentrations of CBZ (0, 0.05, 0.5, 5, and 50 mg/L) for three days with daily passages to replenish nutrients. The changes in the abundances of 26 ARGs and 5 MGEs were quantified via high-throughput qPCR, and the shifts in community compositions were investigated using 16S RNA gene-based amplicon sequencing.

Contrary to previous studies in which high plasmid loads were used, CBZ presence did not result in a robust and all-encompassing spread of ARGs and MGEs in the evolved communities. Only 5 out of 31 genes of interest, two beta-lactam ARGs (bla_{CMY} and bla_{OXA-48}), trimethoprim-resistance *dfrA1*, and two MGE markers (*IS26* and *IncW_trwAB*), showed clear and steady increases across the CBZ concentrations and had a very strong and positive linear relationship with CBZ concentration. For the remaining genes, the effects of CBZ presence on their relative abundances were identified to be inconsistent. This, coupled with the CBZ-induced minor yet significant variations to the microbial diversity, indicates that the sporadic shifts in ARG and MGE levels might be due to the altered fate or selection of ARG/MGE hosts rather than changes in conjugation rates within the community. Furthermore, enrichment analysis of the positively correlating genes based on their levels in the pre-evolved microbial community revealed that for most of them, CBZ exposure merely caused a decrease in the loss rate of the relative abundances during the evolution experiment.

Consequently, the results of laboratory conjugation experiments translate only partly to the effects on the resistome genes levels of natural bacterial communities. Given the poor correlation between laboratory-based and natural community findings, and the rising accumulation of CBZ in various environments, a thorough assessment of its impact on the microbiota that inhabit these areas is necessary.

Abstract ID: 1422

Dose-dependent effects of fungicides on the active microbiome and its functions in agricultural soil

Presenting author: Assoc. Prof. Lea Ellegaard-Jensen, Aarhus University, Roskilde, Denmark

Co-authors: *Marta Gil-Martínez, Lucas Horstmann, Jesper Liengaard Johansen, Athanasios Zervas, Pedro Carvalho, Sanea Sheikh, Kristian Holst Laursen, Flemming Ekelund, Rasmus Kjøller, Carsten Suhr Jacobsen*

The application of chemical pesticides poses the risk of unintended negative consequences on soil biodiversity and ecosystem functioning. In regulatory settings, pesticide risk assessment focuses on soil nitrification as an indicator of negative effects on soil microbial ecosystem function. However, microbial soil communities perform many other important ecosystem functions such as degradation of micropollutants and soil aggregation, which are not considered in the present regulatory framework.

The objective of the present study was to assess dose dependent effects of widely used fungicides the dynamics of agricultural soil microbial biodiversity and functions.

This was investigated in two different experiments: (1) a dose-response soil microcosm experiment applying the commercial pesticide Prosaro (Tebuconazole+Prothioconazole), and (2) a greenhouse experiment testing the effects of fungicide (Axozystrobin+Prothioconazole) application on arbuscular mycorrhiza and soil aggregation w/wo clover. The microcosms were harvested during a 6-month period and subjected to chemical and nucleic acids extractions. The latter were subjected to TotalRNA sequencing to investigate the effects on the active community (rRNA) and its functional gene expression (mRNA). The greenhouse experiment was harvested at two timepoints were hyphal length and aggregate stability were determined in meshbags (placed inside the pots) and bulk soil.

In both experiments, we saw strong dose dependent effects of the fungicide on the soil microbiomes. As expected, soil fungi were negatively affected by the application of the fungicides, seen as decline in hyphal length with increasing fungicide concentrations in the pots with clover (figure 1A), and as a decline in the relative abundance of e.g. Ascomycota in the active microbiome of the microcosm. However, the entire active soil microbiome was also affected by the fungicide concentrations applied in (1) the microcosms, community structure (figure1B) and gene expression profiles annotated by

3 different databases (all ANOVAs $p < 0.05$), and (2) the greenhouse, aggregate stability (ANOVA $p < 0.001$). Datamining of the detailed effects is ongoing.

In conclusion, we have shown that the whole soil microbiome and important functions performed by these can be negatively impacted by application of fungicides, including soil aggregate stability; potentially harming the entire soil health and stability especially upon repeated applications.

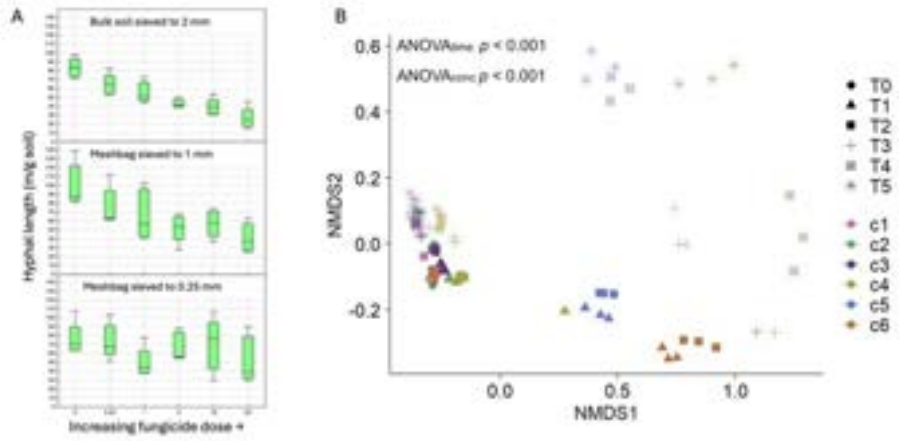


Figure 1: Effects of increasing fungicide dose on A) the hyphal length in the different soil compartments in the clover pots, and B) the entire active microbiome in the soil microcosms. T0-T5 denotes day 0, 3, 8, 24, 65 & 179, and c1-c6 the increasing concentration between 0-242 mg/kg.

Abstract ID: 3362 / Poster Number: 225.17S

Drug repurposing to inhibit Pseudomonas aeruginosa adaptation to the cystic fibrosis lung

Presenting author: Ms. Claudia Ridolfi, Roma Tre University, Rome, Italy

Co-authors: *Marta Mellini, Lavinia Renzi, Paolo Visca, Francesco Imperi, Livia Leoni, Giordano Rampioni*

Introduction: The opportunistic pathogen *Pseudomonas aeruginosa* chronically infects the cystic fibrosis (CF) lung by exploiting the airway mucus (sputum) as a nutritional source during infection and by growing in biofilm, that dramatically increase its antibiotic tolerance. Biofilm formation is finely modulated in response to host-associated stimuli by the 3',5'-cyclic diguanylic acid (c-di-GMP) second messenger. *P. aeruginosa* differs in terms of biofilm formation during growth in the CF sputum compared to standard laboratory media. Moreover, genes essential for *P. aeruginosa* growth in the CF sputum are dispensable in standard media. Hence, unexplored molecular pathways are specifically required for growth and biofilm formation in the CF sputum.

Objective(s): Molecules inhibiting growth and c-di-GMP signaling in a medium mimicking the CF sputum, such as the Synthetic CF sputum Medium (SCFM), may reduce *P. aeruginosa* load and antibiotic tolerance in the CF lung. This project is aimed at identifying and preliminarily characterizing such inhibitors *via* a drug repurposing approach.

Methods: A *P. aeruginosa*-based biosensor strain that allows to simultaneously monitor growth and intracellular c-di-GMP levels has been developed and exploited to screen > 3,000 FDA-approved drugs. The screening has been performed by testing each compound both in SCFM and in MHB-II, a medium commonly used to test antimicrobials. Best hits exhibiting selective efficacy in SCFM have been preliminarily characterized *in vitro* in the reference strain PAO1 and in a small panel of *P. aeruginosa* CF isolates.

Results: This study has led to the identification of hits with promising activity as growth inhibitors against both laboratory-adapted *P. aeruginosa* strains and CF isolates in SCFM, but not in MHB-II, and of one compound that strongly reduces biofilm formation in SCFM.

Conclusion: Drugs with favorable pharmacological properties showing promising antimicrobial or antibiofilm activity in SCFM have been identified. These compounds have the potential to be repurposed to treat *P. aeruginosa* lung infections in people with CF.

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Abstract ID: 2360 / Poster Number: 313.17P

Dual-Culture Assay of Bacillus spp. Antimicrobial Activity Against Fungal Pathogens Relevant to Cereal and Broadleaf Crops

Presenting author: Dr. Simona Chrapaciene, Nando, KAUNAS, Lithuania

Co-authors: *Sidona Sikorskaite-Gudziuniene*

Fungal pathogens such as *Fusarium graminearum*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, and *Bipolaris sorokiniana* are responsible for significant agricultural losses worldwide. Biological control using *Bacillus* strains has emerged as an eco-friendly alternative to chemical fungicides. This study aimed to evaluate the antifungal potential of *Bacillus* spp. against these pathogens under *in vitro* conditions.

Experiments were conducted using a dual-culture assay on tryptone soy agar (TSA) mixed with potato dextrose agar (PDA) media. The fungal pathogens were inoculated alongside selected five *Bacillus* strains, and the inhibition of fungal growth was assessed after 4 and 6 days of incubation at 27°C. Radial fungal growth was measured, and the percentage of inhibition was calculated compared to untreated controls.

Results showed that all tested *Bacillus* strains exhibited antifungal activity, with inhibition rates ranging from 10% to 80% after 4 days and 40% to 65% after 6 days. Among the tested pathogens, *B. sorokiniana* was the most susceptible, with the highest inhibition rates observed. The antifungal effects were attributed to *Bacillus'* production of secondary metabolites, which interfered with fungal growth and mycelial expansion.

In conclusion, the study demonstrates the potential of *Bacillus* spp. as biocontrol agents against multiple fungal pathogens, highlighting their role in sustainable agricultural practices. Further field studies are recommended to validate their efficacy and optimize their application in crop protection strategies.

Abstract ID: 1807 / Poster Number: 238.15P

During Infection, the Insulin-Degrading Enzyme of Vibrio vulnificus Disrupts Host Immune Responses by Breaking Down Chemokines

Presenting author: Yeon-Ran Kim, PhD, Seoul National University, Seoul, South Korea

Co-authors: *You-Jin Kim, Yeong-Jae Seok*

Vibrio vulnificus is a human pathogen causing primary septicemia, a severe bloodstream infection with high mortality. Its virulence relies on strategies that exploit host metabolic and immune pathways, including the insulin-degrading enzyme (vIDE), which is regulated by enzyme IIAGlc of the glucose transport system. This study aimed to elucidate the mechanisms by which vIDE contributes to the virulence of *V. vulnificus*, focusing on its role in metabolic disruption and immune evasion. Using mouse models and in vitro assays, we examined the impact of vIDE on host metabolic processes and immune signaling. We also investigated the interaction between vIDE and IIAGlc in degrading insulin and chemokines. Our results show that vIDE disrupts metabolic processes in infected mice by altering serum insulin and glucose levels, providing *V. vulnificus* with essential nutrients. Additionally, the vIDE-IIAGlc complex degrades specific chemokines, key signaling molecules in the immune system. This degradation weakens the host immune response, facilitating systemic spread and septicemia. vIDE is a multifunctional enzyme critical to *V. vulnificus* virulence. By targeting insulin and chemokines, it disrupts metabolic and immune pathways, enhancing the pathogen's survival and systemic spread. These findings offer insights into *V. vulnificus* pathogenesis and suggest potential therapeutic targets for preventing severe infections.

Abstract ID: 2451 / Poster Number: 440.15P

Dynamic Distribution, Network, and Function of Gut Microbiota in Pigs at Different Growth Stages and Effects of Maternal Microbiome

Presenting author: Yangseon Kim, PhD, Center for Industrialization of Agricultural and Livestock Microorganisms, Jeongeup, South Korea

Co-authors: *Mi Ae Park, Hyun-Jun Jang, Mirieom Park, Dae-Hyuk Kim*

Background: The relationship between intestinal microbiota and growth stages in the swine industry is critical for optimizing health, growth, and overall productivity. This relationship influences various aspects of swine development and health, such as nutrient absorption and metabolism, immune modulation, disease prevention, and physical development. The maternal microbiome significantly impacts the initial colonization and subsequent development of the piglet gut microbiota.

Results: For the dynamic distribution of growth stages, 679 fecal samples were collected from 179 pigs at four time points: the 5th day (lactation), 35th day (nursery), 80th day (growing), and 145th day (finishing) after birth. To discover the effects of the maternal microbiome, 30 maternal fecal samples were collected. The total of 709 fecal samples were analyzed using MGIseq sequencing methods. In the microbiome analysis, microbial richness, diversity, and evenness were significantly lowest on Day 5 and highest on Day 80 compared to other growth stages. The β-diversity of fecal microbiota was significantly separated at different time points. A dynamic shift was observed in the gut microbiota of pigs at different ages and growth stages, with the microbiome of newborn piglets being most similar to that of the mother pigs.

Conclusions: Our study successfully elucidated the dynamic changes in porcine fecal microbiota at various ages and growth stages as stage-associated core microbiota and enterotypes, revealing complex patterns of microbial succession. By mapping these microbiome shifts, this research offers deeper insight into the intestinal health status of pigs at different stages and enhances host-microbe interactions within the swine industry.

Abstract ID: 2038 / Poster Number: 094.15P

Dynamics of Antimicrobial Resistance to Herbicides in Bacteria

Presenting author: Mr. Tuomas Tall, University of Turku, Turku, Finland

Co-authors: *Suni Mathew, Jaime Iranzo, Eero Vesterinen, Lyydia Leino, Miia Rainio, Kari Saikkonen, Irma Saloniemi, Marjo Helander, Pere Puigbò*

Antibiotic resistant (AMR) bacteria are an ever-present threat. AMR spreads rapidly between bacteria. It has been hypothesized that bacteria tolerant to one antibiotic tend to acquire resistance to a second one and (in time) become multi-resistant bacteria. Glyphosate, the most used pesticide globally, has a similar mode of action compared to antibiotics. It targets the key enzyme of the shikimate pathway, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which synthesizes three essential aromatic amino acids. This gene is found in plants, fungi, and bacteria. The widespread use of glyphosate may have an impact on the diversity and composition of microbial communities, including the gut microbiome of farmed animals and humans. Here, we aim to determine the evolutionary dynamics of the *epsps* gene in bacteria by analysing the database of alignable tight genomic clusters (ATGC). We analysed the associations of the evolutionary dynamics of the EPSPS protein with several genomic parameters. The results show that phylogenetic groups and bacterial lifestyles are key factors determining sensitivity to glyphosate. However, previous studies have shown that sensitivity of bacteria may change in a relatively short evolutionary time. Furthermore, it seems that microbes more exposed to the glyphosate are intrinsically more resistant to antibiotics. Future analyses of our dataset should elucidate key factors to gain/loss antimicrobial resistances to glyphosate and other herbicides and pesticides.

Abstract ID: 1611 / Poster Number: 311.16F

Dynamics of bacterial communities in table olive fermentation driven by Lactiplantibacillus plantarum

Presenting author: Dr. Milena Brasca, National Research Council, MILAN, Italy

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Olive fermentation is a crucial step in producing high-quality table olives with desired sensory traits. This study investigated bacterial population dynamics during fermentation by *Lactiplantibacillus plantarum* S61, a strain capable of oleuropein biodegradation and β-glucosidase production, essential for olive debittering. The impact of a heat shock treatment (70 °C for 5 min, repeated three times) applied prior to fermentation, along with the addition of orange peel, on the composition of the microbiota over a 52-day period was also evaluated. Total DNA was extracted from the olives' surface, and the V3-V4 hypervariable region of the bacterial 16S rRNA gene was sequenced using a MiSeq (Illumina) platform with 2×250-base paired-end reads.

Remarkably, the bacterial population demonstrated an increasing trend over the course of the experimental days. Firmicutes along with Proteobacteria were the prevalent groups in samples neither heat-treated nor inoculated and *Clostridium sensu stricto* emerged as the predominant genus, followed by *Lactococcus* and *Lactobacillus*.

The introduction of heat treatment without inoculation resulted in a shift in microbial composition.

Olives i) heat-treated and inoculated with *L. plantarum* and ii) heat-treated, added with orange peel and inoculated with *L. plantarum* emerged as the most species-rich clusters boasting abundant representation of key bacterial species. *Lactobacillus* became the major genus, indicating a successful inoculation. The addition of orange peel, as well as the application of heat treatment and bacterial inoculation, introduced unique dynamics in the microbial community, potentially influencing the overall quality of the olives

Abstract ID: 2163 / Poster Number: 051.15P

Dynamics of vibrio-phage populations under different simulated environmental conditions

Presenting author: Roberto Bastías, PhD, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

Co-authors: *Mauricio Nuñez, Jorge Araya, Daniela Espejo*

Vibrio populations in the environment can be affected by different environmental conditions, including temperature, salinity, and pH. However, the effects of these conditions on Vibrio communities in the presence of phages have been poorly explored. To evaluate this phenomenon, three strains of vibrios, identified by whole genome sequencing as V. splendidus PUCV11, V. splendidus PUCV16, and V. tasmaniensis PUCV18, were isolated, along with three infecting phages named BVM3, BVP20, and BVP25. Short-term microcosm assays (5 hours) were performed under different environmental conditions in the presence and absence of phages. The results showed that in the absence of phages, the V. splendidus PUCV11 strain predominates at 15°C, while the V. splendidus PUCV16 strain predominates at 25°C. In the presence of phages, the V. splendidus PUCV11 strain population decreases at all temperatures. In contrast, the V. tasmaniensis PUCV18 strain increases its proportion to over 60% of the total bacterial population at 15°C and 20°C, and at 25°C, V. splendidus PUCV16 remains nearly 80% of the total population. On the other hand, at different salinities and pH, environmental conditions and the presence of phages also impact the populations of the different vibrios, with a dominance of V. tasmaniensis PUCV18 at 2.3% salinity and pH 5.5. Phages persist at concentrations above 10E5 UFP/mL by the end of most of the assays. These results suggest that alterations in environmental conditions and the presence of phages could favor the proliferation of specific Vibrio strains, highlighting the importance of these studies in the context of global warming.

Abstract ID: 4026 / Poster Number: 246.16P

E. coli as an intratumoural DNA and protein delivery platform

Presenting author: Dr. Munawar Abbas, University College Cork, Cork, Ireland

Co-authors: *Mark Tangney*

Introduction:
Cancer immunotherapy harnesses the immune system to target tumours. Tumour-targeting approaches, such as bacterial delivery systems, offer promising strategies for precise modulation of the tumour microenvironment, given the innate ability of bacteria to selectively replicate within tumours. This study explores the engineering of non-invasive *E. coli* as an intratumoural DNA delivery and protein production platform.

Objective:
To develop *E. coli* as an intratumoural DNA and protein delivery platform, and to validate *in vitro* and *in vivo*.

Methods:
A number of strains of *E. coli* were engineered to i) improve intracellular plasmid delivery to immune cells, and ii) produce and secrete one of a number of murine cytokine proteins. i) To improve plasmid DNA release within phagocytic immune cells, the *hly* gene from *Listeria monocytogenes* (coding for listeriolysin) was introduced and reporter plasmid delivery and expression examined in vitro and in vivo. Ii) A number of murine cytokine sequences (IL-15 and IFN-α) were cloned and expressed in *E. coli*. Protein expression was validated via SDS-PAGE and Western blotting.

Findings:
Hly-engineered *E. coli* mediated reporter plasmid delivery and subsequent expression in murine macrophages was validated *in vitro* and *in vivo*. Cytokine protein production from *E. coli* efficiency varied with protein sequence and secretion signal employed.

Conclusion:
This study establishes a bacterial platform for DNA delivery to tumour-associated phagocytic immune cells and accompanying immune upregulating protein production within tumours. These findings lay the groundwork for bacterial-based immunotherapies involving tumour microenvironment modulation for cancer treatment.

Abstract ID: 3460 / Poster Number: 224.16P

Early detection of frailty syndrome through gut microbiota analysis

Presenting author: Ms. Celia Feria Delgado, Universitat de València (UV), Valencia, Spain

Co-authors: *Vicente Arnau, Wladimiro Díaz-Villanueva, Andrés Moya*

Fragility syndrome (FS) is an age-related disorder characterised by the loss of reserves in numerous organs and systems, thus increasing the individual's vulnerability to even minor external and internal stressors. FS is marked by physiological deterioration and generalised loss of homeostasis, resulting in premature mortality, increased risk of falls, fractures, hospitalisation and institutionalisation in the elderly. Therefore, the ability to diagnose FS is a critical factor that significantly influences patient quality of life and clinical decision-making. Numerous studies have been conducted on microbiota dysbiosis and the development of FS, with the composition of the gut microbiota reported to differ between older people with and without FS [1]. In the present study, a stool sample from a cohort of 200 elderly individuals, composed of both frail and non-frail subjects, was used, given the magnitude and heterogeneity of the data obtained by metataxonomics (16S) and metagenomics (WGS), the use of dimensionality reduction and feature selection techniques was employed to develop predictive models. In addition, an ensemble learning approach has been implemented to improve the predictive capacity. Furthermore, it is possible to obtain clues about the differential microbiota composition that could be related to FS from the characteristics (taxa) selected by the predictive models. This difference can help to detect FS and provide people with the necessary resources.

Abstract ID: 3529 / Poster Number: 298.15P

Ecogenomics of water and sediment Shewanella populations in the Baltic Sea region

Presenting author: Alberto J. Martín-Rodríguez, Karolinska Institutet, Stockholm, Sweden; University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

Co-authors: *Víctor Fernández Juárez*

The genus *Shewanella* includes diverse bacterial species that inhabit environments from oxygen-rich waters to oxygen-depleted sediments. Renowned for their metabolic versatility, *Shewanella* spp. play essential roles in nutrient cycling, global biogeochemical processes, and act as reservoirs of antimicrobial resistance genes, with some species recognized as emerging pathogens[1,2].

Over three years, we isolated and sequenced 130 *Shewanella* strains from sediments and waters in the Baltic Sea region. Approximately 90% were closely related to *S. baltica*, forming a dominant and highly diverse population. Comparative analyses, including phylogenomics, gene flux evaluations, and functional annotations, revealed a species complex of over 20 genospecies with ANI >93.5%, sharing ~2400 core genes.

The evolution of the so-called *S. baltica* complex appears driven by extensive gene gain and loss, elevated mutation rates resulting in over 40,000 single nucleotide polymorphisms (SNPs) across sequenced strains, and horizontal gene transfer (HGT) events contributing to 7.5% of the pangenome. This is significantly higher than other *Shewanella* phylogroups, such as the *S. algae* clade, which exhibits fewer SNPs (32,058) and a lower proportion of its pangenome derived from HGT (4%). Additionally, the *S. baltica* complex harbors a diverse gene pool enriched in functions like sulfite oxidation, Type III and IV secretion systems, or phenylalanine biosynthesis. The accessory genome includes acquired energy acquisition mechanisms, such as DMSO reduction. This underscores that the Baltic Sea region may serve as a hotspot for the diversification and speciation of versatile, ecologically-related strain lineages[3], aligning with the absence of stable ecotype maintenance across the studied populations.

Acknowledgements:

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Abstract ID: 1524 / Poster Number: 350.15F

Ecological interactions drive the assembly of microbiomes exploitable for hydrocarbon bioremediation in polluted soil

Presenting author: Assoc. Prof. Francesca Mapelli, University of Milan, Milan, Italy

Co-authors: *Eleonora Rolli, Gabriele Capriglia, Luigi Bazzana, Elisa Ghitti, Zhang Leilei, Luigi Lucini, Sara Borin*

Introduction: The design of effective nature-based solutions can be deeply impacted by the role of ecological interactions. This can be highly relevant in phyto-rhizoremedation, based on the 'cry-for-help' concept, i.e. the plants' ability to release root exudates and manipulate the rhizosphere microbiome composition.

Objective: We focused on historically polluted sites to identify plant species, bacterial strains and communities aiming to decrease hydrocarbons (HCs) concentration below the legal threshold.

Methods: Metataxonomic, metabolomic, culturomic and qPCR analyses were applied to disentangle the response of the soil microbiome to phyto-rhizoremedation practices.

Findings: Among different tested plant species, sunflower was able to cope with the phytotoxicity of the target polluted soil and was exploited for the recruitment of the rhizosphere microbiome. A bacteria collection was established and the large majority of the strains was able to grow on different aliphatic and aromatic HCs, had the potential to alleviate plant stress by phytohormone balance and to make hydrophobic pollutants more bioavailable.

Molecular analyses showed that the rhizosphere microbiome selected in response to the plant growth (biostimulation) or by the plants inoculated with degrading strains (bioaugmentation) were significantly different in terms of taxonomic composition and degrading potential. Consequently, novel SynComs have been designed as tools for steering the bioremediation potential of the native soil microbiome.

Parallel in vitro experiments revealed that specific molecules were released by sunflower in response to HCs presence, suggesting a possible role in the microbiome recruitment.

Conclusion: Ecological interactions reshape the rhizosphere microbiome in polluted soil selecting specific populations for bioremediation approaches.

Abstract ID: 3500

Ecological interactions of native strains from the apple blossom microbiome that antagonise against the causal agent of Fire Blight in Apples: a phenogenomic approach

Presenting author: Dr. Germán Bonilla-Rosso, Agroscope, Zürich, Switzerland

Co-authors: *Tina Segessemann, Paula Bellés Sancho, Gabriella Pessi, Ueli Von Ah, Franco Widmer, Cosima Pelludat, Christian Ahrens*

Understanding the ecological interactions between bacterial pathogens and their natural antagonists is essential for developing sustainable agricultural practices. Fire blight (FB), a devastating disease caused by the pathogen *Erwinia amylovora* (Ea), infects pome fruits such as apple, pear, and quince through the flower blossom. Consequently, most FB control methods aim to prevent flower infection. However, the most effective measures, such as copper and streptomycin treatments, are unsustainable, while existing biocontrol agents show inconsistent efficacy. Interventions focusing on the native microbiota hold promise but require a deeper mechanistic understanding of the flower microbiome, which is currently lacking.

The flower microbiome of a low-input experimental apple orchard in Switzerland, which has never experienced FB outbreaks, was found to be dominated by strains of *Rosenbergiella* sp. and *Erwinia tasmaniensis* (Et), both non-pathogenic species. To understand their ecological interactions with Ea, we employed an integrative phenogenomic approach, incorporating (i) phenotypic microarrays assessing the utilization of 190 carbon sources, (ii) pairwise competition assays, (iii) genome sequencing, and (iv) genotype-to-phenotype mapping.

Our analyses revealed that despite their close phylogenetic relationship, Ea genomes are smaller, more conserved within strains, and less variable in gene content compared to Et genomes. While both species utilized 30 common carbon compounds, they differed in the efficiency utilising major metabolites from the blossom environment. Ea efficiently metabolized glucose, sorbitol, and D-glucosamine, whereas Et favoured fructose, proline, glutamate, asparagine, arabinose, and gluconate. The presence of identical metabolic pathways for their utilisation suggests the differences likely arise from regulatory mechanisms or overall cellular stoichiometry.

Et strains demonstrated greater versatility in metabolizing alternative carbon sources, such as maltose, turanose, palatinose, maltitol, xylitol, and inositol—substrates

unavailable to Ea. Moreover, all Et strains uniquely utilized amygdalin and arbutin, which Ea could not, potentially due to the absence of a putative glycosyl hydrolase identified in Et genomes. Unexpectedly, both species were capable of pectin utilization despite lacking known pectin degradation pathways, suggesting the existence of a novel pectinolytic mechanism.

Competition assays revealed that Ea growth was significantly inhibited when co-cultured with Et, with the effect being density-dependent and most pronounced in contact-dependent conditions, suggesting direct cell-to-cell killing. Supporting this, variable regions in Et genomes were enriched with genes related to plasmid mobilization and bacterial warfare, including an extensive array of Type VI secretion systems (T6SS), restriction-modification, and toxin-antitoxin systems.

Our findings suggest that Et employs both competitive exclusion through superior resource utilization and direct interference via cell-to-cell killing to suppress Ea in the flower blossom niche. This study underscores the value of integrated functional genomic approaches to obtain mechanistic understanding of ecological interactions in order to develop microbiome-based interventions that leverage native microbial diversity for sustainable crop protection, offering a viable alternative to chemical treatments and enhancing the resilience of agricultural ecosystems.

Abstract ID: 1049 / Poster Number: 016.16P

Ecological Memory of Prebiotic Exposure in a Human Gut Microbiome Model

Presenting author: Mr. Killian Scanlon, APC Microbiome Ireland (University College Cork), Cork, Ireland; School of Microbiology, University College Cork, Cork, Ireland

Co-authors: *Natalia Soledad Rios Colombo, Christopher Jason Richard Turkington, Paul Ross, Colin Hill*

Introduction: Ecological memory describes the capacity of past states or experiences to influence future responses in communities. Community composition and function will normally change in response to an intervention such as the introduction of a prebiotic. The time taken to make this change may be dependent on past exposures to the same or similar interventions. In microbial ecosystems, ecological memory may essentially be a product of hysteresis. This type of memory may have significant implications for how microbial communities respond to recurring stimuli, stressors and environmental changes. It has been shown that ecological memory of prior carbohydrate exposure can be quickly imprinted on gut microbiome composition and this imprint can persist for days (1). However, the full duration and extent of ecological memory is unknown.

Objectives: Using a synthetic highly complex faecal-derived microbial community we set out to examine the extent to which ecological memories can be created to the prebiotic inulin.

Method: Through inulin exposure, memory is seeded by shifting the community to an enhanced inulin-adapted state. After this state is achieved, the community is starved of inulin and daily readings of inulin metabolic capacity are taken to track the duration of ecological memory. Additionally, as the ecological memory of inulin fades, aliquots of the community will be re-exposed to inulin to determine the speed of subsequent adaptations.

Conclusion: Understanding this phenomenon may have consequences for gut health. Specifically, determining how new metabolic capacity can be gained in the human microbiome with habitual fibre intake and how long this ecological memory will persist.

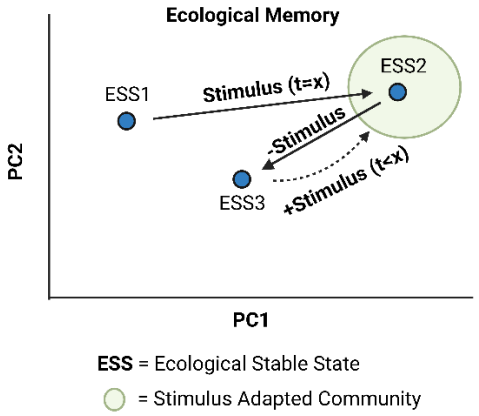


Figure 1: **Ecological memory** is the capacity of past states or experiences to influence present or future responses of the community. Microbial ecological memory may essentially be hysteresis, the phenomenon of a lag in composition and function in response to changes in the environment. In the initial environment, the microbiome occupies an ecologically stable state (ESS1), but after encountering an environmental change or stimulus the community transitions to a second stable state, ESS2. After removal of the stimulus, the community may occupy an intermediate stable state (ESS3) rather than reverting to ESS1. This ESS3 community retains a 'memory' of the initial stimulus and may return quicker to ESS2 upon return of the stimulus.

Abstract ID: 150

Ecology of multiorganism biofilms colonizing outdoor monuments in response to climate change: the case of Assistens Kirkegård

Presenting author: Assoc. Prof. Michela Gambino, The Royal Danish Academy – Architecture, Design and Conservation, Copenhagen, Denmark

Introduction: Stone monuments worldwide are endangered by multiorganism biofilms, well-structured communities of bacteria, fungi and algae, enclosed in a protective matrix. Biofilms can be particularly problematic when the monument is outdoors, because of limited or no possibility of controlling climate, and thus the biofilms' growth. To protect our cultural heritage, conservators clean the monuments, but biofilms promptly grow back. To design more effective and sustainable solutions, we need to understand how multiorganisms biofilms response to treatments and to environmental changes will affect the stone monuments.

Objectives: By studying multiorganism biofilms colonizing six historical stone monuments, we aim to propose an ecological model of organisms succesion on monuments, and to answer the following questions:

1. which pioneer species survive the conservation treatments?
2. how do biofilms respond to the environment and change with time, and which species ensure its stability (keystone)?
3. how do bacteria, fungi and algae organize in a biofilm and how they interact with monuments?

Method: Here, we use a longitudinal study (September 2023 to August 2024) to describe and monitor the evolution of bacterial and fungal biofilms colonizing the stone surface of six tombstones at the Assistens Cementery in Copenhagen. With a conservation treatment in October 2023, the biofilm was removed from the six tombstones, but it is expected to grow again in few years. To monitor the biofilm development and identify the pioneer species, we collected samples for microbiome analysis just before the treatment and for a year after, every two months. Light, temperature and humidity has been measured at each sampling points and environmental parameters were recorded continuously for a year in the Cemementery. In addition, pre-conservation samples have been processed for isolation of phototropic and heterotrophic microorganisms to

reproduce multiorganism biofilms in the laboratory and investigate strategies for biofilm removal with high throughput screening techniques.

Findings: Biofilms colonizing the analyzed stone monuments stones are complex communities of both phototrophic and heterotrophic microorganisms, supporting each other metabolism and resilience to stress. From pre-conservation treatment samples, we could isolate, store and taxonomically identify 18 fungi, 14 bacteria and 17 algae from the six tombstones. We are now processing all samples collected at six time point during one year for metagenomic analysis (16S for bacteria, ITS for fungi and 18S for algae) in order to identify the pioneer species that survive the conservation treatments and reproduce and validate the observation in the laboratory with multiorganism biofilms growing on stone mockup samples. Metagenomic data will be also used to identify specific time point to further process with shotgun sequencing and add details to identify keystone species and gaining insights on the interaction between biofilms, environment and material.

Conclusion: The project is expected to shed light on the interactions within multiorganisms biofilms and in response to environmental changes and conservation treatments. we will link the acclimation and evolution of multiorganism biofilms with material deterioration and investigate their resistance to treatments for biodeteriogens removal and inactivation, currently used in conservation.

Abstract ID: 3240 / Poster Number: 227.16P

Ecotoxicological and metabolic impacts of anti-seizure medications on faeces-derived microcosms and microbial modulation of drug bioavailability

Presenting author: Dr. Zehra Esra Ilhan, Université Paris-Saclay, INRAE, AgroParisTech, Jouy-en-Josas, France

Co-authors: *Catherine Philippe, Andrea Marchetto, Philippe Gérard, Stéphane Auvin, Patricia Lepage*

Anti-seizure medications (ASMs) are commonly prescribed to manage symptoms of epilepsy, yet one-third of the patients resist treatment with the underlying reasons remaining elusive. Investigating the bi-directional interactions between the gut microbiome and ASMs may provide insights into their pharmacokinetics and pharmacodynamics. Our study aimed to characterize the impact of ASMs and their formulations on the gut microbiome and metabolism, and examine the microbiome's role in ASMs bioavailability. We exposed five faeces-derived microcosms to 10 ASMs (20 – 1000 µM) and used a multi-omics approach, including 16S rRNA gene sequencing, high-performance liquid-chromatography, and global metabolomics, to analyse microbial community structure, metabolism, and the fate of ASMs (carbamazepine and lamotrigine). Our results showed that lamotrigine and carbamazepine had the largest impact on microcosms, while phenytoin and topiramate had the lowest. The effect size was more pronounced for *Bacteroides*- and *Bifidobacterium-dominated* microcosms while *Clostridium*-dominated microcosms were resilient. Syrup formulations had larger impact than active ingredients, particularly depleted *Eggerthella* genus and increased *Anaerostipes* by 50%. Lamotrigine decreased the relative abundances of *Escherichia*, *Faecalibacterium*, and *Gemmiger* while increasing *Enterococcus* and *Clostridium* in a dose-dependent manner. Lamotrigine altered microbial metabolism, reducing the production of 28 metabolites (mainly amino and nucleic acids) and increasing the consumption of 18 metabolites (mainly dipeptides). Lamotrigine dissipation varied between 5-26% corresponding to 25-130 µM whereas carbamazepine dissipation remained <5%. The highest lamotrigine-degrading cultures were enriched in *Enterococcus* and *Eggerthella* sp. Our results indicate that ASMs can disrupt gut microbiome composition and function and that ASM bioavailability may depend on microbiome composition.

Abstract ID: 2676 / Poster Number: 101.15F

Effect of a constructed technosol on the microbial functional diversity in an urban afforestation site

Presenting author: Simon Masson, PhD, University of Turin, Turin, Italy

Co-authors: *Matteo Chialva, Luisa Lanfranco*

The increasing expansion of urban environments around the world highlights the need to apply nature-based solutions (NBS) that can support the development of green urban areas, mitigate environmental and health risks and enable the recovery of degraded soils. Constructed technosols (CT), i.e. artificial soils composed of mineral materials and by-products (technogenic materials, soil from urban excavation) and organic biomasses designed to support plant growth, are promising tools to remediate contaminated sites and reduce the exploitation of natural soils.

In this study, using a multidisciplinary approach we aimed to characterise the long-term impact of a CT on soil microbial communities at the taxonomic and functional levels in a site located in the city of Turin (Northern Italy) and linked it to soil physico-chemical features and vegetation. The microbial functional diversity in the CT was profoundly different from that of the surrounding control soil, and the application of compost and microbial inoculum in the original CT appears to have contributed significantly to the microbiota assembly. These differences include an increase in prokaryotic diversity and an impact on nitrogen, phosphorus and sulphur metabolism. These results indicate that tailored design CTs can be powerful tools for NBS solutions in urban afforestation interventions that can have significant impact on soil microbiota composition and functioning with potential implications on plant growth and health.

Abstract ID: 4059 / Poster Number: 305.16S

Effect of aeration mode on the activity of nitrogen-cycling microorganisms and nitrous oxide emission in the wastewater treatment plant

Presenting author: Ms. Sahar Babazadeh, University of Stuttgart, ISWA, Stuttgart, Germany

Co-authors: *Sergry Abramov, Quirin Goetzke, Martin Reiser, Sara Klinedinst*

Wastewater treatment plants (WWTPs) contribute approximately five percent of annual anthropogenic nitrous oxide (N_2O) emissions to the environment, with N_2O having a global warming potential 265 times greater than carbon dioxide and a long lifetime in the atmosphere (121 years). Incomplete denitrification is one of the primary sources of N_2O emissions in WWTPs; however, the impact of aeration regimes and denitrification on N2O emission by activated sludge remains unclear. To address this knowledge gap, we applied a combination of hydrochemical, molecular biological (i.e., 16S rRNA gene amplicon sequencing, RT-qPCR) analyses and gas emission quantification by infrared spectroscopy to quantify (i) N_2O emissions, (ii) concentrations of dissolved nitrogen species (NO_2^-, NO_3^-, NH_4^+, N2O), and (iii) copy numbers of functional genes involved in denitrification (i.e., *napA, narG, nirK, nirS, norB,* and *nosZ*) in the aeration tank of a WWTP in southwestern Germany. Preliminary results indicate that NO_3^- accumulation correlates with increased N_2O emissions. N_2O emissions by activated sludge decreased during aeration phases, while sufficiently long nonaerated phases enabled complete denitrification. Optimizing operational strategies, such as adjusting aeration regimes, could reduce N_2O emissions, contributing to more sustainable and climate-friendly wastewater treatment practices.

Abstract ID: 3946

Effect of biostimulation from Limnospira indica polysaccharides on microbiome modulation and plant resilience

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Co-authors: *Alice Delacuvellerie, Ruddy Wattiez*

Introduction Abiotic stress, such as hydric stress, threatens food security under challenging environmental conditions. Cyanobacteria-based biostimulants, such as *Limnospira indica*, have emerged as promising tools for enhancing plant resilience (Renaud et al., 2023). This study investigates how *L. indica*-based biostimulants influence tomato (*Solanum lycopersicum*) performance under hydric stress and explores their impact on the rhizosphere microbiome. This research is conducted within the framework of the European Space Agency's MELiSSA (Micro-Ecological Life Support System Alternative) project (Lasseur et al., 2010), which aims to develop regenerative life support systems for long-term space missions.

Objective We aimed to (i) evaluate the physiological responses of tomato plants treated with *L. indica* under normal and under hydric stress conditions, (ii) characterise the influence of *L. indica* on plant-microbe interactions, and (iii) analyse shifts in the rhizospheric and endospheric microbial community induced by the biostimulant treatment.

Methods Tomato plants were subjected to two watering regimes: well-watered (80% field capacity) and drought-stressed (40% field capacity). Plants were treated with *L. indica*-based biostimulants (from culture biomass (BM) or culture media (CM) in normal growth condition (N+) or with nitrate deficient (N-) environment) known for their beneficial metabolites production such as their polysaccharides, and physiological measurements, size, number of leaves and flowers, chlorophyll content, flavanol and anthocyanin indices, and stomatal conductance were recorded at multiple time points (early morning, noon, night). Nanopore sequencing of *L. indica* was performed to identify genus linked to plant growth promotion (PGPB). Metagenomic analysis was further conducted using shotgun sequencing to assess bacterial function in response to biostimulant application. The results were analysed in the context of space agriculture, aligning with MELiSSA's goals of optimising biological resource recycling for extraterrestrial habitats.

Findings Physiological data revealed that biostimulant-treated plants exhibited improved flower production and a better stress resistance in case of hydric stress. Genomic analysis highlighted key plant growth promoting bacteria (Figure 1) development in presence of *L. indica*-based biostimulants which may contribute to plant growth improvement and better stress tolerance. Metagenomic functional annotation of metagenomic reads suggested enhanced microbial activity related to plant growth promotion through nutrient cycling.

Conclusion Our findings demonstrate that *L. indica*-based biostimulants increased the number of flower produced by *S. lycopersicum* and confer a hydric stress resilience. This study provides novel insights into the functional role of *L. indica* polysaccharides in plant-microbe interactions, supporting its potential application in sustainable agriculture and controlled-environment farming for space missions. The integration of these results into the MELiSSA framework highlights the feasibility of using cyanobacteria-based biostimulants in closed-loop ecosystems designed for long-duration space travel while cyanobacteria are also present for their food and oxygen supply to the station.

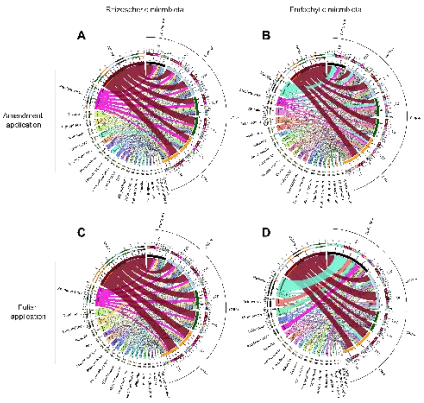


Figure 1: Circos graphs (Krzywinski et al. 2009) representing the relative abundance of rhizospheric microbiota (A and C) and endophytic microbiota (B and D) at T0, T20 and T35 following the application of L. indica culture media-based biostimulant via amendment (A and B) and foliar (C and D) treatment.

Abstract ID: 3381 / Poster Number: 279.15P

Effect of Carbon and Nitrogen on growth of an oligotrophic and extremotolerant fungus

Presenting author: Mr. Abolfazl Dehkohneh, Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany; Freie Universität Berlin, Berlin, Germany

Co-authors: *Ruben Gerrits, Julia Schumacher, Jan-Ulrich Kreft, Anna Gorbushina*

Introduction: Rock-inhabiting black fungi are renowned for their constitutive melanin production, extremotolerance and ubiquitous colonization of oligotrophic, natural or anthropogenic subaerial surfaces, including marble monuments and solar panels. *Knufia petricola* is a model for these fungi as it is genetically amenable and has their typical characteristics. It is capable of colonizing and deteriorating minerals and produces extracellular polymeric substances that facilitate attachment.

Objectives: The objective of this study is to understand how such an organism is able to grow in environments depleted of C and/or N.

Method: *K. petricola* growth parameters (colony extension and thickness, biomass, CO_2 respiration and carbon-use efficiency (CUE)) were analysed for different C to N ratios (C:N), changing both the C and N concentrations and sources. The study utilized a dual-scale approach, examining biofilm formation on agar (macroscale) and growth of a single cell into a microcolony in a microfluidic device (microscale). This methodology provided insights into the nutritional requirements and growth behaviours of *K. petricola* across different spatial scales.

Findings: Our macroscale analysis indicated optimal growth at the C:N ratio of 60. The morphological analysis demonstrated that when nitrate is utilized as the N source, colonies tend to exhibit filamentous growth at their edges, especially when glucose is available as C source. In contrast, when ammonium is the N source, the colony surface appears smooth (Fig. 1A). Moreover, C or N depletion led to higher substrate penetration, mostly through filaments at the edge of the biofilm. Notably, the lowest carbon concentration yielded reduced biomass C:N ratio and the peak biomass C:N ratio was observed at the medium C:N ratio of 60, suggesting a potential correlation between C:N ratio and optimal growth (Fig. 1B). The CUE was however quite low, ca. 0.4-0.6, being lowest when C and N were limiting. Concurrently, microscale analyses of single cells revealed that increasing the C:N ratio from 0.6 to 60 caused (1) shorter cell cycles and (2)

more branching, no branching formed without C and N (Fig. 1C). This led to a denser colony at optimal conditions and a rather extended morphology under C or N limitations.

Conclusion: The findings demonstrate that the depletion of either carbon or nitrogen results in enhanced substrate penetration, predominantly through edge filaments. Notably, the optimal growth and peak biomass C:N ratio of 60 suggest both a correlation with and importance of the medium C:N ratio. Specifically, the lowest carbon input led to a reduced biomass C:N ratio, while the peak biomass C:N ratio was achieved at the medium C:N ratio of 60.

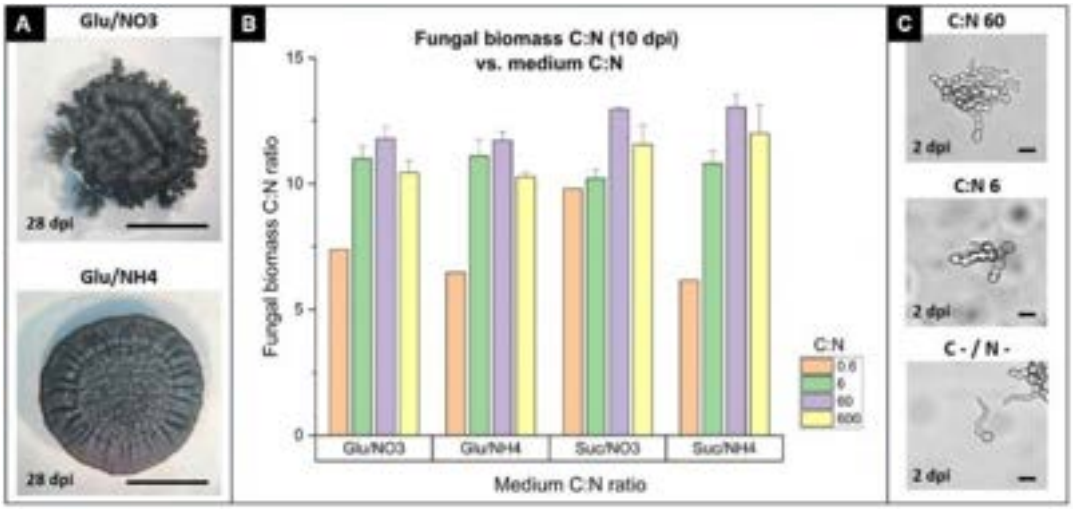


Figure 1: The effects of various sources and concentrations of C and N on the growth of *K. petricola*. (A) Colony morphology at a C:N ratio of 60. With ammonium, the colonies were smooth and flat, with nitrate, the colonies had filaments on the edge. Scale: 5 mm. (B) Fungal biomass C:N ratio against medium C:N ratio. The lowest biomass C:N ratio was observed at a medium C:N of 0.6, while the peak biomass C:N ratio was observed at a medium C:N ratio of 60. (C) Single cell growth of *K. petricola* in different media. Shorter cell cycles and more branching were observed at a C:N of 60. Scale: 20 µm. dpi: days post inoculation.

Abstract ID: 2972 / Poster Number: 062.15P

Effect of cationic and anionic surfactants on nitrification rate in activated sludge obtained from a municipal wastewater treatment plant in southwest Germany

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Co-authors: *Sergey Abramov, Raul Barahona, Peter Maurer, Katharine J. Thompson, Sara Kleindienst*

Background: Over 70% of surfactants are used in household applications and the majority ends up in municipal wastewater. Before being discharged into the environment, wastewater is treated in a wastewater treatment plant (WWTP). One of the key steps in wastewater treatment is ammonia removal via nitrification.

Objective: To quantify the effect of anionic and cationic surfactants on the nitrification rate in activated sludge.

Methods: Two nitrification inhibition suspension experiments based on DIN EN ISO 9509 were performed with activated sludge obtained from an aerated tank of the WWTP (LFKW, Stuttgart, Germany). Linear alkylbenzene sulfonates (LAS), an anionic surfactant, previously detected in the inflow and primary sedimentation tank of the LFKW, were tested in concentrations ranging from 0.2 to 100 mg/L. Additionally, dodecyl trimethylammonium chloride (DTAC), a cationic quaternary ammonium surfactant, was tested in concentrations ranging from 0.1 to 200 mg/L.

Findings: LAS did not cause inhibition of nitrification in concentrations up to 20 mg/L. DTAC demonstrated a dose-dependent inhibitory effect on nitrification in concentrations as low as 1 mg/L. The nitrification rate decreased by 30.4% in a 4-hour test with 1 mg/L DTAC and by 44.3% with 10 mg/L DTAC. The addition of 200 mg/L DTAC caused sludge coagulation, inhibiting nitrification by 99.2%.

Conclusion: The results indicate a prominent dose-dependent inhibitory effect of DTAC on nitrification. The lack of an observed inhibitory effect of LAS may partly be attributed to adaptation of the activated sludge microbial community to LAS due to prior exposure to trace amounts in the nitrification tank.

Abstract ID: 1030 / Poster Number: 204.15P

Effect of CRP global regulator on Escherichia coli ATPase activity under fermentation of different glucose concentrations

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Cyclic AMP receptor protein (CRP) of *Escherichia coli* is a master regulator that primarily functions under glucose-depleted conditions. It regulates the expression of hundreds of genes, helping *E. coli* adapt to changing environmental conditions. This study investigated the role of the CRP regulator on ATPase activity of *E. coli* during fermentation of different glucose concentrations. BW25113 wild-type (WT) and the *Δcrp* strain lacking CRP were used.

Bacteria were grown in peptone media without glucose, with the addition of low (2 g l^{-1}) and high (8 g l^{-1}) glucose concentrations. ATPase activity was determined by measuring the amount of inorganic phosphate (P_i) produced during the ATP hydrolysis reaction by bacterial membrane vesicles.

When cells were grown in low glucose media, ATPase activity of the WT was ~30% lower compared to cells grown without glucose or with high glucose. In the absence of glucose, ATPase activity may increase due to stress adaptation, while low glucose reduces the metabolic rate, leading to decreased enzyme activity. In high glucose, ATPase activity is upregulated due to a higher metabolic rate and the need to expel protons and acidic byproducts. When grown with low glucose, ATPase activity was ~40% higher in the *Δcrp* strain compared to the WT.

Taken together, it can be concluded that CRP plays a critical role in cell signaling and helps regulate metabolic pathways according to environmental conditions. The absence of this regulator disrupts cell metabolism, resulting in the *Δcrp* strain not exhibiting any differences in ATPase activity based on glucose concentration.

Abstract ID: 1499 / Poster Number: 155.16P

Effect of garlic extract on regional cerebral blood flow and peripheral blood flow changes

Presenting author: BYUNG-CHEUL SHIN, Pusan National University Yangsan Hospital, Yangsan, South Korea

Co-authors: *Jisoo Baik, SEONG-JANG KIM, CHERRIE AHN, YONG-IL SHIN*

Regional cerebral blood flow (rCBF) issues can lead to serious conditions such as ischemia and cell necrosis. Improving rCBF is a global health priority, often targeting vascular inflammation reduction. Garlic extract (GE), known to stimulate nitric oxide (NO) production, offers a promising alternative to drugs with potentially fewer side effects, but clinical trials are limited. This study evaluated the effects and safety of GE on rCBF and peripheral blood flow (PBF).

Approved by Pusan National University Yangsan Hospital (Approval No. 04-2020-036), the study included 28 healthy participants (15 in the GE group and 13 in the placebo group). The GE group consumed 450 mg encapsulated GE, while the placebo group took a placebo. rCBF was measured using SPECT, and PBF was assessed via body heat changes using Digital Infrared Thermographic Imaging (DITI).

Results showed significant rCBF improvements in the GE group in the right frontal lobe (Brodmann areas 6, 9, 10) and right parietal lobe (Brodmann area 2). DITI analysis indicated increased body heat in the forearm, palm, and plantar regions, reflecting improved PBF. These findings suggest GE positively affects systemic blood flow, aligning with its NO-mediated effects on circulation.

In conclusion, GE demonstrates potential in enhancing rCBF and PBF. Future studies with larger sample sizes, long-term dosing, and diverse populations are recommended to validate these findings and further explore GE's therapeutic benefits.

Abstract ID: 1773 / Poster Number: 301.17P

Effect of Lactobacillus curvatus-based feed additive on growth performance and fecal microbiota in finishing pigs

Presenting author: Dr. Yangseon Kim, Center for Industrialization of Agricultural and Livestock Microorganisms, Jeongeup, South Korea

Co-authors: *Soyeon Park, Yu Rim Shin, Dae-Hyuk Kim,*

Introduction: *Lactobacillus*-based feed additives are known to enhance the nutritional value of feed, benefiting pig health and production. However, the effects of *Lactobacillus curvatus*-based supplementation on growth performance and fecal microbiota are not yet fully understood.

Objective: The study aimed to evaluate the effect of *L. curvatus* CACC879 supplementation on growth performance and intestinal microbiota in finishing pigs.

Methods: A total of 20 finishing pigs were randomly divided into two groups, with 4 pens/group and 5 pigs/pen: (1) a basal diet without supplementation (control), and (2) a basal diet supplemented with 0.25% *L. curvatus* CACC879 (1.2×10^{10} CFU/g). The gut microbiome of both groups was analyzed using a 16s rRNA amplicon sequencing.

Results: The average daily gain of the CACC879 group tended to increase compared to the control group, while the average daily feed intake showed a tendency to decrease ($P > 0.05$). Moreover, energy digestibility increased, and the feed conversion ratio was reduced in the CACC879 group compared to the control group. The gut microbiome remained stable in the CACC879 group, as confirmed by the alpha and beta diversity analysis. Analysis of the microbial community revealed that gut healthy-related genera, *Butyrivibrio, Lactobacillus,* and *Bifidobacterium*, were higher in the CACC879 group compared to the control. Additionally, the levels of short-chain fatty acids were higher in the CACC879 group compared to the control. Overall, the inclusion of CACC879 in the diet positively impacted gut health, with additional benefits observed in growth performance.

Abstract ID: 482 / Poster Number: 377.15P

Effect of particulate matter size on airborne microbial community structures

Presenting author: Prof. Kyung-Suk Cho, Ewha Womans University, Seoul, South Korea

Co-authors: *Sookyung Kang, Hee Wook Ryu*

Particulate matter (PM) is a major source of air pollution, causing a range of health problems related to the skin, bronchial tubes, and lungs. The concentrations of PM with a diameter of 2.5 μm or smaller (PM2.5) may have a more severe effect on human health due to its smaller size. This study aims to determine whether there are quantitative or qualitative differences between the microbial communities in PM2.5 and PM with a diameter of greater than 2.5 μm (PM>2.5) and whether environmental factors affect these differences. The PM2.5 and PM>2.5 samples were collected in Seoul from fall 2022 to summer 2023. Microbial concentrations and diversity were consistently higher in PM>2.5 samples, while correlations with environmental factors, including temperature, varied depending on the PM size. Differences in the behavior of different sizes of PM in response to wind speed and direction led to varying concentrations of PM2.5 and PM>2.5 microbes. Functional gene analysis revealed that fungal communities exhibited a higher prevalence of pathotrophs in PM2.5 samples compared to PM>2.5 samples. In terms of PM microbial community structure, the dominant species compositions of the PM2.5 and PM>2.5 microbial communities were remarkably similar. Furthermore, even under high wind speeds such as during Asian dust events, the microbial communities of the PM2.5 and PM>2.5 exhibited similar shifts in their community structure, suggesting minimal differences in overall community composition. This suggests that characterizing the PM2.5 microbial community structure provides valuable insights into the microbial ecosystem associated with atmospheric PM.

Abstract ID: 3729 / Poster Number: 109.16P

Effect of Surface Characteristics on Oral Biofilm Development and Microbial Composition

Presenting author: Mr. Srinivas Kore, Haleon UK, Weybridge, United Kingdom; University of Portsmouth, Portsmouth, United Kingdom

Co-authors: *Marta Roldo, Rob Howlin*

Introduction: Biofilm is a structured community of microbial cells adherent to surfaces, enclosed in a self-produced polysaccharide matrix. Previous studies reported that surface characteristics play a major role in influencing biofilm growth.

Objective: This research aims to analyse oral biofilm growth on different surfaces both quantitatively and qualitatively.

Methods: Oral biofilm was grown for 5 days on different substrates, namely hydroxyapatite (HA), polymethyl methacrylate (PMMA) and stainless steel (SS), which are commonly used materials in dental appliances. Surface characterisation was performed using profilometry, contact angle measurement, SEM and imaging of biofilm via confocal microscopy. DNA sequencing was performed to study the species composition.

Results: The results indicated that despite HA being the most hydrophilic (contact angle θ 76°) and having the highest surface roughness (Ra 10-2.99μm), the greatest biofilm growth was observed on stainless steel (contact angle θ 79°, volume 365 μm^3, surface area 26936 μm^2)

Hydrophobicity (contact angle θ 92°) of the PMMA impacted initial bacterial growth. On day 1, biofilm growth was low (volume 154 μm^3, surface area 14009 μm^2) compared to HA or SS. However, by day 5, it was comparable to HA.

Sequencing indicated that beta diversity between the bulk and all three surfaces is significantly different. Beta diversity of HA is different compared to PMMA and HA.

Conclusion: The study highlights significant impact of surface characteristics on biofilm growth and microbial diversity in dental appliances. Selecting materials with favourable surface has the potential to reduce biofilm, promote a healthier oral microbiome, and enhance dental health.

Abstract ID: 3799 / Poster Number: 358.17P

Effect of the application of Sargassum muticum extract and PGPR in combination on soft wheat growth and their roles in induced systemic resistance

Presenting author: Prof. Jamal Ibijbijen, Faculty of Sciences, University Moulay Ismail, Meknes, Morocco

Co-authors: *Mohamed KHABCHA, Fatima Zahra ALIYAT, Brahim SABOUR, Laila NASSIRI*

Global agriculture is facing major challenges such as the extensive use of chemical fertilizers and climate change which poses a challenge for maintaining soil microbiome and environmental health. This study aims to investigate an eco-friendly approach by utilizing *Sargassum muticum* extract (SME) and Plant Growth-Promoting Rhizobacteria (PGPR) as biostimulants to enhance soft wheat growth, while also evaluating their potential roles in inducing systemic resistance against photogenes through in vitro analyses. In this study four bacterial strains (*Serratia* sp, *Enterobacter* sp, *Pantoea* sp, *Pseudomonas* sp) and SME were used. The strains were tested for their PGP traits and their antifungal activities against *Fusarium* sp and *Septoria* sp. An *in vitro* germination test of soft wheat with different SME concentrations was carried out. In greenhouse, four treatments were applied to wheat plants: T0 (control), T1 (PGPR), T2 (SME), and T3 (PGPR+SME). After two months the growth parameters are analysed. As results, the four PGPR strains showed significant performance ($p \leq 0.05$) for P solubilization, IAA secretion, HCN and siderophores, *Pseudomonas* sp. strain demonstrated 40% inhibition of *Fusarium* and *Septoria*. Likewise, SME at 2% showed approximately 70% reduction of *Fusarium* sp. The application of SME demonstrated a significant enhancement in seed germination at 2%. Greenhouse experiments demonstrated significant increases in the morphological and physiological parameters of treated plants, with T3, producing the highest yield compared to T1 and T0. In conclusion, the combined approach of SME and the studied PGPR represents a promising strategy to improve soft wheat growth and resistance against pathogens.

Abstract ID: 2164 / Poster Number: 052.15P

Effect of the plant growth-promoting bacteria (PGPR) Paraburkholderia phytofirmans PsJN abundance in the rhizosphere microbiota of the plant Arabidopsis thaliana

Presenting author: Dr. Bernardo González, Universidad Adolfo Ibáñez, Santiago, Chile

Co-authors: *Felipe Maturana, Angela Cantillo González, Alejandra Serrano*

Introduction: Microbial interactions in the rhizosphere are essential in plant development. Among these interactions, plant growth promoter rhizobacteria (PGPR) can stimulate and protect plant growth from stress, making it greatly interesting for agricultural uses. However, translation from lab to field frequently fails because key ecological issues are generally not considered. **Objective**: In this work, we addressed the effects of the PGPR abundance, bacterial competence, and the impact on the rhizosphere microbiota, using *Paraburkholderia phytofirmans* PsJN and *Arabidopsis thaliana* as a PGPR/plant model system. **Methods**: Agar Petri plates or peat/perlite/vermiculite pot systems were selected for plant growth, using Murashige and Skoog ½ sucrose medium at 21ºC and a 16h/8h day-night cycle for up to 21 days. **Findings**: we found optimal inoculation abundance for the maximal rosette and root development. Successive inoculations do not improve plant growth parameters, and simultaneous inoculations (competence) with other PGPR and non-PGPR taxonomically related or not have no apparent effects on Arabidopsis growth mediated by strain PsJN. Bacterial community structure analyses showed significant changes in the relative abundance of the major taxonomic groups (*Pseudomonadota*, *Actinomycetota*, and *Bacillota*) in plants inoculated with strain PsJN compared with non-inoculated plants. **Conclusions**: PGPR relative abundance is more relevant than competence when effects on plant growth and bacterial community structure are tested.

Abstract ID: 3754 / Poster Number: 009.16S

Effect of various bio-phospho-compost fertilizers on the dynamics of soil bacterial

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Co-authors: *BOUEUNAN Wongbe Beralex, BAHAN Franck Lemounou M*

Phosphorus availability in agricultural soils is often limited due to the low solubility of phosphate compounds, leading farmers to use inorganic and organic fertilizers. Fertilization influences bacterial community abundance and diversity, with chemical fertilizers having a well-documented impact on soil microbes.

This study assessed the effects of bio-phospho-compost fertilizers on soil bacterial communities in Man, western Côte d'Ivoire. A three-year field experiment was conducted in maize and rice cropping systems with four bio-phospho-compost fertilizers applied during the first cultivation cycle. Soil samples were collected after each cycle to quantify total and cultivable phosphate-solubilizing bacteria (PSB) and analyze bacterial community structure and α-diversity (Shannon, Evenness, and Chao1 indices).

Results showed a consistent bacterial community composition across treatments, dominated by Firmicutes, Actinobacteria, Proteobacteria, Halobacterota, and Chloroflexia. Bio-phospho-compost significantly increased total aerobic bacteria (1.08×10^9 to 3.87×10^{12} bacteria/g dry soil) and PSB (1.00×10^9 to 8.16×10^{10} PSB/g dry soil). NK fertilizer reduced Proteobacteria (-63.5%) and Firmicutes (-55.9%) while increasing Actinobacteria (+15%) and Chloroflexia (+18.8%). Conversely, bio-phospho-compost alone enriched all major phyla.

Combining bio-phospho-compost with NK fertilizer significantly ($p < 0.05$) reduced total bacteria and PSB. However, the highest bacterial abundance, crop yield, and nutrient uptake occurred with C3 compost (450 kg RPM/ha) compared to lower doses. These findings confirm that 450 kg RPM/ha is an optimal dose to enhance soil bacterial communities and improve acidic soil fertility

Keywords: NK fertilizers, natural phosphate rock, bacteria phylum, bio-phosphocompost fertilizer, maize, rice, Man.

Abstract ID: 3028

Effect of Vibrio tapetis, temperature and antibiotics on Manila clam microbiota within three tissues: digestive gland, extrapallial fluid and hemolymph

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Co-authors: *Cynthia Oliveira, Morgan Perennou, Emilie Vanpeene, Adeline Bidault, Gwenaelle Le Blay,*

Vibio tapetis is the causative agent of the Brown Ring Disease (BRD) on the Manila Clam, *Ruditapes philippinarum*, which lead to a significant decrease of the Manila clam production in Northwestern Europe. While the pathogen leads to higher BRD at 14°C, at 21°C clam immunity seems stimulated. Research on Manila clam microbiota is relatively recent and most of them have focused on the digestive gland microbiota, but none has ever compared several tissues under a microbial challenge nor under temperature change.

The objective of this study was to improve our understanding of the interactions between Manila clam microbiota, a clam pathogen and environmental factors.

In a nested-design lab experiment (Figure 1), we studied the potential effect of *V. tapetis*, temperature increase and antibiotics (ATB) on clam health and microbiota diversity.

Microbiota from three tissues (digestive glands DG, extrapallial fluids EPF, and hemolymph HLPH) were analysed over a two-month period.

During the experiment, the proportion of ill clams remained relatively low. The three tissues exhibited specific microbiota: both fluids exhibited relatively similar microbiota (dominated by α and γ-*Proteobacteria*, *Flavobacteriales* and *Spirochaetales*), but were very different from the DG (largely dominated by *Mycoplasmatales* followed by α-*Proteobacteria*). Despite high variability between individuals, significant impacts on β-diversity were highlighted for all the tested parameters. At 14°C, *V. tapetis* exposures were associated with an increase in *Crocinitomicaceae*, which did not persist at 21°C. Therefore, the temperature might partially mitigated some *V. tapetis* effects. However, temperature increase was also a structuring factor including by increasing *Nitrospinales* in fluids. ATB exposure modified clam microbiota and made clams more sensitive to both the *V. tapetis* challenge and the temperature increase.

Our results suggest that pathogens and environmental parameters can modify microbiota even before influencing clam health.

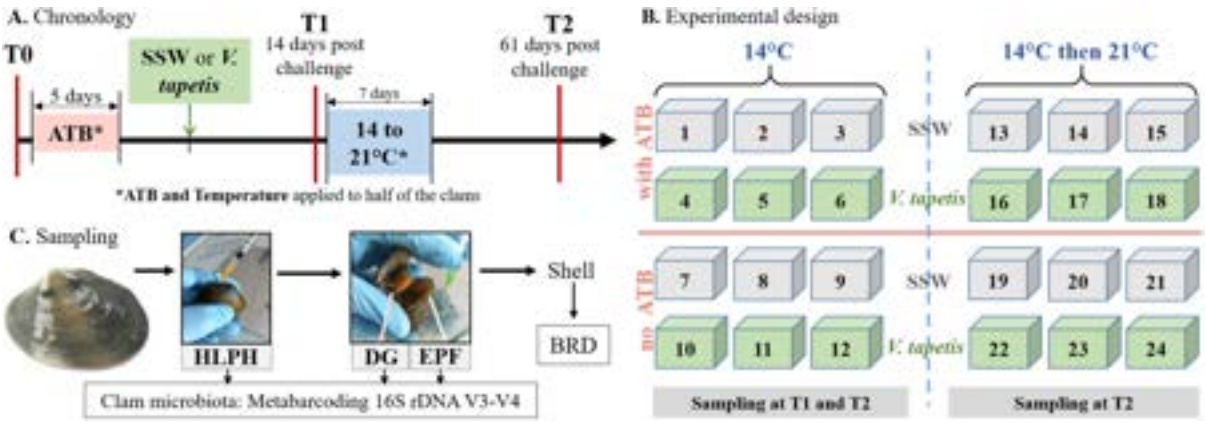


Figure 1: Chronology, experimental design of the experiments combining three conditions (antibiotics, Vibrio tapetis infection and temperature) and sampling. A. Chronology of the experiment from T0 (day 0, before any treatment) to T2 (2 months after T0). B. Experimental design. Clams were split between 24 trays represented by numerated cubes. ATB: antibiotic; SSW: sterile seawater. C. Sampling of the three tissues for metabarcoding and shell conservation for BRD diagnostic.

Abstract ID: 1596 / Poster Number: 367.17P

Effectiveness of plasma-activated fog treatments for Listeria monocytogenes decontamination

Presenting author: Daniele Chieffi, PhD, National Research Council of Italy (CNR), Bari, Italy

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Plasma-activated media, such as plasma-activated water (PAW), are reported to be effective against microbial contamination of agrifood products. To achieve higher antimicrobial efficacy, we propose a new method based on the direct plasma treatment of fog (plasma-activated fog (PAF)), which allows more efficient delivery of plasma-generated reactive oxygen and nitrogen species to target samples. *Listeria monocytogenes* is a ubiquitous pathogenic bacterium found in soil, water and food. Most severe infections affect infants, pregnant, immunocompromised and elderly individuals, resulting in febrile gastroenteritis, sepsis, meningoencephalitis, and perinatal disorders.

In this work, PAF was tested for *in vitro* treatments of animal-, human- and food-associated strains of *L. monocytogenes*.

Different concentrations of each *L. monocytogenes* strain ranging from ca. 5.0×10^4 to 5.0×10^1 colony forming units (CFUs) were inoculated on solid culture medium and subjected to PAF for 30 sec, 1 min and 3 min. In addition, to assess the PAF effectiveness on *L. monocytogenes* multi-strain culture, a five-strain mixture was inoculated at the same concentrations and subjected to the same treatment times.

All *L. monocytogenes* strains and the five-strain mixture showed statistically significant reduction ($p<0.05$) compared to untreated controls at all treatment times and concentrations tested. In particular, the highest mean Log CFU reduction (ca. 2.08-2.95 Log) was observed after 3 min treatments in both single- and multi-strain cultures.

PAF could be an effective medium for *L. monocytogenes* decontamination, and ongoing research is focusing on its applicability to fresh produce for exploitation in the agrifood sector.

Abstract ID: 1377 / Poster Number: 432.15P

Effector diversity and structural variations of the type III secretion system in plant-beneficial Pseudomonas isolates

Presenting author: Ms. Marija Nedeljković, University of Belgrade – Faculty of Biology, Belgrade, Serbia; Centre for Biological Control and Plant Growth Promotion, University of Belgrade, Belgrade, Serbia

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Introduction: The type III secretion system (T3SS) is a well-characterised virulence factor, but its function in non-pathogenic bacteria remains poorly understood. This knowledge gap limits insight into plant-microbe interactions involving beneficial *Pseudomonas* species.

Objective: The aim of this study is to characterise structural variations and effector repertoires of T3SS in plant-associated, non-pathogenic *Pseudomonas* and to identify novel effectors associated with beneficial interactions.

Methods: We performed genome sequencing of T3SS-positive *Pseudomonas* isolates from healthy sugar beet plants and created a comprehensive genome database of T3SS-positive *Pseudomonas* from different plant hosts. Using a customised effector prediction pipeline, we identified effectors that are only present in non-pathogens and absent in the pathogen *P. syringae*. The structures of the T3SS operon were mapped and functional effector domains were annotated.

Findings: We discovered structural variations in the T3SS operon, with some isolates lacking the needle filament and tip components. The repertoire of effectors associated with beneficial interactions contains 93 different functional domains. Among them, we identified a novel ankyrin repeat-containing effector (ARE) present in both isolates from the sugar beet collection and genomes in the expanded database. ARE was expressed together with T3SS genes in response to sugar beet signalling. Ankyrin repeats, known for their role in eukaryotic protein-protein interactions, suggest a function in host manipulation.

Conclusion: Our results demonstrate the structural and functional diversity of T3SS and effectors in beneficial *Pseudomonas*, laying the foundation for future research on T3SS-

mediated promotion of plant growth and expanding the known roles of T3SS beyond pathogenicity.

Abstract ID: 1284 / Poster Number: 051.16P

Effects of BNT162b2 vaccine on the microbiome of chronic lymphatic leukemia and follicular lymphoma patients

Presenting author: Gaia Vertillo Aluisio, PhD, University of Catania, Catania, Italy

Co-authors: *Roberta Pecora, Adriana Antonina Tempesta, Roberta Battaglia, Alessandra Romano, Grete Privitera, Maria Santagati*

Immunotherapy in patients with chronic lymphatic leukaemia (CLL) and follicular lymphoma (FL) determines B-cell depletion and temporary suppression of humoral immunity, which can affect the vaccination response in patients. Yet, the vaccine could shape the microbiome composition in these patients.

This prospective, longitudinal study aimed to assess changes in the gut microbiome composition and functionality of 7 CLL and 8 FL patients after BNT162b2 vaccination during the COVID-19 pandemic. Faecal samples were collected before vaccination and 15 days after vaccination. After DNA extraction, microbiome sequencing was performed by Illumina MiSeq and analysed with the QIIME2 pipeline. PICRUSt2 was used for the prediction of metagenomic functions on the basis of the marker gene sequencing profiles.

After BNT162b2 vaccination, similar shifts in the abundance of bacterial populations were found in CLL and FL. The genera *Collinsella*, *Gemmiger*, *Blautia*, *Ruminococcus*, *Lactobacillus* and family *Lachnospiraceae* increased in both cohorts, whereas *Faecalibacterium*, *Enterococcus* and *Enterobacteriaceae* decreased. Moreover, after vaccination several statistically significant catabolic pathways increased: in CLL pathways for sugar degradation and formaldehyde assimilation, which could be involved in cross-feeding between formate- and acetate-producing bacteria in the circuitry of SCFA production; similarly, in FL pathways for TCA cycle, whose intermediate metabolites can contribute in SCFA production, and hexitol fermentation that produces formate among other metabolites.

Despite the small number of patients, the obtained results could suggest that BNT162b2 vaccination might affect the microbiome and its metabolic functions in CLL and FL, highlighting the importance of a comprehensive analysis to understand immune function in these patients.

Abstract ID: 1007 / Poster Number: 404.17P

Effects of CO2 and H2 Limitations on Methanogenesis

Presenting author: Prof. Wenyu Gu, Swiss Federal Institute of Technology Lausanne, Lausanne, Switzerland

Co-authors: *Jie Xue*

Power-to-methane (PtM) presents a promising technology for the long-term storage of surplus renewable energy in form of CH_4. The biocatalytic conversion of H_2 and CO_2 to CH_4 and water is carried out by hydrogenotrophic methanogens. Successful application of the technology can be promoted by a better understanding of methanogen physiology, specifically the metabolic response of methanogenesis to fluctuations in substrate supply, including H_2- or CO_2- limitations and intermittencies. In batch cultures of the mesophilic *Methanococcus maripaludis* and the thermophilic *Methanothermobacter marburgensis*, we determined the apparent K_m for dissolved inorganic carbon (DIC) to be 0.6 ± 0.3 mM and 1.1 ± 0.8 mM, respectively, indicating microbial capacity to access and reduce CO_2 at pH 7.0 at rates close to μ_{max} when CO_2 is above 340 Pa at 30 °C and 620 Pa at 60 °C. Cultivating *M. maripaludis* in chemostats under either H_2- or CO_2- limitation did not affect the yield or methanogenesis activity. However, differences were observed when *M. maripaludis* was completely starved in the presence of either excess CO_2 or H_2. *M. maripaludis* exhibited a higher tolerance to H_2 starvation compared to CO_2 starvation, resulting in shorter lag times and higher methanogenesis activity upon revival with replenished substrates. CO_2-starved cells exhibited higher NADH/NAD^+ ratios and were more susceptible to O_2 inactivation, indicating a reductive stress from the accumulation of reducing equivalents. These insights can help to stabilize the operation of PtM processes by adjusting the ratio of H_2 to CO_2 feed in case of intermittent operation or shutdowns.

Abstract ID: 2875

Effects of Elite Controllers metabolites on Tight Junction Protein Expression in epithelial cell lines

Presenting author: Itziar Chapartegui-González, PhD, Karolinska Institutet, Stockholm, Sweden

Co-authors: *Aswathy Narayanan, Anders Sönnerborg, Shilpa Ray*

HIV is a pathogenic virus that attacks the immune system, targeting CD4+ T cells, and persists in cellular reservoirs where the virus can stay latent despite antiretroviral therapies, contributing to chronic infection and progression to AIDS. Among the infected people, there is a small subset of individuals called Elite Controllers (EC), which exhibit unique genetics, microbiome, and immune profiles that limit viral replication and maintain $CD4^+$ T-cell counts.

Previous study has identified unique metabolites in ECs, and we hypothesize that some of these metabolites, such as dipeptides (DP), might have a role in affecting the low levels of inflammation in ECs. TJs are key structures in epithelial tissues that regulate paracellular permeability and are involved in several cellular signalling pathways, which is often compromised during inflammation. Disruption of TJs, contributes to systemic inflammation, immune activation, microbial translocation, and disease progression in HIV and other inflammatory diseases.

We hypothesize that some of these ECs metabolites, such as dipeptides (DP), might have a role in affecting the low levels of inflammation in ECs. We investigated the role of these EC specific metabolites on both vaginal and gut epithelial integrity, particularly on tight junction (TJ) proteins, which play pivotal roles in controlling inflammation and microbial translocation.
In this work, cells were treated with different DP, followed by gene expression analysis of key TJ proteins (*cld1, cld3, cld4, cld7, cld14, ocld, tjp1, tjp2*). Immunofluorescence was used to evaluate protein expression under select conditions.

We found that different DP modulate TJ protein expression in a tissue- and time-dependent manner, reflecting the unique regulatory networks of different epithelial cells. These findings highlight the potential of dipeptides as therapeutic agents for enhancing epithelial barrier integrity in diverse tissues.

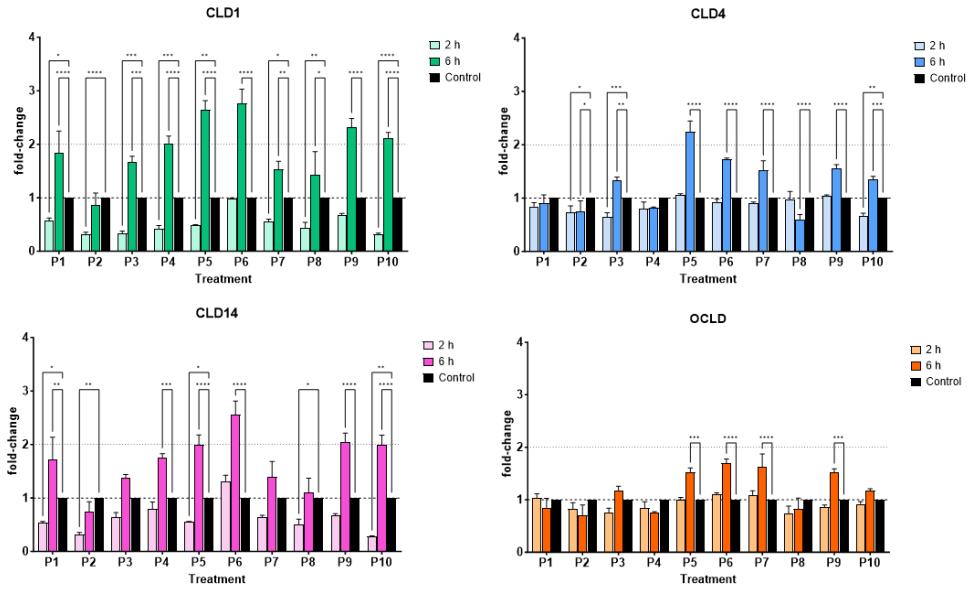


Figure 1: **tight junction (TJ) protein genes expression in HCT116 cells.** Gene expression measured by RT-qPCR in HCT116 cells treated with different dipeptides (P1-P10) from elite controllers (EC) during 2 or 6 h. Control was considered untreated cells kept in culture for the same incubation time as the treated ones. Bars show the average of at least 3 independent replicates quantified in triplicate, and error bars the standard error. Significance was established through two-way ANOVA (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; **** $p<0.0001$).

Abstract ID: 1951 / Poster Number: 048.15P

Effects of high and low temperature on the hindgut microbiome and virome of Blattella germanica

Presenting author: Vicente Pérez-Brocal, PhD, FISABIO-Public Health, Valencia, Spain; Biomedical Research Networking Center for Epidemiology & Public Health- CIBEResp, Madrid, Spain

Co-authors: *Rebeca Domínguez, Carlos García-Ferris, Rosario Gil, Andrés Moya*

Introduction: Metagenomic sequencing enables the study of potentially billions of microbes from different environments, including animal hosts. The cockroach *Blattella germanica* constitute an excellent experimental model for climate change effects to study the symbiosis between the host and its gut microbiota, allowing determining the effects of stressing factors (i.e. high/low temperatures) on its microbiota, which, in turn, may affect the host fitness.

Objective: We have assessed the impact of optimal and suboptimal high and low temperature conditions on the microbiome from *B. germanica*.

Method: We have conducted the metagenomic sequencing of the hindgut content of insects from laboratory populations reared using three temperatures (12, 25 and 37ºC). Reads identified as bacterial and viral hits were separately analysed for the composition and abundance as well as the alpha and beta diversity at taxonomic genus and species ranks. Results from these conditions were compared.

Findings: Few viral genera were present in all groups (<10%), compared to bacterial genera (>90%). Additionally, significant differences were observed in the viral alpha diversity, with populations reared in colder condtions showing higher diversity than those reared under optimal or even hot conditions, suggesting that a greater mobilization of bacteriophages (i.e. induction of lytic cycle) could be taking place. In bacteria, we did not observed those differences. Finally, we determined which genera differed more significantly between pairwise comparisons.

Conclusion: Our results show that high temperature do not stress the phage communities from *B. germanica* as much as low temperature, and this effect is measurable.

Abstract ID: 3928 / Poster Number: 115.16P

Effects of prenatal antibiotics on breast milk immune function and on neonatal fecal microbiota. Preliminary results from a case-control study

Presenting author: Dr. Carolina Carlosama, IRCCS Humanitas Research Hospital, Rozzano, Italy

Co-authors: *Carlo Pietrasanta, Michela Lizier, Elisa Carbone, Giulia Fornasa, Sara Carloni, Silvia Giugliano, Paola Brescia, Martin Mihula, Alessandro Bernardinello, Lorenza Pugni, Fabio Mosca, Giuseppe Penna, Monica Fumagalli, Maria Rescigno*

Introduction: A well-established gut microbiome in early life is essential for the proper development of the immune system, creating a bidirectional relationship in which disturbances in one component can negatively impact the other. It has been demonstrated on a murine experimental model[1] that antibiotic (Abx) treatment of pregnant mice reduces the amount of breast milk secretory IgA (SIgA) and alters the neonatal fecal microbiota, with perturbations persisting even after weaning. Evidence of this phenomenon in humans is limited[2].

Objective: to evaluate in human dyads of pregnant women and their neonates the impact of prenatal Abx on the concentration and characteristics of breast milk IgA, on intestinal IgA of neonates, and on the microbiota of breast milk and neonatal feces.

Methods: ongoing case-control cohorts of 41+41 mother/infant pairs (Fig. 1), enrolled if mothers breastfeed and received any antibiotic for > 7 days consecutively after 32 weeks of gestation. In the control group, mothers had no exposure to any antibiotic treatment during pregnancy. At 1 week, 1 – 3 – 8 months of life fecal and breast milk IgA are quantified by ELISA, while breast milk and fecal microbiota DNA is extracted withDNeasy PowerSoil Pro Kit (Qiagen) and sequenced by shotgun metagenomics on the Illumina HiSeq 2500 platform.

Findings: on a preliminary subset of patients, concentration of breast milk and fecal IgA (Fig. 2) was not significantly different between the two groups at any timepoint. At 1 week of life, neonatal fecal microbiota was shaped by prenatal Abx, with increased abundance (%) of Escherichia and Enterococcus genera and diminished Bifidobacterium genus (Fig.3). Biodiversity richness (observed features) was lower at 1 week of life in neonates born to Abx-treated mothers (Fig. 4). Differences in fecal microbiota composition waned at subsequent timepoints, together with a significant increase of biodiversity in neonates born to Abx-treated mothers over time.

Conclusion: prenatal Abx negatively shape the composition of neonatal fecal microbiota during the first week of life, but breastfeeding supports the recovery of a more physiological biodiversity. Preliminary results need to be confirmed with full sample size.

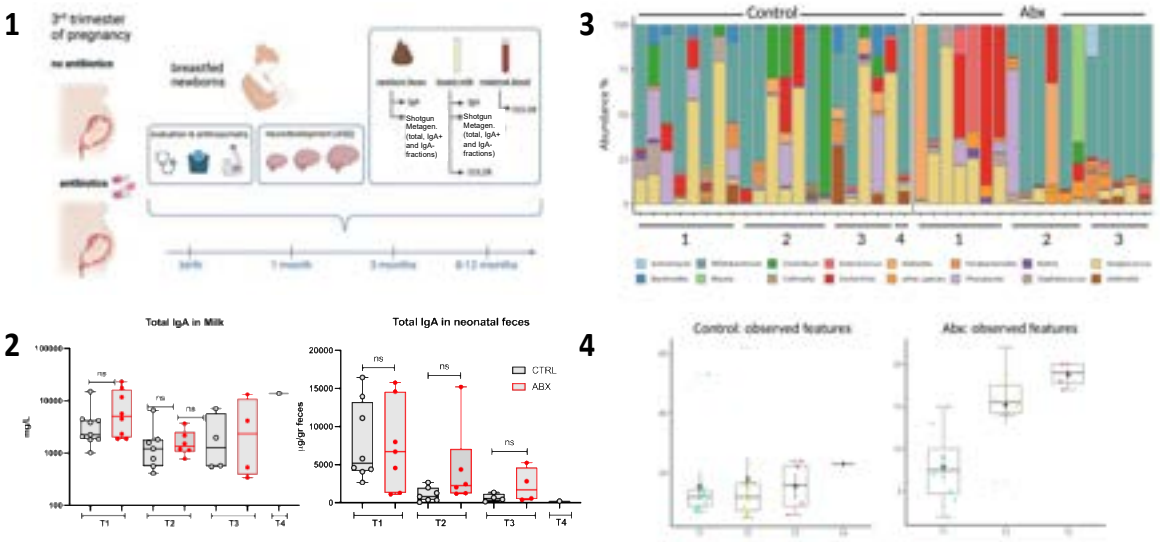


Figure 1: study design. Figure 2: Concentration of IgA in breast milk and neonatal feces. Figure 3: bacterial genera in neonatal feces. Figure 4: fecal microbiota diversity (observed features)

Abstract ID: 1365 / Poster Number: 370.15F

Effects of progressive agent and light treatment on carotenoid production of Rhodotorula toruloides from adaptive laboratory evolution by using pineapple peel hydrolysate

Presenting author: Assoc. Prof. Yen-Hui Chen, National Ilan University, Yilan, Taiwan

Co-authors: *Tzu-Chin Chen*

Rhodotorula toruloides is a yeast that can produce carotenoids (β-carotene, γ-carotene, torulene, torularhodin) and lipids. Carotenoids with antioxidant, anti-bacterial, and anti-cancer properties could be used as food pigments and dietary supplements. Pineapple peel is composed of lignocellulose, which contains 10-25% cellulose and 20-30% hemicellulose, and these can be hydrolyzed to reduce sugars as an alternative carbon source. This study aimed to investigate the effects of progressive agents and light on the carotenoid production of *R. toruloides* from adaptive laboratory evolution (ALE) and to analyze the carotenoid production and composition changes in a bioreactor. The results showed that the addition of 2% methanol as a progressive agent in the growth stabilization period did not affect the growth and utilization of reducing sugars, and the biomass reached 8.32±0.06 g/L on day 2, and carotenoid production was significantly 1.3 times higher than that of the control group, and also the carotenoid productivity was improved. The 6000 lux LED light wouldn't affect the growth and utilization of reducing sugars, but the carotenoid content was significantly increased by applying the light, which was about 1.5 times of the dark group, and the light treatment on day 3 had the highest content, which was 1.9 times of the dark group. In conclusion, the carotenoid production and productivity of ALE *R. toruloides* could be enhanced by adding 2% methanol and light treatment, and the results of this study could be used as a reference for further commercial carotenoid production by ALE *R. toruloides*.

Abstract ID: 3672

Effects of Pseudomonas aeruginosa on Gut Microbiota and Short-Chain Fatty Acid Production: Insights from Akkermansia muciniphila and Faecalibacterium prausnitzii

Presenting author: Dr. Azam Tayabali, Health Canada, Ottawa, Canada

Co-authors: *Kevin Curtin, Houman Moteshareie*

Introduction: *Akkermansia muciniphila* (Am) *and Faecalibacterium prausnitzii* (Fp) are abundant components of the gut microbiota, playing key roles in gut health by producing short-chain fatty acids (SCFAs). This is accomplished in a process known as cross-feeding between Am and Fp. Mucin produced by colonocytes is metabolized by Am to acetate, which is used by Fp as a substrate to produce butyrate. Butyrate is a vital energy source for colonocytes, which in turn respond by producing more mucin to strengthen the gut barrier.

Objectives: We hypothesized that the balance of SCFA production would be disrupted by opportunistic pathogens like *Pseudomonas aeruginosa* (Pa). The goal of this study was to investigate the effects of Pa on the stability of Am and Fp, as well as downstream effects of SCFA production. Additionally, we investigated if the presence of non-pathogenic bacteria could attenuate the pathogenic effects of Pa.

Method: An anaerobic bioreactor was used to establish cross-feeding co-cultures of Am and Fp capable of producing SCFA and butyrate. The co-cultures were treated with (a) Pa culture filtrates (Pa CFs) from a culture pre-grown for 24-h, (b) Pa CFs from a culture pre-grown for 24-h in the presence 11 non-pathogenic microorganisms (approved for biotechnology), (c) CFs from a culture of 11 non-pathogenic microorganisms pre-grown for 24-h (negative control), and (d) filtrates of growth media alone (vehicle control). SCFA and butyrate levels were monitored by ELISA, and bacterial growth was tracked using optical density of cultures and 16S-rRNA PCR specific to Am and Fp.

Findings: To generate the anaerobic cross-feeding culture, Am and Fp were initially grown independently. Growth and stability of Am was dependent on availability of porcine mucin and maintenance of a strict anaerobic environment (≤0.1% dissolved oxygen). Once Am and Fp cultures reached stationary phase, cultures were combined with fresh media containing mucin. Growth of both bacteria was confirmed by PCR and was accompanied with elevated SCFA and butyrate. Culture filtrates of Pa added to the co-cultures reduced Am and Fp growth. This inhibition occurred regardless if non-

pathogenic bacteria were included during CF production. PCR analysis of Am and Fp corroborated the optical density data. Correspondingly, SCFA production and butyric acid decreased significantly in the presence of Pa, with butyric acid levels being the first to drop. Control treatments with only media or non-pathogenic bacteria alone did not demonstrate any effects on Am and Fp growth or levels of SCFAs.

Conclusion: These findings demonstrate that the beneficial cross-feeding interaction between Am and Fp could be mimicked in an *in vitro* anaerobic system. The opportunistic pathogen, Pa, caused significant reductions in Am and Fp growth and SCFA production, but was unaffected by presence non-pathogenic bacteria. This disruption may have stemmed from Pa virulence factors such as pyocyanin or elastases which could ultimately compromise the gut's mucus layer to cause downstream health effects. This research demonstrates how animal-free systems can further our mechanistic understanding of how pathogens like Pa can disrupt the gut microbiota and lead to downstream adverse health effects.

Abstract ID: 944 / Poster Number: 337.16P

Effects of seasonal and nutrient management on soil properties and bacterial communities: Insights for sustainable agriculture

Presenting author: Ms. Katarina Kruščić, University of Belgrade, Faculty of Biology, Belgrade, Serbia

Co-authors: *Tamara Janakiev, Nenad Antić, Nikola Unković, Milica Ljaljević Grbić, Ivana Đokić, Aleksandar Knežević, Maja Radulović, Milan Gavrilović, Peđa Janaćković, Dalibor Stanković, Ivica Dimkić*

Soil health, composition and microbial activity are crucial for sustainable agriculture and the functionality of ecosystems. This study investigates how nutrient management and seasonal variations affect soil properties and bacterial communities in spring and summer in four different localities, comparing nutrient-depleted (ND) and nutrient-overloaded (OL) soils. The composition of the bacterial community varied according to treatment and season. Bacillus was the predominant genus in all samples, but decreased in summer. ND soils favored nutrient-utilizing genera such as Priestia and Peribacillus, while OL soils supported nutrient-rich, adapted genera such as Streptomyces and Paenibacillus. Seasonal shifts introduced taxa such as Alkalicoccobacillus in OL soils together with Arthrobacter and Nocardia in ND soils. ICP-OES analysis revealed significant differences in macro- and microelements. In spring, ND soils contained higher microelement concentrations, including arsenic (3.21 µg/g), barium (63.84 µg/g) and zinc (59.72 µg/g), while OL soils showed increased macroelements such as calcium (20,763.16 µg/g vs. 9,604.67 µg/g), magnesium (10,445.68 µg/g vs. 8,513.81 µg/g), potassium (29.14 mg/100g vs. 23.27 mg/100g) and phosphorus (27.24 mg/100g vs. 22.84 mg/100g). Due to seasonal changes, certain microelements (As, Cd, Co) decreased in both soil types in summer. ND soils also had higher pH values, while OL soils were enriched with organic matter. Nutrient overload in OL soils increases nutrient content but risks acidification and imbalance, whereas ND soils with fewer nutrients have greater microbial diversity and resilience. The seasonal changes highlight the need for sustainable strategies to balance nutrients, promote microbial diversity and avoid over- or under-fertilization.

Abstract ID: 2178 / Poster Number: 064.16P

Effects of the artificial sweetener sorbitol on gastrointestinal colonization resistance against Salmonella Typhimurium

Presenting author: Mr. Yves Steiger, ETH Zürich, Zürich, Switzerland

Co-authors: *Sanne Kroon, Jennifer Carry, Bidong Nguyen, Wolf-Dietrich Hardt*

The human gastrointestinal tract is colonized by a complex bacterial consortium, the microbiota, which provides numerous benefits, including protection against enteropathogenic bacteria (colonization resistance). However, infections caused by diarrhoea-inducing bacteria remain common, and their underlying causes are not fully understood. While previous studies have shown that high-fat diets can transiently impair colonization resistance, we hypothesized that food additives, such as artificial sweeteners, might have similar effects.

Here, we investigated the impact of the widely used artificial sweetener sorbitol on colonization resistance against the foodborne pathogen *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium). Our experiments revealed that sorbitol significantly enhances *S*. Typhimurium gut infections in mice with a complex microbiota. Unlike antibiotic-mediated microbiota disruption or germ-free models, sorbitol application delayed luminal pathogen growth and disease progression. However, infection levels eventually reached comparable endpoints after four days.

We characterized the effects of sorbitol treatment on microbiota composition, host inflammatory responses, and the challenges S. Typhimurium faces to overcome colonization resistance. The pathogen critically relies on virulence factors for epithelial invasion and tissue replication. Mutants lacking these factors failed to proliferate or cause disease in sorbitol-treated mice.

Altogether, this sorbitol-based infection model provides a valuable alternative to classical antibiotic pre-treatment approaches for studying *S*. Typhimurium infections. It offers insights into the role of virulence factors *in vivo* and may more closely mimic natural infection dynamics.

Abstract ID: 2010 / Poster Number: 402.16P

Efficacy of Spike Protein-Based Subunit Vaccines Against Porcine Epidemic Diarrhea Virus

Presenting author: Prof. Chun Yen Chu, International Degree Program in Animal Vaccine Technology, NPUST, Pingtung, Taiwan

Co-authors: *Hsing Chieh Wu, Doan Thi Thu Dung, Yu Hsi Chen,*

Porcine epidemic diarrhea (PED) is a highly contagious intestinal disease in pigs, causing significant economic losses in the global pig industry. The etiologic agent, porcine epidemic diarrhea virus (PEDV), belongs to the genus *Alphacoronavirus* with the family *Coronaviridae*. Frequent genetic mutations in PEDV, akin to other coronaviruses, limit the effectiveness of vaccines derived from traditional strains, underscoring the need for more effective solutions. The PEDV spike (S) protein, a key determinant of viral entry and a major target for host antibody responses, represents a promising candidate for protein-based vaccines. This study evaluated the immunogenicity and efficacy of a subunit vaccine based on the full-length recombinant PEDV S protein, expressed using the baculovirus expression system, in a mouse model. Vaccinated groups exhibited normal body temperatures and weight gain, confirming vaccine safety with no observed adverse reactions. Immunological analysis revealed significantly elevated serum IgG levels that persisted for at least 8 weeks post-vaccination, indicating a robust humoral immune response. Cellular immunity assessments demonstrated increased IFN-γ levels at week 4, suggesting activation of Th1 and NK cells. Although IL-12 and IL-4 secretion showed an upward trend post-vaccination, individual variability prevented statistical significance. Flow cytometry further revealed a significant expansion of $CD4^+$ and $CD8^+$ T cell populations at week 4 ($P < 0.05$). In conclusion, this study highlights the strong immunogenicity and efficacy of the subunit vaccine, emphasizing its potential to address emerging PEDV variants and mitigate economic losses in the swine industry.

Abstract ID: 904

Efficient Pretreatment of Lignocellulosic Biomass by MOF-based Nanozymes and Subsequent Production of BioHydrogen through Dark Fermentation

Presenting author: Dr. Arpita Nandy, TCG-CREST, Kolkata, India

Co-authors: *Anusha Rai, Ayan SinghaRoy*

Introduction: Agricultural waste generally has a high energy conversion efficiency compared to fossil fuels, and has 60-70% sugars, consisting of hemicellulose, lignin, and cellulose. Lignin readily forms covalent bonds with proteins and hemicellulose, embedding cellulose within them and making depolymerization difficult. Diverse physical, chemical, and biological processes have been standardized for the pre-treatment phase; however, they are not sustainable methods and contribute to environmental hazards. To address the limitations of the pre-treatment methods, novel procedures, such as "nanozymes" which act as biological enzymes due to their intrinsic properties, have been investigated for bioenergy related applications. In this context, Metal-organic frameworks (MOFs) based magnetic nanoparticle (NP) was assessed for the pretreatment of rice straw, and subsequent biohydrogen production from pre-treated biomass through dark fermentation, using *Clostridium* sp. and *Thermoanaerobacterium* sp. were evaluated. The results show hydrogen yield comparable to conventional alkali treatment methods.

Objectives:

- Efficient pre-treatment of the lignocellulosic biomass (rice straw) using the MOF based nanomaterial (MM-53) for application towards second generation biofuels.
- Evaluation of the sugar rich hydrolysate resulting from the pretreatment for hydrogen production in batch mode in dark fermentative conditions.
- Establishment of sustainable technology through the use of recyclable nanomaterial, bypassing the environmentally hazardous acid/alkali treatments

Method: This work is performed in two steps, where the first step is the synthesis and pre-treatment of the lignocellulosic biomass. The MOF based nanomaterial (MM-53) is synthesized, characterized, and is analyzed for its' enzymatic activities for removal of the lignin.

The second step is about the application of the pretreated biomass for second generation biofuels. Sugar hydrolysates resulting from the pretreatment of rice straw were subjected to saccharification, and biohydrogen production through dark fermentation.

Findings:

- ***Efficient Degradation of Lignin from Rice Straw (RS):*** To optimise the lignin degradation process, MM-53 nanoparticles were evaluated under various conditions, namely positive control (1% NaOH treated), Negative control (0.75 M H_2O_2 treated), and T_1 to T_3 (Tests, rice straw treated with 1, 3, and 5 g L^{-1} NP), with respect to solid loading of 7.5% (w/v) of RS for 24 hours. Out of all the treatments, positive control and T_2 (3 g L^{-1}) exhibited the highest lignin degradation, achieving comparable values of 38%, and 33.7%, respectively.
- ***Biohydrogen production:*** After the pretreatment, rice straw samples were subjected to fermentation by *Clostridium diolis* X3 and *Thermoanaerobacterium sp*. under specific conditions. Figure 1a displays the biohydrogen production profiles for *C. diolis* X3 over a 48 hours (2 days) fermentation period at an incubation temperature of 37°C. The cumulative gas volumes produced from untreated, positive control, negative control and test samples reached maximum values of 160.67, 202, 152.67, and 196 mL g^{-1} total solids (TS) RS, respectively at the 48-hour mark. Notably, the NP-treated RS achieved a hydrogen yield of 42.83%, representing 1.14-fold and 1.05-fold increase compared to the untreated and H_2O_2-treated rice starw, respectively. Figure 1b shows the biohydrogen production profiles for *Thermoanaerobacterium* sp. over a 216 hours (9 days) fermentation period at 50°C.

Conclusion: In this study, RS was pretreated with magnetic MM-53 for biofuel applications. The pretreatment with the nanomaterial produced comparable result to those of alkali treated, in terms of delignification (34%), and biohydrogen production (1.14-fold increase). The findings show that the proposed method of pretreatment with magnetic NPs is promising to enhance the hydrolysis of biomass, which resulted in the efficient downstream processing of the pre-treated biomass to produce biohydrogen.

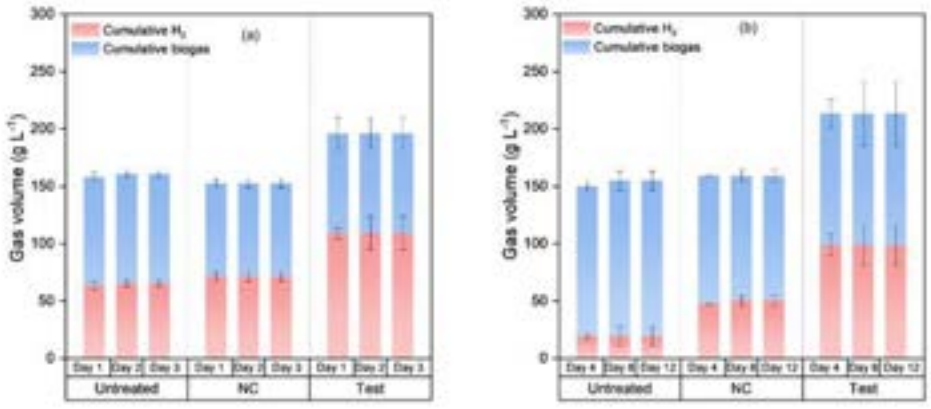


Figure 1: Profile of biohydrogen production, cumulative gas volume and H_2 volume (ml g^{-1} TS) (a) *Clostridium diolis* strain XTS051-3 (b) *Thermoanaerobacterium* sp. strain CMT5567-1.

Abstract ID: 1548 / Poster Number: 206.15P

Elucidating differential expression of Shiga toxins across Escherichia coli serotypes

Presenting author: Ms. Samantha K Tucker, University of Glasgow, Glasgow, United Kingdom

Co-authors: *David R Mark, Rebecca E McHugh, Andrew J Roe*

Shiga toxin-producing *Escherichia coli* (STEC) are a group of enteric pathogens that carry phage-encoded Shiga toxins (Stx). The association between Stx production by STEC and renal damage was first described in 1985, and its mechanism of action has since been described. STEC can produce two types of Stx: Stx-1 and Stx-2, both toxins function identically but have distinct expression systems. Classical antibiotics activate the RecA response and upregulate expression of both toxins, so they are contraindicated in treating STEC. Consequently, there is no treatment for STEC, and patients rely on supportive therapies. We aim to reveal how nucleotide sequence variations in the Stx promoter region impacts expression of the toxin in different bacterial strains.

We curated a collection of twenty-two complete chromosomes across *E. coli* serotypes from NCBI and subsequently identified eight variations of the Stx promoter regions. The eight variants were cloned into a dual transcriptional reporter system to enable simultaneous analysis of RecA expression and the Stx promoter-driven expression. The dual reporter has been inserted into four STEC outbreak strains and *E. coli* K-12 MG1655 to elucidate whether the bacterial strain influences stress-induced Stx expression. Results show that the bacterial strains exhibit differential activation of the Stx promoter and differential responsiveness to mitomycin C, a classical DNA damaging agent. Overall, this work seeks to unravel the interplay between bacterial strains and differences in the sites of variation within the promoter that may have a critical impact on toxin expression.

Abstract ID: 1062 / Poster Number: 380.15P

Elucidating SigB-mediated growth delay that occurs in Listeria monocytogenes under conditions of isoleucine starvation

Presenting author: Mr. Yubin Xue, University of Galway, Galway, Ireland

Co-authors: *Conor O'Bryne*

Listeria monocytogenes exhibits a deficiency in isoleucine biosynthesis despite possessing an intact isoleucine biosynthetic operon (*ilv*) (we refer to this as an isoleucine pseudo-auxotrophy). It seems counterintuitive to maintain all the biosynthetic genes but not to use them, even when isoleucine is absent from the medium. SigB negatively influences the isoleucine biosynthesis by repression of *ilvA* expression through an unknown mechanism. To elucidate this regulatory mechanism, we set up a series of *in-vitro* evolution experiments (IVEE) to identify mutations that suppress the growth lag in isoleucine-depleted media.

First, we reconfirmed that *sigB* deletion mutants do not display the isoleucine pseudo-auxotrophy seen in both wild-type EGD-e and 10403S. Following the IVEE, we observed at least four distinct colony morphologies associated with various suppressors of the isoleucine pseudo-auxotrophy among 15 isolates across 3 biological replicates. Whole-genome sequencing analysis of 5 suppressor isolates revealed SigB loss-of-function mutations (*rsbT* A42D and *sigB* G156*) in 2 isolates. We then investigated whether SigB loss-of-function mutations contributed to the decreased growth lag phenotype observed in these isolates through Congo red staining and acid survival experiments. The remaining three suppressors, displaying less Congo red staining and higher acid tolerance, had intact *sigB* operon and are currently under further investigation. These results allowed us to systematically classify the suppressor mutants. Collectively, these data show that the pseudo-auxotrophy for isoleucine can be rapidly overcome by suppressor mutations that arise when cells are deprived of isoleucine.

Abstract ID: 3136 / Poster Number: 269.15P

Elucidating the Metabolic Function of Microbial Communities in the Phycosphere of the Red Alga Gracilariopsis lemaneiformis Using Meta-Omics Approaches

Presenting author: Mr. Jeong Min Kim, Chung-Ang University, Seoul, South Korea

Co-authors: *Byeong Jun Choi, Hülya Bayburt, Che Ok Jeon*

Marine macroalgal phycosphere is dynamic environments in which microbial communities participate in symbiotic interactions critical to algal health. In this study, we used metagenomic and metatranscriptomic approaches to characterize the metabolic potential of the phycosphere microbiome associated with *Gracilariopsis lemaneiformis* harvested from seaweed farms off the southern coast of Korea in June (normal temperature) and July (high temperature). Metabolic pathway reconstructions revealed that microbes were involved in nitrogen fixation, sulfur cycling, phytohormone biosynthesis, carotenoid production, and vitamin B synthesis, all of which are related to algal growth and stress resilience. Comparative analyses between the two samples highlighted changes in microbial activity associated with seasonal temperature changes.

Metagenomic data identified *Pseudomonadota* (e.g., *Litorimonas*, *Hellea*, *Sulfitobacter*, *Arenicella*, *Erythrobacter*) and *Bacteroidota* (e.g., *Aquimarina*, *Maribacter*, *Tenacibaculum*) as dominant taxa, representing the majority of the community. Functional profiling revealed that these members were enriched in pathways related to nitrogen and sulfur metabolism, and associated with polysaccharide degradation. In particular, metatranscriptome analysis identified active expression of genes involved in these metabolic processes, suggesting a role in maintaining algal-microbial symbiosis. Changes in the relative abundance of specific taxa were observed in the July samples when temperatures were elevated, suggesting possible impacts on nutrient supply.

This study highlights the complexity of the metabolic network within the *G. lemaneiformis* phycosphere, where diverse microbial taxa cooperatively support algal physiology and environmental adaptation. Integrating a metaomics approach provides fundamental insights into the functional dynamics of marine microbial communities and their responses to environmental variability, advancing our understanding of algal-microbial interactions.

Abstract ID: 2799 / Poster Number: 281.16F

Elucidating the structural role and bioengineering potential of docking domains in histone deacetylase inhibitor biosynthesis

Presenting author: Dr. Munro Passmore, University of Warwick, Coventry, United Kingdom

Co-authors: *Xinyun Jian, Matthew Jenner, Lona Alkhalaf, Gregory Challis*

Bacterial natural products are chemically diverse compounds with a wide range of biological activities. Many are biosynthesised by multi-domain megaenzymes such as modular polyketide synthases and nonribosomal peptide synthetases. Accurate interfacing of subunits in these remarkable biosynthetic machines is critical for maintaining their fidelity, and is often mediated by docking domains. These promote productive subunit interactions and show considerable promise as synthetic biology tools.

A Short Linear Motif (SLiM) and a β-Hairpin Docking (βHD) domain mediate the interaction between conserved machinery for pharmacophore assembly and divergent machineries for variable peptidic cap construction in the biosynthesis of the histone deacetylase (HDAC) inhibitor family, exemplified by the chemotherapeutic agent Romidepsin. Our objective was to better understand how SLiM-βHD domain interactions mediate engagement of the conserved pharmacophore machinery with various peptidic cap machineries using a combination of intact protein mass spectrometry, mutagenesis, and bioinformatics.

We developed in vitro and in vivo assays to probe the role of the SLiM and βHD domain in mediating these interactions, showing that unlike analogous systems, the βHD domain, but not the SLiM, is critical for productive engagement [1]. Structural characterisation using AlphaFold and carbene footprinting indicated this is likely due to direct interaction of the βHD domain with the pharmacophore biosynthetic machinery.

In vitro reconstitution experiments showed that the pharmacophore and peptidic cap biosynthetic machinery from different systems can productively crosstalk. Future research will focus on probing the role played by individual residues in mediating subunit interactions and harnessing βHD domain promiscuity to create novel HDAC inhibitors.

Abstract ID: 3290 / Poster Number: 326.16F

Elucidating trophic interactions in anammox bioreactors through time resolved protein-SIP

Presenting author: Dr. Christian White, Argonne National Laboratory, Lemont, United States

Co-authors: *Chang Ding, Simon Klaes, Martin Taubert, Lisa Alvarez-Cohen, Lorenz Adrian*

Anaerobic ammonium oxidizing (anammox) bioreactors, utilized for wastewater treatment, harbour metabolically and phylogenetically diverse microorganisms that form both mutualistic and competitive interactions[1]. In many anammox systems no organic carbon is supplied suggesting that anammox bacteria, which have never been isolated in pure culture, supplies organic carbon through inorganic carbon fixation to other heterotrophic bacteria[2]. In this study, we sought to assess the transfer of carbon between the anammox bacteria and the rest of the microbial community to ascertain the trophic interactions that govern community stability and function. This was accomplished through a time series ^{13}C protein stable isotope probing (SIP) experiment. A stable continuous stirred reactor tank (CSTR) anammox bioreactor[3] with *Candidatus* Kuenenia stuttgartiensis as the dominant anammox strain was loaded with ^{13}C bicarbonate until all inorganic carbon in the system was replaced with labelled carbon. 4087 unique peptides were identified from shotgun proteomic analyses and 2155 of those peptides belonged to Kuenenia. Protein-SIP analysis demonstrated that the relative isotope abundance (RIA) of anammox peptides increased from 0.92±0.08 on day 0 to 1.05±0.02 on day 4 to 95.18±0.03 on day 13. Non-anammox peptides displayed similar labelling patterns to anammox peptides in the early and latter stages of the experiment when ^{13}C incorporation was low and high respectively. However, all non-anammox peptides had less ^{13}C incorporation on day 4 ($p<0.001$) indicating a lag in carbon assimilation indicative of trophic interactions. These results suggest that metabolically diverse microorganisms exhibit different carbon acquisition strategies that influence the microbial ecology of the system.

Abstract ID: 4151

Emergence of *B. pertussis* macrolide-resistant isolates in France in 2024: out of China?

Presenting author: Dr Valérie Bouchez, Institut Pasteur, Université Paris Cité, Biodiversity and Epidemiology of Bacterial Pathogens, Paris, France; National Reference Center for Whooping Cough and other Bordetella infections, Institut Pasteur, Paris, France

Co-authors: *Noémie Lefrancq, Nora Zidane, Nathalie Armatys, Annie Landier, REMICOQ study group, Julie Toubiana, Carla Rodrigues, Sylvain Brisse*

Introduction: In 2024, an intense post–COVID-19 resurgence of pertussis, a highly contagious vaccine-preventable respiratory disease mainly caused by *Bordetella pertussis* (*Bp*) and potentially lethal in young infants, was observed in Europe and elsewhere. Macrolides remain the first-line treatment, reducing bacterial carriage and transmission, yet macrolide-resistant *Bp* (MRBP) is widespread in China, exceeding 90% in some regions. Elsewhere, only sporadic MRBP were reported, including a case in France in 2011. We now describe 14 MRBP cases identified in France between February and November 2024.

Objective: We compared the 14 MRBP at the genomic level with strains circulating in China to investigate whether the study isolates originated from macrolide-susceptible *Bp* (MSBP) in France or were imported.

Methods: Among 354 Bp isolates cultivated in 2024, 14 MRBP carried the A2047G mutation in all three *23S rRNA* gene copies, concordant with their antimicrobial resistance phenotype. We compared their whole-genome sequences with 1,572 isolates from France (1993–2024, including the 2011 MRBP strain) and 824 from China (2018–2024, 596 MRBP). Phylogenetic relationships were assessed to investigate possible importation paths and evolutionary patterns.

Findings: Whole-genome SNPs analysis showed that the 14 new MRBP clustered within a global group of 349 MRBP isolates (the *ptxP3*-MRBP clade), primarily from China. They formed three discrete branches nested among Chinese strains, suggesting at least three independent introductions into France (**Figure 1**). Two branches contained multiple isolates from geographically distinct French regions, indicating onward transmission. The *ptxP3*-MRBP lineage emerged in China around 2016, likely exhibiting higher fitness than older *ptxP1*-MRBP strains, and has become predominant since 2020.

Conclusions: This unexpected rise of MRBP in France underscores the importance of global surveillance and intervention. Direct introductions from China to France are likely, although cryptic transmission through intermediary unsampled countries cannot be excluded. Delayed MRBP emergence in France may reflect lower fitness of *ptxP1* variants in populations using acellular vaccines, whereas *ptxP3*-MRBP appears more adaptable. Representative sampling and genomic analyses are crucial for managing dissemination. With macrolides central to pertussis transmission control, preserving their efficacy is crucial, yet defining effective alternative treatments, particularly for newborns, remains essential for managing future macrolide-resistant cases.

Abstract ID: 2969 / Poster Number: 307.16P

Emerging Alternatives to Antibiotic Therapy Evaluated in a Galleria mellonella Model: Aptamers Targeting Fimbrial Adhesion of Enterotoxigenic Escherichia coli

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Enterotoxigenic *E. coli* (ETEC) infections lead to significant costs for the swine industry worldwide. Fimbriae are the virulence factors responsible for the initial adhesion of ETEC to intestinal epithelial cells, with F4 and F18 types being the most prevalent. These infections are traditionally prevented or treated with antibiotics, but their use is highly restricted due to antimicrobial resistance. Thus, emerging alternatives, such as DNA aptamers with target-binding capacities, seem to be a promising approach to block the initial adhesion of ETEC.

This study was divided into three main steps for the two ETEC strains encoding distinct types of fimbriae (F4/F18) separately: (1) selection of specific aptamers using the cell-SELEX approach; (2) evaluation of affinity (K_D)/ specificity *in vitro*; (3) evaluation of the anti-ETEC performance of the most promising aptamers *in vivo* (*Galleria mellonella* model).

From the cell-SELEX methodology, a pool of millions of aptamers was sequenced for F4/F18 ETEC strains individually. Aptamers with high K_D for F4/F18 were obtained. Regarding the *in vitro* specificity evaluation, among the most representative aptamers targeting F4, one aptamer stood out due to its higher specificity. Conversely, none of the most representative aptamers targeting F18 demonstrated specificity. However, the *in vivo* studies highlighted the anti-ETEC potential of two aptamers, one for each fimbria. Both aptamers at 500 nM increased the survival and health index of *Galleria mellonella* compared to the control group (only F4/F18 ETEC inoculation).

Therefore, this work demonstrates the potential of aptamers in the treatment of ETEC infections as an alternative to antibiotics.

Abstract ID: 2405 / Poster Number: 408.16P

Emphasising the need for further identification of Salmonella beyond biochemical assays

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Salmonella remains a leading cause of foodborne illnesses. The culture-based-biochemical assays outlined in ISO 6579-1:2017 are widely used but prone to false positives due to phenotypic similarities with closely related genera like *Citrobacter* and *Proteus*. These similarities stem from shared genetic traits, including metabolic activities, leading to cross-reactivity in biochemical assays. Although ISO guidelines advocate for additional serological or molecular verification, numerous studies continue to depend solely on culture-based techniques for *Salmonella* identification. While some others opt to further characterize only a small subset of representative strains from a larger collection of isolates, potentially overlooking important variants. This study on *Salmonella* in poultry samples from UK processing chains demonstrated the potential for error in relying solely on culture-based-biochemical methods. Following the ISO culture-based-biochemical assays, a total of 82 presumptive *Salmonella* isolates were recovered from the poultry samples. Genomic characterisation confirmed only 70.7% as *Salmonella enterica*. The remaining 29.3% were misidentified urease-negative mutants of *Proteus mirabilis* (18.3%), *Citrobacter werkmanii* (6.1%), and *Citrobacter braakii* (4.9%). Average nucleotide identity comparisons showed 89.1-94.6% similarity between *Citrobacter* and *Salmonella* strains, and 85-90.1% between *Proteus* and *Salmonella* strains. The misidentification of *Salmonella* could have several significant downsides such as food safety concerns and public health risks, inaccurate epidemiological data, compromised outbreak investigation and flaws in developing regulatory and compliance policies. These downsides highlight the need for precise and reliable characterization methods, such as cost-effective gene amplification targeting *Salmonella*-specific genes, to ensure accurate identification of the pathogen.

Abstract ID: 3397 / Poster Number: 362.16P

Employing basidiomycetes and their enzymatic machinery for synthetic polymer degradation

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The recalcitrant nature of plastic waste has led to its accumulation, posing a significant global environmental challenge[1]. Fungi, known for their metabolic versatility and role in decomposing organic matter in soil, are highly effective in the degradation of polymers. Among them, white-rot fungi are especially renowned for their bioremediation capabilities, primarily attributed to their ligninolytic enzymatic system[2,3]. This study investigates a strain of the order Agaricales (Basidiomycota), previously isolated from a Greek habitat and identified through ITS rRNA gene sequencing, which has shown significant potential in degrading plant litter but remains largely unexplored in its ability to break down xenobiotic polymers such as plastics. The strain was evaluated for its capacity to grow on polyester- and polyether-polyurethane substrates (Impranil® DLN-SD, Impranil® DL 2077) as the sole carbon source, exhibiting effective substrate degradation and significant biomass production. To explore the biodegradation mechanism, extracellular enzymatic activities (hydrolases and oxidative enzymes) were assessed spectrophotometrically in the culture supernatants of Impranil® DLN-SD, revealing significant oxidative enzyme activity. Substrate modifications were confirmed using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), while Gas Chromatography-Mass Spectrometry (GC-MS) identified the formation of biodegradation products, including ester and amide compounds, indicative of polymer breakdown. Proteomic analysis of the culture supernatants was performed to identify and quantify enzymes associated with polymer degradation. This study underscores the effectiveness of this strain as a biocatalyst for polymer degradation, offering a sustainable solution for managing plastic waste and valorizing its byproducts.

Abstract ID: 2874 / Poster Number: 061.15P

Endemic Reservoirs and Aquaculture Spread of Renibacterium salmoninarum: Genomic Insights from Norway and Beyond

Presenting author: Alejandra Villamil-Alonso, PhD, Technical University of Denmark, Kongens Lyngby, Denmark

Co-authors: *Torfinn Moldal, Cathrine Arnason Bøe, Birkir Þór Bragason, Lone Madsen, Argelia Cuenca, Karin Lagesen*

Renibacterium salmoninarum is the causative agent for bacterial kidney disease (BKD) and has been isolated from northern European salmonid farms since the 1980s. The bacteria have been only sporadically detected in Norway during the last decades, but different production areas within the country experienced a sudden increase of BKD incidence since December 2022. The ongoing BKD outbreaks on the west coast and in Mid-Norway were explored using a whole genome sequencing approach to trace the patterns of transmission of *R. salmoninarum*. In the global context, gaining a broader overview of the phylogeography of the pathogen was allowed though sequencing and analysis of a collection of isolates from Norway, Iceland, Denmark, and the Faeroe Islands. The accuracy of SNP-based analyses allowed us to discriminate between two distinct active hotspots of BKD where the disease emerged and locally spread. Both clusters are part of the expanding, aquaculture-associated Lineage 1 of *R. salmoninarum*. The spread aligns with contemporary aquaculture practices, raising critical questions on the effectiveness of current disinfection protocols for *R. salmoninarum*. Here, Lineage 3 is described for the first time consisting mainly of *R. salmoninarum* from Iceland, where BKD has been considered endemic since historical times. This study supports the hypothesis that different endemic reservoirs of *R. salmoninarum* exist in the European water systems, as suggested by Lineages 2 and 3, and underscores the importance of further investigating pathogen persistence in different environments, especially in those related to the aquaculture industry.

Abstract ID: 4092 / Poster Number: 292.17P

Engineering *Amycolatopsis mediterranei* for Enhanced Rifamycin Analog Production through Targeted Biosynthetic Modifications

Presenting author: Dr. Utkarsh Sood, Kirori Mal College, University of Delhi, Delhi, India

Co-authors: *Moritz Mueller, Andreas Bechthold, Rup Lal*

Introduction: The emergence of rifampicin-resistant and multidrug-resistant *Mycobacterium tuberculosis* strains necessitates the development of novel rifamycin analogs with improved bioactivity and therapeutic potential. This study focuses on optimizing the biosynthesis of rifamycin analogs, such as 24-desmethyl rifamycin B, through targeted genetic modifications in *Amycolatopsis mediterranei*. Building on prior success with the deletion of the *rifQ* gene and overexpression of *rifO*, the study aims to further optimize analog production by combining these genetic strategies.

Objective: To enhance the production of rifamycin analogs by employing metabolic and genetic engineering strategies, including modifications to key biosynthetic and regulatory enzymes.

Generate newer analogs through the exchange of *rifAT6* with *rapAT2* and *DH1* with *DH10* simultaneoulsy, facilitating structural modifications that may enhance antibiotic activity and bioavailability.

Methods: We employed homologous recombination using a modified PCR-targeted *Streptomyces* gene replacement method to introduce genomic modifications in *A. mediterranei*. Structural diversification was achieved by exchanging *rifAT6* with *rapAT2* and *DH1* with *DH10* to generate novel rifamycin derivatives. To enhance yield, we deleted *rifQ*, overexpressed *rifO*, and modified the biosynthetic pathway by removing 3-dehydroquinate synthase (DHQS) and 3-dehydroquinate dehydratase (DHQD) in the shikimate pathway. These changes redirect precursor flux toward rifamycin biosynthesis, optimizing production efficiency.

Findings: The engineered strains demonstrated improved yields of rifamycin analogs. Structural modifications enhanced bioavailability and activity against resistant *M. tuberculosis* strains.

Conclusion: This study provides a novel approach for rifamycin analog diversification and yield enhancement through rational genetic modifications, paving the way for the development of effective therapeutic agents against resistant tuberculosis strains.

Abstract ID: 2129

Engineering an intercellular communication toolbox for biological computation and spatial patterning in a consortium of Escherichia coli

Presenting author: Dr. Astri Kusumawardhani, University of Lausanne, Lausanne, Switzerland

Co-authors: *Florian Zoppi, Théodora Steiner, Yolanda Schaerli*

Recent advances in synthetic biology have focused on embedding logical functions within living cells. However, challenges in engineering single cells, such as complexity and metabolic burden[1,2], have led to the growing interest in multicellular systems, where computation is distributed among different cell types. Distributing functions across a multicellular consortium can overcome these issues and unlocking applications including biocomputing, biomedical therapy and bioprocess technology. However, a major challenge remains in establishing orthogonal intercellular communication, or "wires", within synthetic bacterial consortia.

In this project, we engineered multiple intercellular communication systems to enable distributed computing within a consortium of *Escherichia coli* (Figure 1). First, we established a DNA-based communication system by transferring single guide RNAs (sgRNAs) on plasmids via M13 phages between donor and receiver cell populations [3]. In receiver cells, the transferred sgRNAs mediated gene regulation through CRISPR interference, allowing the construction of one-, two-, and four-input logic gates. Additionally, we demonstrated the use of conjugation as another DNA-based communication mechanism.

We also developed a chemical-based communication system utilizing the volatile organic compound indole. We identified a promoter region and its corresponding repressor protein capable of detecting and responding to indole, along with the gene responsible for its overproduction. By characterizing this promoter-repressor pair and optimizing the *E. coli* host, we achieved a robust dynamic range with a 100-fold activation. Furthermore, we implemented quorum-sensing-based intercellular communication channels.

By integrating DNA-based and chemical-based communication modes, we created a versatile communication toolbox that spans contact-dependent and diffusion-based communications (Figure1). This toolbox enables distributed computing across diverse

setups. Ongoing work focuses on combining the different communication modes to achieve spatial patterning and two-way communication.

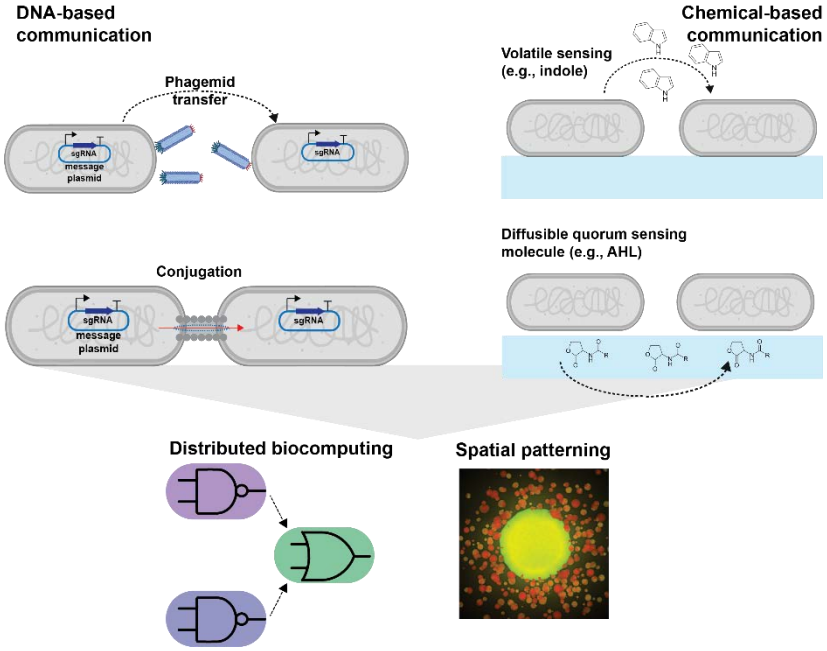


Figure 1: Design of intercellular communication using DNA-based and Chemical-based communication system. Intercellular communication can be achieved through transferring message plasmid using M13 phage or conjugation as well as through volatile compounds and quorum sensing molecules. Ongoing work focuses on combining the different communication modes to achieve spatial patterning and two-way communication.

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Abstract ID: 167 / Poster Number: 470.15S

Engineering Non-pathogenic Bacteria to Secrete Functional Interferon Using Autotransporter

Presenting author: Assoc. Prof. Neta Sal-Man, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Co-authors: *May Tfilin*

In recent years, various strategies have been developed to enable the oral administration of protein-based drugs (biologics) with the aim of overcoming the degradation and inactivation of these drugs that can occur as they traverse the gastrointestinal tract (GIT). In this study, we investigated bacteria as a delivery vehicle for biologics, harnessing their ability to withstand the harsh gastric environment and deliver therapeutic drugs directly to the intestine. Specifically, we explored using the type 5 secretion system (T5SS) to secrete therapeutic cargoes under simulated gut conditions. Our research focused on EspC, a T5SS protein from enteropathogenic Escherichia coli, and its potential to secrete interferon-α (IFNα), a cytokine with immunomodulatory and antiviral properties widely used in the clinic. We demonstrated that EspC can facilitate the secretion of IFNα variant when expressed in non-pathogenic bacteria. Moreover, this EspC-secreted IFN was able to activate the JAK-STAT pathway, upregulate IFN-stimulated genes, and induce a robust antiviral response in cells. Collectively, these findings provide proof of concept supporting the utilization of the EspC protein as a novel delivery platform for protein-based therapeutics.

Abstract ID: 1637

Engineering sequential cell differentiation in Escherichia coli leads to self-organized spatial patterning

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Co-authors: *Hélène Siboulet, Giacomo Ceracchini, Içvara Barbier, Gábor Holló, Hadiastri Kusumawardhani, Yolanda Schaerli*

In nature, complex multicellular structures originate from a single cell that contains all essential information for differentiation, patterning and development. Despite being genetically identical, cells can morphologically and functionally diverge as the result of a complex interplay of several factors. Synthetic biology allows researchers to adopt a bottom-up approach, studying individual cell differentiation building blocks in isolation and then combining them to progressively increase the system's complexity.

In this study, we engineer a multi-step differentiation program in the model prokaryote *Escherichia coli*. Starting from genetically identical cells and without providing any external positional information, we generate complex spatial patterns. More specifically, we first employ a toggle switch to break the homogeneity in the initial population and stochastically differentiate into two subpopulations: senders and receivers. Next, we enable further differentiation of a subset of receivers that are in close proximity to a sender through quorum sensing based cell-to-cell communication. The cells destiny and their mutual influence can be represented via a differentiation landscape (Fig 1A). Wiring each cell state with a fluorescent reporter gives rise to patterns at the Petri dish level, characterized by red areas surrounding green centres, corresponding to sender colonies (Fig 1B). By tuning the colony abundance and the ratio of senders and receivers, the 2-step differentiation system can generate a variety of spatial patterns that we can successfully recapitulate with simulations of a mathematical model.

Currently ongoing work focuses on expanding the system with a third sequential differentiation step. In conclusion, we engineer a multi-step differentiation program to produce completely self-organized patterns from unicellular bacteria and provide a promising approach to pattern engineered living materials.

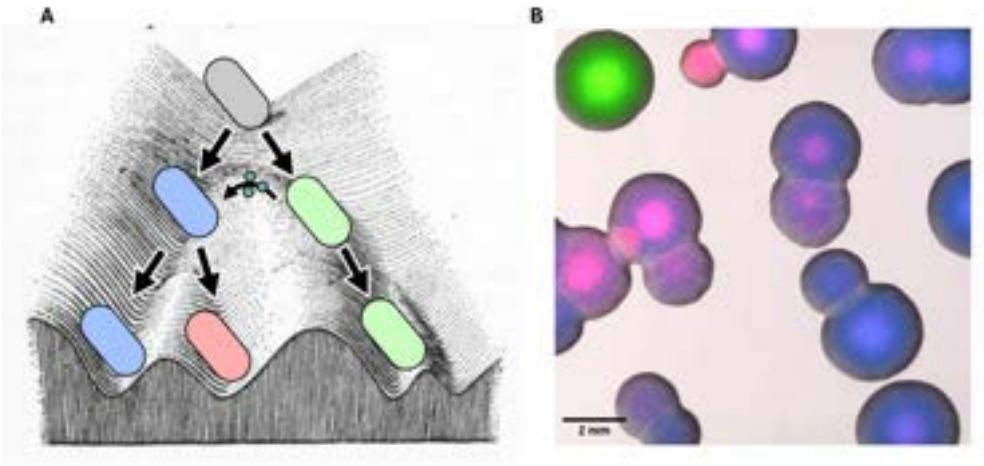


Figure 1: **A.** Schematic representation of the sequential differentiation system inspired by Waddington's landscape. An undifferentiated cell can become a green sender or a blue receiver. Receivers close enough to senders can, in turn, become red. **B.** Microscopy image of the 2-step sequential differentiation system grown on regular Petri dishes. Channels: brightfield, Red (mCherry), green (GFP), blue (mCerulean).

Abstract ID: 926 / Poster Number: 416.15P

Engineering surface display in Debaryomyces hansenii

Presenting author: Ms. Antonia Paić, University of Zagreb Faculty of Food Technology and Biotechnology, Zagreb, Croatia

Co-authors: *Tea Martinić Cezar, Renata Teparić, Bojan Žunar*

Surface display is an engineering technique that facilitates the covalent binding of proteins to the microbial exterior, such as the yeast cell wall, thereby anchoring proteins and transforming the cell wall into a catalytically active interface. A particularly compelling aspect of this technique is its ability to covalently bind biotechnologically relevant enzymes to the outer cell surface. To date, most surface display systems in fungi have been developed by using model yeas *Saccharomyces cerevisiae*. However, this species does not tolerate high salinity and therefore cannot be used for seawater-based bioprocesses or the treatment of saline wastewater. To address these drawbacks, we extended the surface display methodology to extremophilic yeast *Debaryomyces hansenii*, which is able to grow in media supplemented with up to 4 M NaCl. To utilize this organism's potential, we engineered a new genetic marker, constructed expression vectors with some of its strongest constitutive promoters, and investigated several of its promoters responsive to changes in salinity. Building on these advances, we have developed constructs that covalently bind several biotechnologically relevant enzymes to *D. hansenii*'s cell surface, and thus, for the first time, transformed *D. hansenii*'s cell wall into an engineered, catalytically active surface.

Abstract ID: 3356 / Poster Number: 408.17P

Engineering the Marine Pseudoalteromonas haloplanktis TAC125 via pMEGA Plasmid Targeted Curing Using PTasRNA Technology

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Co-authors: *Concetta Lauro, Marzia Calvanese, Christopher Riccardi, Andrea Colarusso, Marco Fondi, Ermenegilda Parrilli, Maria Luisa Tutino*

Introduction

Marine psychrophilic bacteria, that thrives in extreme cold environments, are valuable resources in industrial biotechnology. Among them, *Pseudoalteromonas haloplanktis* TAC125 (*Ph*TAC125) is a model organism to study psychrophilic physiology and proved to be an exceptional host for recombinant production of challenging human proteins. The discovery of its endogenous megaplasmid, pMEGA, raises questions about its physiological and functional role in *Ph*TAC125. Understanding the role of megaplasmids in psychrophilic bacteria can contribute to the knowledge of bacterial genetic mechanisms and expand the potential applications of psychrophiles in biotechnology processes.

Objective(s): This study aims to provide new insights into the functional roles of megaplasmids by developing a strategy to cure *Ph*TAC125 of pMEGA plasmid. The resulting strain KrPL^2 allows exploration of pMEGA contributions to *Ph*TAC125 physiology, focusing on the evaluation of growth performances, oxidative stress response and biofilm formation ability. Removal of pMEGA might enhance *Ph*TAC125 performance achieving a more suitable strain for biotechnological applications.

Method: Genomic and transcriptomic landscape of the pMEGA plasmid was explored using bioinformatic tools (NCBI, BLAST, MaGE). To cure pMEGA, we designed a two-step genetic approach. Firstly, homologous recombination was employed by integrating the pAT suicide vector carrying a selection marker into pMEGA, necessary to distinguish wild type from cured bacteria. Next, we used the PTasRNA gene silencing technology to target and interfere with the replication machinery of the plasmid. The successful removal of pMEGA resulted in the construction of a novel strain named KrPL^2. Thorough phenotypic characterization was performed assessing: growth performances in different media and temperature conditions, response to oxidative stress through a disk-diffusion assay, biofilm formation ability, replicability, stability and copy number of heterologous plasmids.

Findings and conclusion: The results highlight the successful removal of the pMEGA plasmid achieved through a precise genetic modification strategy, demonstrating the potential for targeted manipulation of megaplasmids. Our findings show that PTasRNA technology is effective in silencing plasmid replication genes, extending its application to other genetic manipulations in psychrophilic bacteria, and demonstrating its broad potential for future genetic studies. The cured KrPL^2 strain acquired novel phenotypic changes exhibiting enhanced resistance to oxidative stress, which is a critical feature for biotechnological applications, especially in industrial fermentation systems where oxidative stress is a limiting factor. Additionally, we observed a reduced capability of biofilm formation, offering potential advantages in industrial settings by improving process efficiency and reducing contamination risks. This study underscores the value of studying *P. haloplanktis* TAC125 as a model organism for cold-adapted microorganisms, revealing its potential for biotechnological applications such as the production of "difficult proteins" and environmental stress responses. The successful removal of the pMEGA plasmid opens new opportunities for utilizing this strain in recombinant protein production and other industrial applications.

Abstract ID: 492 / Poster Number: 265.17P

Enhanced ectoine production in Paludifilum halophilum through a pulsed glucose feeding fermentation approach in a 7-L bioreactor

Presenting author: Prof. Sami Maalej, Faculty of Sciences, SFAX, Tunisia

Co-authors: *Houda Ayadi, Ahlem Ammar*

Introduction: The halophilic bacterium Paludifilum halophilum naturally produces ectoine, a compound with strong moisturizing, protective, and stabilizing properties, highly valued in the cosmetic and pharmaceutical industries (1,2).

Objective: This study aimed to enhance ectoine production by analyzing the effects of nitrogen and carbon sources in batch flask cultures and employing a pulsed fed-batch fermentation strategy in 7L bioreactor.

Method: Fresh mycelium was cultivated in sea water medium and cultures were analyzed for growth, ectoine, glucose, and nitrogen levels. In the fed-batch fermentation, 3.8 L of seawater medium supplemented with ammonium chloride (NH4Cl) and glucose were inoculated with 0.2 L preculture. Once glucose dropped below 1 g/L, fresh medium was added to maintain a glucose concentration of 10 g/L and a C/N ratio of 5. Samples were taken every 24 hours to measure dry cell weight (DCW), residual glucose, ammonia-nitrogen (NH4+-N), and ectoine content.

Findings: Ammonium chloride was identified as the most effective nitrogen source, enabling maximum ectoine production (82.2 mg/g DCW) and biomass growth (3.2 g/L). During fermentation, glucose and nitrogen levels declined gradually, while biomass increased to 5.69 g/L over 162 hours. Subsequent glucose feeding further boosted dry cell weight and intracellular ectoine concentration, achieving a maximum ectoine content of 243 mg/g DCW and productivity of 0.216 g/(L·day).

Conclusion: The findings demonstrate that P. halophilum is a promising candidate for industrial-scale ectoine production, especially when optimized with a controlled C/N ratio and pulsed glucose feeding strategy. This scalable approach highlights its potential in meeting growing industrial demands for ectoine

Abstract ID: 1937 / Poster Number: 323.16F

Enhanced Hydrogenase Activity in Cupriavidus necator H16 Cultivated on a Mixture of Whey and Glycerol under Microaerophilic Conditions

Presenting author: Mrs. Ofelya Karapetyan, Yerevan State University, Yerevan, Armenia

Co-authors: *Meri Iskandaryan, Anna Poladyan*

Cupriavidus necator H16 is a chemolithoautotrophic bacterium with significant biotechnological potential. It synthesizes oxygen tolerant [NiFe] hydrogenase (Hyd) enzymes that serve as promising anodic catalysts in enzymatic fuel cells (EFCs). Cheese and curd whey (SW and AW) of the dairy industry are rich in proteins, sugars and minerals; consequently, they are a suitable source for cultivation of microbes. In this study, AW and SW, both individually and in combination with glycerol (G), were evaluated for the cultivation of *C. necator* H16 and the investigation of H_2-oxidizing total Hyd activity. Bacterial growth, oxidation-reduction potential (ORP) kinetics and Hyd activity were followed under microaerophilic conditions for 7 days, at 30 °C, pH 7.0. During bacterial growth, the drop of ORP from positive (+ 250 ± 10 mV) to negative (- 115 ± 5 mV) values was shown. H_2-oxidizing total Hyd activity of whole cells quantified by monitoring H_2-dependent methylene blue reduction. Maximum biomass formation was observed on the 5th day of bacterial growth with AW (OD_{600} 3.13) and on the 6th day with SW+G (OD_{600} 3.3). Prolonged bacterial growth was observed in glycerol-supplemented samples. With the decrease of pH and ORP Hyd activity was detected in all conditions; however, compared to AW and SW, enhanced 0.17 ± 0.03 U / min/g CDW H_2-oxidizing Hyd activity was shown in the sample with SW+G. The results will facilitate the production of economically valuable Hyd enzymes from industrial side streams and advance hydrogen-based EFC technologies.

Abstract ID: 3309 / Poster Number: 378.16P

Enhanced Hydrogenase Activity in Cupriavidus necator H16 Through the Utilization of Recycled Whey as a Substrate

Presenting author: Lusine Baghdasaryan, PhD, Yerevan State University, Yerevan, Armenia; Scientific-Research Institute of Biology, Yerevan, Armenia

Co-authors: *Tatev Asatryan, Ofelya Karapetyan, Anna Poladyan*

Cupriavidus necator and *Escherichia coli* are valuable organisms in biotechnology. *C. necator* is known for producing O_2-tolerant [NiFe]-hydrogenases (Hyds) and polyhydroxyalkanoates, whereas *E. coli* generates H_2 and bacterial biomass during mixed acid fermentation. A combination of curd and cheese whey (CW) was employed to investigate the biphasic growth of *E. coli* BW25113 and *C. necator* H16. The curd and cheese whey mixture, prepared in equal volumes, underwent physicochemical treatment and served as the microbial culture medium. The growth of *E. coli* BW25113 wild type, hydrogen production, and redox potential (ORP) kinetics were analyzed using CW at pH 7.5. After 24 h, the Pt electrode readings significantly decreased from positive to slightly negative (-540±10 mV in glycerol-containing medium and -490±10 mV without glycerol), indicating the production of H_2 during bacterial growth. Glycerol supplementation enhanced both hydrogen production and Hyd activity. After *E. coli* cultivation and cell removal, the remaining growth medium was used to culture *C. necator* H16 at pH 7.0. *C. necator* H16 showed peak growth after 72 hours in the glycerol-supplemented medium, reaching an OD_{600} of about 2.9 ± 0.02. Moreover, the H_2-oxidizing hydrogenase activity in whole cells reached a maximum of 1.2 ± 0.05 U/min/mg CDW. Polyhydroxyalkanoate synthesis was also detected, particularly in the presence of glycerol. This study demonstrates that cost-effective, industrial by-products like whey can be reused as a substrate for producing *C. necator* H16 biomass and Hyd enzymes. Furthermore, two-phase cultivation offers a highly efficient approach to maximizing outputs.

Keywords: Dairy industry by-products$_1$, biphasic cultivation$_2$, hydrogenases$_3$, *Cupriavidus necator*$_4$.

Abstract ID: 395 / Poster Number: 048.17P

Enhanced multi-species biofilm formation and antimicrobial resistance of Salmonella Typhimurium to sodium hypochlorite

Presenting author: Se-Wook Oh, Kookmin University, Seoul, South Korea

Co-authors: *Unji Kim*

Introduction: The widespread use of sodium hypochlorite as a disinfectant in food processing environments has raised concerns about its potential role in promoting bacterial biofilm formation.

Objective: The objective of this study was to evaluate the minimum inhibitory concentration (MIC) of sodium hypochlorite on S. Typhimurium and understand the sodium hypochlorite resistance mechanism in dual- and multi-species biofilms based on S. Typhimurium in relation to aggregation, cell leakage, morphology, and gene expression.

Methods: MIC was evaluated for the assessment of antimicrobial resistance of S. Typhimurium to sodium hypochlorite. Biofilm was formed on 96-well microtiter plate and was assessed with crystal violet assay. To understand the mechanism of antimicrobial resistance, aggregation, cell leakage, propidium iodide uptake, extracellular polymeric substances (EPS) quantification, FE-SEM, and relativegene expression were performed.

Findings: Exposure to sub-lethal concentrations of sodium hypochlorite triggered oxidative stress responses in S. Typhimurium, enhancing the production of EPS and increasing bacterial adhesion to stainless steel and other food-contact surfaces. Multi-species biofilms, co-cultured with Listeria monocytogenes, exhibited significantly greater biomass and structural complexity under sodium hypochlorite treatment compared to untreated controls. Transcriptomic analysis revealed upregulation of stress response and biofilm-associated genes, including those linked to quorum sensing and EPS production.

Conclusion: The findings indicated that the formation of dual- and multi-species biofilms with L. monocytogenes has significantly increased the resistance to sodium hypochlorite. These results emphasized the importance of targeting multi-species biofilm for sanitization to ensure food safety.

Abstract ID: 992 / Poster Number: 391.16P

Enhancement of hydrogen photoproduction by Parachlorella kessleri using a mixture of brewery and ethanol production wastes

Presenting author: Jemma Manoyan, PhD, Yerevan State University, Yerevan, Armenia

Co-authors: *Armine Petrosyan, Lilit Gabrielyan*

Introduction: The importance of renewable energy sources has become crucial in the search of sustainable energy solutions. One promising approach is the use of microalgae for hydrogen (H_2) production. Application of microalgae for industrial waste recycling offers a promising way to reduce waste and improve sustainability in industries facing increasing pollution levels. These wastes contain substantial amounts of organic material, which microalgae can effectively utilize to produce high yields of H_2.

Objective: The current study aims to investigate H_2 photoproduction by microalga *Parachlorella kessleri* during utilization of a mixture of brewery and ethanol production wastes.

Methods: *P. kessleri* MDC6524 (Microbial Depository Center, NAS, Armenia) was used in this study. H_2 production assays were performed under anaerobic conditions and continuous illumination. H_2 yield was determined using an electrochemical method and gas chromatography.

Findings: The highest H_2 yield in *P. kessleri* was observed in media containing mixed waste, which was 2-3 times higher than the H_2 yields of microalgae cultivated in sole ethanol and brewery production waste, and 7 times higher compared to the control grown in standard Tamiya medium. H_2 generation in this microalga occurred only in diluted waste containing media. H_2 yield was not observed when algae cultivated in undiluted waste containing media, which is due to the high concentration of organic compounds in the waste that inhibit the H_2 production by microalgae.

Conclusion: The obtained data suggest that a mixture of diluted ethanol and brewery production waste is a promising substrate for increasing H_2 yield in microalgae.

Abstract ID: 745 / Poster Number: 075.17P

Enhancement of oxidative stress improve treatment efficiency against Enterococcus faecium

Presenting author: Ms. Julie Plouhinec, University of Caen Normandy, CBSA UR4312, Caen, France

Co-authors: *Axel Hartke, Abdellah Benachour, Eliette Riboulet-Bisson*

Introduction: Since the 70's-80s, enterococci are a major concern for public health because of their ability to cause hospital acquired infection (HAI). The species E. faecium is categorized as high priority to find new therapeutic treatments because of its multidrug resistance. A recent study showed a link between oxidative stress and activity of tigecycline (TIG), a bacteriostatic antibiotic in E. faecium.

Objective: Our research goal is to potentiate tigecycline efficiency to make it bactericidal instead of bacteriostatic. We developed two strategies: either preventing bacterial defense against oxidative stress or sharpen cellular oxidative stress.

Methods: We used molecular docking to identify potential inhibitors of NADH peroxidase (Npr) an antioxidant enzyme which degrade H2O2. We used menadione (MEN), a prooxidant, to increase oxidative stress during TIG treatment. A treatment combining TIG and MEN was evaluated by determining TIG MICs in presence or absence of MEN, FIC index, effect on E. faecium AUS0004 survival and oxidant production.

Findings: Based on molecular docking, we tested 14 molecules in an enzymatic assay which allowed us to identify six molecules with in vitro inhibitory activity against Npr. E. faecium was killed by the treatment TIG + MEN. We showed that these molecules have an additive effect and that this effect relies on H2O2 as addition of extracellular catalase prevents bacterial killing.

Conclusion: These results constitute a first step towards our goal of potentiating tigecycline efficiency either by combining the antibiotic with menadione to increase intracellular H2O2 level, or by inhibiting NADH peroxidase to prevent H2O2 degradation.

Abstract ID: 1467 / Poster Number: 418.15P

Enhancing Biological Activities with Hybrid Vesicles by Fusing EVs and OMVs from Rhodobacter sphaeroides

Presenting author: Ms. Subin Lee, Jeonbuk Natioal University, Jeonju, South Korea

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This study explores the development of hybrid vesicles by fusing extracellular vesicles (EVs) and outer membrane vesicles (OMVs) derived from *Rhodobacter sphaeroides*, aiming to enhance biological activities and stability. EVs naturally exhibit antioxidant properties by scavenging reactive oxygen species (ROS), anti-inflammatory effects by inhibiting pro-inflammatory cytokine production, cell protection through the modulation of oxidative stress, and immune regulation by influencing immune cell responses. These characteristics make EVs ideal candidates for various therapeutic applications. On the other hand, OMVs, as nanoscale structures derived from bacterial outer membranes, are enriched with immunogenic components such as lipopolysaccharides and proteins, providing robust immunostimulatory and adjuvant-like properties. The combination of these vesicles was achieved using various fusion methods, including mixing, sonication, extrusion, and freeze-thaw cycles, to optimize fusion efficiency. Key findings revealed that hybrid vesicles demonstrated enhanced antioxidant, anti-inflammatory, and anti-aging properties, offering significant potential for applications in skincare and pharmaceutical industries. Comparative analyses using NTA and Bio-TEM confirmed successful hybrid vesicle formation, while cellular uptake experiments showed enhanced internalization. The hybrid vesicles exhibited a synergistic effect by reducing iNOS expression and mitigating inflammation, particularly under LPS-induced conditions. Furthermore, they demonstrated enhanced wound healing capabilities and stability compared to individual EVs and OMVs. This study provides a novel platform for the development of hybrid vesicles with therapeutic applications, particularly in oxidative stress, inflammation modulation, and skin health. Future optimization of fusion methods and exploration of clinical applications hold promise for advancing nanovesicle-based technologies.

Abstract ID: 3120 / Poster Number: 376.16P

Enhancing Food Safety in Plant-Based Milk Alternatives: The Role of High Hydrostatic Pressure and Nisin Against Listeria monocytogenes

Presenting author: Ana Rita Sousa, PhD, University of Reading, Reading, United Kingdom

Co-authors: *Nikolaos Giannoulis, Kimon-Andreas Karatzas*

Introduction: Plant-based milk alternatives (PBMAs) are increasingly popular due to their nutritional benefits, lactose-free composition, and lower environmental impact. High Hydrostatic Pressure (HHP) is a non-thermal technology commonly used in the food industry to extend shelf life and ensure food safety. Combining HHP with natural antimicrobials, such as nisin, offers a promising approach to achieving food safety goals under milder processing conditions, contributing to sustainable food processing practices.

Objectives: This study aimed to investigate the potential synergistic effect of combining HHP with nisin to enhance food safety by controlling *Listeria monocytogenes* in PBMAs. Additionally, the study sought to optimize the decontamination process and improve the overall efficiency of HHP technology.

Method: This study focused on the effect of three pressure levels (300-350-400 MPa) combined with nisin (500 IU/mL) on the inactivation of a 5-strain cocktail of *L. monocytogenes* in five commercially available UHT PBMAs derived from oat, almond, soya, coconut and hazelnut. Samples were prepared with and without nisin (controls), inoculated with *L. monocytogenes* (10^7 CFU/mL), and subjected to HHP for 10 min. Three independent biological replicates were conducted, and post-hoc statistical analysis ($p<0.05$) was performed to assess inactivation levels and synergistic effects across PBMA types.

Findings: *L. monocytogenes* was reduced by more than 5 log CFU/ml in oat- and almond-based PBMAs when HHP (400 MPa) was combined with nisin. In soy, coconut, and hazelnut PBMAs, reductions ranged from 3.4 to 4.8 log. Pressures higher than 400 MPa are required to achieve significant synergistic effect in PBMAs from coconut and hazelnut ($p<0.05$). Notably, almond-based PBMA demonstrated significantly higher inactivation with HHP alone at 350 and 400 MPa, whereas soy PBMA showed a protective effect during HHP treatment at 400 MPa, resulting in only a 2.2 log reduction.

Conclusion/Significance: This research highlights the potential of combining HHP with nisin to enhance the inactivation of *L. monocytogenes* and optimize processing conditions for PBMAs. The plant-based protein source appears to significantly influence HHP resistance. These findings contribute to improved risk assessments and support the development of safer and more sustainable food processing technologies.

Abstract ID: 1189 / Poster Number: 285.17P

Enhancing Methane Production through DIET: The Role of GAC, Magnetite, and Substrate Variability

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Methane production via Direct Interspecies Electron Transfer (DIET) presents a promising avenue for improving anaerobic digestion efficiency. This study investigates the effects of conductive materials and substrate variability on methane production. Anaerobic batch experiments were conducted using a 280mL serum bottle with a working volume of 180mL, and the anaerobic digester sludge was used as an inoculum. Granular activated carbon (GAC) and magnetite were supplemented as conductive materials combined with two substrates (3gCOD/L), sodium acetate and acetic acid, respectively, at a fixed food-to-microorganism (F/M) ratio of 5. Acetic acid outperformed sodium acetate, in terms of methane production, producing significantly higher methane production and exhibiting a shorter lag phase—indicative of faster microbial adaptation and activity. The enhanced performance of acetic acid may stem from its greater bioavailability, which facilitates more efficient microbial metabolisms. GAC performed better among the conductive materials tested, yielding higher methane production the control. The GAC and magnetite were shown to enhance different microbial community compositions together with syntrophic microbial communities for accelerating methane generation. However, substrate variability had a more pronounced impact, with acetic acid proving to be the optimal substrate for boosting methane production via DIET mechanisms. Future research will delve into microbial community analysis to identify the specific populations involved in DIET under varying substrate and conductive material conditions.

Abstract ID: 3903 / Poster Number: 260.16P

Enhancing molecular detection of Candida auris. Boosting sensitivity through selective broth enrichment for improved diagnosis and surveillance

Presenting author: Mrs. Chiara Senti, Copan Italia S.p.A., Brescia, Italy

Co-authors: *Federico Degiovanni, Matteo Luigi Beffa, Cristiano Sabelli,*

Introduction: The diagnosis of *Candida auris* infections remains challenging. Rapid and accurate identification of *C. auris* is essential for effective patient management and infection control in healthcare settings. Molecular methodologies have gained interest as they offer improved sensitivity and specificity over standard techniques.

Objective: This study evaluates the performance of Candida Auris Broth (CAB™) (Copan Italia, Brescia), designed for selective *C. auris* enrichment, in combination with a CE-IVD-marked molecular detection assay.

Methods: The *C. auris* CDC*B11-903 strain was tested in CAB™ and eSwab® (0.5 McF:10 dilution). eSwab® was analyzed independently and combined with CAB™ (30 µL inoculated into 2 mL CAB™). CAB™ samples were incubated at 37°C for 24h (T24h) and 48h (T48h), while eSwab® alone was analyzed at T0 as a control. DNA extraction (PrepSEQ, Applied Biosystems) was performed at T0, T24h, and T48h, followed by qPCR (AurisID, OLM) on an AB7500 system. Additional *C. auris* strains (ATCC*B11103, B11220, B11221, B11244) were tested exclusively in CAB™.

Findings: CAB™ enrichment reduced Ct values for *C. auris* CDC*B11-903, with ΔCt of -2.68 (T24h) and -4.83 (T48h). eSwab® in CAB™ shown decreased ΔCt to -4.93 (T24h) and -7.36 (T48h) compared to T0. Additional strains showed similar ΔCt reductions, confirming CAB™'s enrichment potential alone and combined with eSwab®.

Conclusions: CAB™ demonstrated significant *C. auris* enrichment and compatibility with AurisID molecular assay, reducing Ct values at T24h and T48h compared to T0, suggesting *C. auris* molecular detection sensitivity improvement that may facilitate patient surveillance and management.

Abstract ID: 3448 / Poster Number: 340.17P

Enhancing PHBV Production with Lignocellulose-Derived Carboxylic Acids and PHA Hydrolysates

Presenting author: Dr. Michael Egermeier, Wood K plus – Kompetenzzentrum Holz GmbH, Linz, Austria

Co-authors: *Norbert Hohenauer, Gunnar Spiegel*

Polyhydroxyalkanoates (PHAs) are a versatile family of linear bio-polyesters whose properties are strongly influenced by the composition of their monomer units. Despite their potential, widespread application remains limited due to high production costs.

This study investigates the co-polymer poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), focusing on tailoring its structural composition and physical properties by varying the polyhydroxy valerate content. Renewable and cost-effective substrates, including lignocellulose-based carboxylic acids and PHB hydrolysates, are explored for microbial PHBV production.

Levulinic acid serves as a co-feed to achieve a valerate content of 0–45% (w/w) in the co-polymer. The acid-catalysed production of carboxylic acids from lignocellulose forms the basis for fermentation processes using *Cupriavidus necator*. Additionally, PHA hydrolysates, composed of 3-hydroxybutyric acid and crotonic acid, are generated via chemical solvolysis and tested for microbial PHBV production.

This work provides new insights into the production and circular usability of PHB hydrolysates. Carbon source screening has assessed the growth performance of *C. necator*, while bioreactor fermentations demonstrate the feasibility of PHBV production from lignocellulose-based acids and PHB hydrolysates. Furthermore, the use of PHB hydrolysates in microbial processes has been successfully implemented.

Future efforts will focus on optimizing PHBV formation through the combined use of hydrolysates and renewable acids, with the resulting polymers characterized and tested for material applications.

Abstract ID: 1438 / Poster Number: 212.15P

Enhancing Salt Resilience in Phaseolus vulgaris with Microbacterium testaceum and Arthrospira platensis

Presenting author: Ms. Giovanni Pascucci, University of L'Aquila, L'Aquila, Italy

Co-authors: *Rihab Djebaili, Beatrice Farda, Giancarlo Pagnani, Michele Pisante, Marika Pellegrini*

Introduction: Salinity is a critical challenge to agriculture in arid and semi-arid regions, where excessive salt accumulation disrupts plant water uptake, nutrient balance, and growth. Addressing this issue necessitates sustainable approaches to enhance plant resilience under saline conditions.

Objective: This study aimed to evaluate salt stress mitigation in *Phaseolus vulgaris* (green bean) by integrating a halotolerant bacterial strain, *Microbacterium testaceum* (MT), with *Arthrospira platensis* (AP).

Materials and Methods: The MT strain was selected for its plant growth-promoting attributes (i.e., phosphate and potassium solubilization, indole-3-acetic acid production, drought tolerance, siderophore production, and ACC deaminase activity) and used as tanning agent on seeds (10^6 UFC/g). SP powder was applied independently and in combination with MT by leaf spray method. Treatments included: MT, AP, MT+AP, and control. A greenhouse randomized block experiment was set up. Each treatment was challenged to incremental salinity stress levels (0-500 mM NaCl).

Results: The HB tanning improved significantly plant growth performance under salt stress than stressed control (e.g., fresh leaf weight, stem diameter). The combination of SP treatment and HB tanning promoted a higher proline accumulation (1.90 µmol g^{-1} FW), essential for plant osmotic adjustment under salinity stress. The HB treatment alone increased flavonoid contents (29.33 µg mL^{-1}), a marker of stress resilience.

Conclusion: The integration of HB with SP provides a cost-effective, eco-friendly solution to enhance plant resilience and productivity under saline conditions. This combined approach holds significant promise for promoting sustainable agriculture in salt-affected regions.

Abstract ID: 3063 / Poster Number: 267.15P

Enhancing soil CH4 uptake by microbiome engineering

Presenting author: Dr. Jana Täumer, GFZ Helmholtz Centre for Geosciences, Potsdam, Germany

Co-authors: *Susanne Liebner*

Introducing methanotrophs to soils (i.e., microbiome engineering) may promote microbial methane oxidation in high and low-CH4-emitting soils, potentially reducing soil methane emissions or increasing the soil methane sink. However, although microbiome engineering is used in agriculture and has shown promising results in enhancing soil Carbon sequestration, its potential for microbial methane oxidation in soils has yet to be investigated. We tested different type II methanotrophs (*Methylocapsa* and *Methylocistis* strains) for their potential to decrease CH_4 emissions from soils. Therefore, soil samples from wetland (CH_4 emissions) and upland (CH_4 uptake) soils were inoculated with methanotrophs and incubated for 120 days at 15°C in dark conditions at their natural water content. During the incubations, soil GHG concentrations and fluxes were measured regularly, and the methanotroph community was monitored after 7, 30, 60, and 120 days. GHG fluxes (CH_4, CO_2, and N_2O fluxes) of the microcosms were measured in a closed system with a Picarro G2508 gas concentration analyzer. The microbial community was monitored by marker gene sequencing and quantitative PCR (qPCR). Shifts in the methanotroph and microbial community composition were assessed by sequencing a functional marker gene for methanotrophy (i.e., the *pmoA* gene) and the 16S rRNA gene marker, respectively. With qPCR, we quantified changes in the absolute abundances of methanotrophs. In conclusion, our study investigated the potential of introducing methanotrophs as a microbiome engineering strategy to enhance microbial methane oxidation in soils. This may be promising a way to reduce methane emissions in soils.

Abstract ID: 3417 / Poster Number: 087.16P

Enrichment and characterization of Candidate Phyla Radiation bacteria from human and environmental ecosystems

Presenting author: Dr. Daniele Ghezzi, University of Bologna, Bologna, Italy

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Candidate Phyla Radiation (CPR) represents a diverse and largely uncultivated group of bacteria that plays a significant yet still poorly understood role in both human and environmental microbiomes. Despite their genetic and ecological diversity, many CPR exhibit unique adaptations, such as reduced genome sizes, unconventional metabolic pathways, and symbiotic/parasitism relationships with other microorganisms. These interactions can influence the expression of specific metabolic genes in the host microorganism, affecting physiological processes, including nutrient metabolism, immune function, and host-microbe co-metabolism. Some CPR also appear to harbour resistance genes and exhibit adaptive strategies to survive in challenging environments. In this study, we cultivated and characterized CPR from several human and environmental ecosystems, including vaginal and nasal environments, broad bean roots, and contaminated peat-rich aquifers. The bacterial host cells employed for cultivation/enrichment included either reference actinobacterial strains or other bacterial strains isolated from each ecosystem. Molecular characterization revealed distinct CPR taxa associated with each ecosystem. Co-cultivation procedures with actinobacterial host cells were particularly successful in enriching CPR, which belonged to *Candidatus* (*Ca.*) Saccharibacteria, *Ca.* Nomurabacteria, and *Ca.* Roizmanbacteria. However, human-derived *Lactobacillus* host strains were less effective in hosting CPR, suggesting group-specific symbiotic relationships. Finally, preliminary functional assays indicated that CPR interaction reduced antibiotic production in one actinobacterial host. The study of CPR is reshaping our understanding of evolutionary processes, offering novel insights into microbial resilience, the evolution of resistance traits, and the re-evaluation and improvement of antimicrobial strategies.

Abstract ID: 570

ENTELLI-02: Standardised front-line phage product for nosocomial enterobacter cloacae complex infections

Presenting author: Dr. Dinesh Subedi, Monash University, Clayton, Australia

Introduction: Antimicrobial resistance (AMR) is one of the greatest health challenges of our time. Phage therapy is a promising alternative to treat antimicrobial-resistant (AMR) infections. Currently, phage therapy applications are either individually tailored for a given patient's infection or utilise pre-established cocktails of lytic phages with activity against clinically relevant bacterial species. However, existing approaches are either too specific with limited utility, or too broad and lacking antimicrobial efficacy. We need a standardised approach that combines fundamental phage research, clinical insights and AMR data to create institution-specific phage cocktails with front-line efficacy and on-demand availability.

Objective(s): The Alfred Hospital Melbourne (Australia) has experienced an outbreak of nosocomial infections caused by *Enterobacter cloacae* complex, including highly emergent carbapenemase-resistant clones associated with high mortality rates. This project aims to develop a phage-based product to combat these nosocomial isolates. Our objectives are:

1. *Development of targeted phage combinations:* Create a phage cocktail specifically targeting Enterobacter cloacae complex strains prevalent in the institution to address the immediate outbreak.
2. *Personalization for broader efficacy:* Optimise and adapt the phage product to extend its capacity, positioning it as a near-first-line treatment against carbapenemase-resistant pathogens.

Method: Targeting an Enterobacter cloacae complex (ECC) collection (n=206) originating from nosocomial infections at The Alfred Hospital (Melbourne, Australia) over the last decade, we established a baseline phage collection with antibacterial activity against several strains. Through phage-host co-evolution and targeted phage isolation, we systematically improved and refined this cocktail to enhance killing efficiency and host spectrum against challenging ECC strains for which the phage coverage was originally low. The efficacy of the final phage product was validated through in vitro and in vivo infection models.

Findings: This iterative optimisation process led to the creation of “Entelli-02”, a product containing five well-characterised virulent phage, that targets 99% of ECC strains from The Alfred Hospital. Each phage in this product infects their bacterial host through distinct cellular receptors, thereby reducing the emergence of phage-resistance. Entelli-02 reduced bacterial load by >99% in ECC septicaemic mice and provided broad host coverage. We produced a clinical-grade “Entelli-02” product for frontline treatment at The Alfred.

Conclusion: Our approach integrates fundamental phage research and clinical insights to produce a phage product that demonstrates front-line efficacy and ensures its availability for rapid clinical deployment as required. This strategy provides a template for developing tailored phage therapies against challenging nosocomial pathogens at an institution-level.

Abstract ID: 3170 / Poster Number: 169.17P

Enterococcus faecalis Genomic Overview: Striking Balance Between Resistance and Virulence in Infectious Endocarditis

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Co-authors: *Simone Giuliano, Michela Bulfoni, Grete Francesca Privitera, Alfredo Pulvirenti, Alessandra Giardina, Sebastiano Alberto Fortuna, Corrado Pipan, Francesco Curcio, Carlo Tascini, Floriana Campanile*

Enterococcus faecalis, a key cause of infective endocarditis (IE), poses treatment challenges and high recurrence rates. This study aimed to characterize the genomic diversity of 19 *E. faecalis* clinical isolates from IE patients treated with dual beta-lactam therapy, emphasizing on identifying resistance and virulence factors that contribute to treatment challenges and recurrence.

E. faecalis exhibited 13 distinct multilocus-sequence types (STs), among which ST102 and ST72 (3 strains each) were more frequent. ST21 showed an adenine insertion in *pbp*4 promoter region, associated to increased beta-lactam MICs.

All strains possessed six common antimicrobial resistance determinants to macrolide [(MLS)mph(D)], trimethoprim (dfrA), drugs and biocides (efrA/B, emeA), lincosamides and streptogramins [(MLS)lsaA)]; roughly half presented tet(M), ant(6)-Ia and erm(B) genes were less common.

9 core virulence factors encoding biofilm formation (bopD), immunomodulation (cpsA/B), adherence (ebpA/B/C, srtC), endocarditis antigen (efaA), surface fibrinogen-binding-protein (fss1); Q/S-associated genes (fsrA/B/C-gelE-sprE agr-like) were commonly present. 9 strains showed *cps* genes coding for capsular polysaccharide enzymes, with immune-modulation properties.

5 strains showed prgB gene coding for an adhesin with a major role in cellular aggregation and robust biofilm formation. 3 strains carried the cyl-operon/asa1, coding for a cytolysin/hemolysin toxin and aggregation substance. Eight isolates were *ace* defective, lacking an adhesin involved in host-cell attachment and immune-stimulation, suggesting immune-system evasion, persistence and IE relapse.

Most frequent replicon families were repUS43 and rep6 (9 and 5 strains respectively).

This study illustrates IE *E. faecalis* genomic features and variations, endowing phenotypic characteristics which help bacteria overcome antibiotic activity and immune challenges, potentially fostering relapses.

Abstract ID: 2414 / Poster Number: 125.17P

Enterohemorrhagic E. coli O157 continuously acquiring streptomycin- and ampicillin-resistant isolates from healthy Japanese foodhandler from 2006 to 2021

Presenting author: Prof. Tatsuya Nakayama, Hiroshima University, Higashi-Hiroshima, Japan

Co-authors: *Natsuki Ohata, Mamoru Noda, Kenji Ohta, Moritaka Hatta,*

The increasing prevalence of antibiotic-resistant foodborne pathogens is a serious public health concern. Enterohemorrhagic Escherichia coli O157 (EHEC-O157) poses a high risk of food contamination. This study aimed to investigate changes in the prevalence of EHEC-O157 among food handlers in Japan from 2006 to 2021. A total of 279 randomly selected isolates from the years 2006 to 2009, 2019 and 2021 were analysed for their antimicrobial resistance patterns and virulence genes.

The result showed that EHEC-O157 isolation rates, from 2012 to 2022, ranged from 0.0011 to 0.0037%, with an average isolation rate of 0.0024% (659/27,848,713). Antimicrobial susceptibility testing showed that EHEC-O157 isolates from all years were most resistant to streptomycin (>34%) and ampicillin (>7.7%), followed by tetracycline (<5.1%). Since 2019, EHEC-O157 isolates resistant to chloramphenicol (>9.4%) and kanamycin (>2.6%) have been isolated. Furthermore, the detection of Shiga toxin-related genes revealed that STX2 (57.4%) was more prevalent than STX1 (6%) in all sampling years. Whole genome analysis revealed the presence of aph(6)-ld, aph(3'')-ld and blaTEM-1B in the multidrug-resistant isolates, suggesting that these genes contribute to streptomycin and ampicillin resistance. Furthermore, one strain was found to be an extended-spectrum beta-lactamase producer, harbouring the IncFII plasmid that encodes blaCTX-M-15. This resistance plasmid was transferred to other enterobacteria.

Consequently, it can be concluded that EHEC-O157 from healthy individuals have exhibited uninterrupted streptomycin and ampicillin resistance for the past decade. In recent years, there has been a discernible trend towards multidrug resistance in EHEC-O157.

Abstract ID: 2635 / Poster Number: 396.15P

Environmental diversity of Candidatus Babelota and their relationships with protists

Presenting author: Assoc. Prof. Vincent Delafont, Université de Poitiers, Poitiers, France

Co-authors: *Louis Weisse, Lucile Martin, Bouziane Moumen, Yann Héchard,*

Ca. Babelota is a phylum of strictly intracellular bacteria whose representatives are commonly detected in various environments through metagenomics, though their presence, ecology and biology have never been addressed so far. As a group of strict intracellular, we hypothesize that their presence, occurrence and abundance heavily depend on their hosts, which are known as heterotrophic protists, based on few described isolates. Here, we conducted a sampling campaign allowing to characterize protists and associated bacterial communities, using high throughput sequencing. In parallel, a systematic enrichment of protists from samples was performed, to attempt characterization and isolation of new *Ca*. Babelota within native hosts. We found that *Ca*. Babelota are amongst the most widespread phylum among the rare ones. Protists enrichments allowed in certain cases to enrich as well for *Ca*. Babelota, which could be visualized *in vivo* infecting protist cells. Though cosmopolitan, *Ca*. Babelota diversity was highly site-specific. Cooccurrences analyses allowed to retrieve well-known as well as new putative associations involving numerous protists of various trophic regimes. The combination of approaches developed in this study further our understanding of *Ca*. Babelota ecology and biology, while paving the way for future isolation of new members of this elusive phylum, which could have huge impact on protists -and ecosystems- functioning.

Abstract ID: 1947 / Poster Number: 223.15P

Environmental risk assessment at the microbial level: utilizing total RNA sequencing to evaluate the non-target impact of nitrification inhibitors in agriculture

Presenting author: Dr. Erkin Gözdereliler, Aarhus University, Roskilde, Denmark

Co-authors: *Lucas Horstmann, Athanasios Zervas, Jonathan Donhauser, Louise Feld, Cecilie Skov Nielsen, Anders Prieme, Carsten Suhr Jacobsen, Lea Ellegaard-Jensen*

The use of chemicals in agriculture is unavoidable to meeting the growing global demand for food production. Fertilizers and pesticides enhance crop productivity, while nitrification inhibitors can potentially reduce the environmental impacts associated with excessive fertilizer use. Although their targeted effects on specific microbial taxa are well-documented, their broader environmental risks to microbial communities remain poorly understood.

To address these risks, total RNA extraction and deep sequencing provides a promising method for uncovering non-target effects without bias toward specific taxa or incorporating signals from dead biomass. Simultaneous analysis of rRNA and mRNA enables the evaluation of negative impacts, not only on microbial activity but also on critical ecosystem functions. Moreover, this approach provides an opportunity to identify previously unknown effects and better understand the ecological roles of microbial taxa.

In the amoA project, a field trial employing total RNA analysis investigated the non-target effects of nitrification inhibitors nitrapyrin and DMPP on microbial soil communities, both of which are known to reduce emissions of the greenhouse gas N_2O. Specifically, mRNA analysis was used to assess potential impacts on enzymes closely associated with ammonia monooxygenase, the primary target of nitrification inhibition. Ongoing in-depth analyses aim to identify affected taxa, link them to their ecological roles and uncover suppressed genes to pinpoint ecosystem functions potentially disrupted by their use.

Refining this method will strengthen its application in agrochemical studies, making total RNA analysis an essential tool for integrating microbial community data into environmental risk assessments and promoting sustainable agricultural practices.

Abstract ID: 2324 / Poster Number: 128.15F

Epidemiology and inactivation of Tomato Brown Rugose Fruit Virus (ToBRFV) in Commercial Greenhouses

Presenting author: Mr. Emilien Di Rosa, Institut National de la Recherche Scientifique (INRS), Laval, Canada

Co-authors: *Audrey-Anne Durand, Caroline Provost, Philippe Constant*

Tomato brown rugose fruit virus (ToBRFV), a recently identified member of the *Tobamovirus* genus, was first reported in Israel in 2014. This virus primarily infects solanaceous crops, with tomato (*Solanum lycopersicum* L.) and bell pepper (*Capsicum annuum* L.) as its main hosts. Its rapid spread and capacity to remain infectious on various surfaces for extended period causes significant economic losses, posing a significant threat to greenhouse production systems. Our research aimed to examine the distribution of the virus in relation to greenhouse conditions and management practices across several tomato production companies in Québec (Canada) and evaluate the performance of disinfection methods. A total of 311 tomato samples were collected in 31 commercial greenhouses across three sampling periods (February, July and September 2022) and submitted to ToBRFV detection via RT-PCR and genome sequencing using the Nanopore technology. The virus was detected in seven commercial greenhouses, with 11 positive samples out of the 311 analysed. No correlation was found between greenhouse conditions and ToBRFV detection, suggesting that no specific greenhouse profile is more susceptible to infection than others. Retrieved partial genome sequences were affiliated to ToBRFV variants from North America, suggesting cross-border propagation through commercial trades. A strict control in the seed's importation would thus be a way to avoid virus propagation across continents. Various chemical disinfectants were integrated in a ToBRFV tomato plant infection assays. The product Bioxy was proven as the most efficient to inactivate the virus, with 77% reduction compared to control.

Abstract ID: 3583 / Poster Number: 185.17P

Epidemiology and phenotypical characterisation of Staphylococcus aureus in a Portuguese healthcare setting

Presenting author: Ms. Ana Catarina Rodrigues, Coimbra's Healthcare Integrated Delivery System, Coimbra, Portugal; University of Coimbra, Coimbra, Portugal

Co-authors: *Isabel Henriques, Catarina Chaves*

Antimicrobial resistance is a significant public health concern, with Staphylococcus aureus as a major healthcare-associated pathogen, requiring surveillance and effective management strategies.

In this study, 779 *Staphylococcus aureus* isolates were randomly collected in 2023 from Coimbra's Healthcare Integrated Delivery System, Portugal, and analysed for their phenotypic profiles and associated epidemiological data. Among these, 219 isolates (28.1%) were obtained from samples collected at the emergency room, 117 (150%) from ambulatory services, and 443 (56,87%) were from inpatient services. Regarding patient gender, 324 isolates (41,6%) were from female patients and 455 (58.4%) from male patients.

The age distribution revealed a higher prevalence in patients over 65 years, accounting for 424 isolates (54.4%), followed by 265 isolates (34.0%) from patients aged 18 and 64 years and 90 isolates (11.6%) from patients under 17 years. After duplicate removal according to EUCAST guidelines, 657 isolates were phenotypically evaluated. Of these, 217 (33.1%) were classified as MRSA, 438 (66.9%) as MSSA, and 2 remained unclassified. Notably, in five cases, the acquisition of resistance to oxacillin was demonstrated.

All isolates were found to be sensitive to vancomycin; however, 27 (4.1%) presented a borderline minimum inhibitory concentration of 2 mg/L, comprising 17 MSSA (3.9% of the total number of isolated MSSA) and 10 MRSA (4.6% of isolated MRSA). This raises concerns regarding alternative treatment options.

This evaluation provides critical insights to ensure that antimicrobial resistance control programs are aligned with global objectives to combat antimicrobial resistance effectively.

Abstract ID: 1905 / Poster Number: 461.15P

Epigenetic Gene Regulation by Restriction Modification Systems in Streptococcus pneumoniae and Escherichia coli

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Restriction modification systems (RMSs) provide means of genetic isolation to bacterial clones and lineages. In addition to allowing preferential recombination with DNA from highly related isolates, chromosome methylation is hypothesised to have epigenetic effects on gene regulation, particularly for phase variable RMSs.

Promoters containing SpnIII or EcoKI target recognition sites were cloned into a luciferase reporter system within their respective hosts, *Streptococcus pneumoniae* and *Escherichia coli*, to investigate promoter activity based on methylation status.

In both bacterial species, promoter methylation significantly influenced reporter gene expression. These changes corresponded to the observed differences in promoter activity between hosts with and without a functional methylation system. These findings were validated by RNA sequencing and quantitative PCR assays, which yielded consistent results. Testing the role of histone-like proteins in this context, suggests that a significant portion of the epigenetic effect is mediated through alterations in histone-like protein binding and thus DNA conformation.

Our data clearly demonstrate that epigenetic regulation of gene expression in prokaryotes is driven by DNA methylation and mediated through histone-like protein binding, which induces conformational changes in chromosomal DNA.

Abstract ID: 2093 / Poster Number: 184.16P

Escherichia coli in urosepsis – genetic and proteomic studies for the identification of new markers for the risk assessment of urosepsis

Presenting author: Assoc. Prof. Beata Krawczyk, Gdańsk University of Technology, Gdańsk, Poland

Co-authors: *Paweł Wityk, Michał Markuszewski, Joanna Raczak-Gutknecht*

Introduction: Depending on the geographical region, 9%-31% of severe sepsis cases are reported to be caused by a urinary tract infection. Early identification of bacterial markers is important in host-pathogen interaction and may be useful in assessment sepsis risk.

Objective: Our study aimed to analyse virulence factors and proteomic profiles of *E. coli* strains isolated from patients with uncomplicated UTI (85 isolates) and patients with urosepsis (64 isolates) who were admitted to the Emergency Department of the Medical University of Gdansk (Poland) between 2019 and 2022.

Method: Exclusion criteria were hospital-acquired infections confirmed by genotyping (PCR melting profile method). The PCR assay was used to detect 36 genes encoding virulence factors for UPEC strains: fimbriae type, afimbrial adhesins, invasins, toxins, autotransporters, capsules and iron uptake systems. Phylogenetic groups were also described using the Clermont method. The *E. coli* strains cultivated in artificial urine were subjected to proteomic analysis using LC-MS technologies.

Finding: Based on binary classification, two genes - *sfa* (fimbriae S) and *upaG* (autotransporter) were significantly differentiated between the urosepsis and control groups. Key findings included also bacterial iron uptake systems such as Aerobactin and the Iha protein. The B2 phylogroup was dominated by *upaG* and *ag43* autotransporters for the urosepsis group. Proteomic analyses indicated 17 bacterial proteins found in urosepsis only, mainly related to Fe uptake and oxidative stress.

Conclusion: The identification of bacterial biomarkers offers promising tools for early diagnosis and risk stratification in urosepsis.

Abstract ID: 4090 / Poster Number: 247.16P

Escherichia Coli Isolates From Sputum Samples In The Background Of Others Sources: A Comparative Study

Presenting author: Ms. Anna Dziuba, Jan Kochanowski University Of Kielce, Kielce, Poland

Co-authors: *Julia Dulębska, Monika Wawszczak-Kasza, Wioletta Adamus-Białek*

Escherichia coli is one of the most frequently isolated clinical bacteria which exhibit the increasing antibiotic resistance.

The aim of this study was to characterize the antibiotic resistance profiles (ARP), phylogenetic classification, biofilm formation, and virulence-associated genes (VAGs profiles) of *E. coli* isolates from sputum in comparison to other clinical materials.

E. coli isolates were collected from: sputum (N=22), urine (N=194), lower respiratory tract (N=91), wounds (N=75), body fluids (N=161), blood (N=132), and other sources (N=44). The low sputum sample count is due to rare *E. coli* isolation. Biofilm formation was assessed using crystal violet staining and *agn43, iha, ibeA* were identified via PCR. Data on ARP, phylogenetic groups, and *papC, sfaD/sfaE, cnf1, usp, fimG/H, hlyA* were reused. Statistical analysis included the chi-square test with Fisher's correction and Kruskal-Wallis ANOVA with post-hoc tests ($p<0.05$).

The majority (86%) of sputum isolates belonged to the phylogenetic group B2. These isolates exhibited significantly higher antibiotic resistance compared to other groups, except for blood. Among them, 64% were ESBL-positive, and 41% multi-drug resistant. Sputum isolates formed significantly larger biofilms than isolates from body fluids, wounds, and blood. Additionally, they more frequently carried the *agn43* gene compared to all other groups and the *iha* gene more often than isolates from the lower respiratory tract, wounds, and other sources. Other VAGs were either less prevalent or did not show statistical significance.

Sputum-derived *E. coli* isolates exhibit distinct resistance and virulence profiles, emphasizing the need for enhanced monitoring and targeted treatment strategies.

Abstract ID: 4100 / Poster Number: 202.17P

Escherichia marmotae – an Emerging Pathogen or Overlooked Species?

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Introduction: In 2015, a new species *E. marmotae* was described. It was originally isolated from the Himalayan marmot and includes strains from *Escherichia coli* cryptic clade V. In 2021, two additional species were proposed: *E. withamii* for strains from *E. coli* cryptic clade II, and *E. ruysiae* for the strains from cryptic clades III and IV. All three new species are considered emerging opportunistic pathogens with zoonotic potential.

Objective: The aim of our study was to investigate the occurrence and characteristics of *E. marmotae* in aquatic ecosystems and karst cave environments.

Methods: Fifty *E. marmotae* isolates were obtained from various environmental aquatic and bat guano samples, identified by MALDI-TOF mass spectrometry, and sequenced using the Illumina platform. All isolates were tested for hemolytic activity, lactose metabolism, and antimicrobial resistance by the disc diffusion method.

Findings: Only tree out of fifty *E. marmotae* isolates exhibited the Lac^- phenotype, a characteristic of the type strain. Whole genome analysis revealed that none of the isolates were resistant to standard antimicrobial agents. This was also confirmed by disc diffusion antibiograms. Further sequence analysis identified several virulence genes characteristic of *E. coli*, with significant variability in sequence similarity and the presence of multiple plasmids. Although hemolytic activity was confirmed, no known hemolysin genes were detected.

Conclusion: Based on our results and available data, it remains uncertain whether *E. marmotae* is an overlooked, environmentally adapted species or an emerging pathogen.

Abstract ID: 1683 / Poster Number: 288.16F

Essentiality of recombinational repair factors in Thermus thermophilus

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Co-authors: *Marc Gost, Marta Failde, Alba Blesa, Verónica Pulido, Bruna Fernanda Silva de Sousa*

Recombinational repair is a critical DNA repair mechanism in living organisms. In the extremophilic bacterium *Thermus thermophilus* (Tth), a traditional source of proteins for crystallographic studies, an integrative understanding of the recombinational repair routes operating *in vivo* has been lacking.

Tth's repertoire of recombination factors includes most conserved bacterial proteins known to be involved in recombination, such as AddAB, RecA, F, O, R, J, RuvABC and more. In addition, Tth contains recombination proteins typical of archaea, such as the NurA-HerA complex, and also a RecO-like protein characteristic of the *Deinococcus/Thermus* phylum. We performed a systematic deletion study on many of the corresponding genes to gain information on their essentiality and possible biological role in Tth.

The results indicate that AddAB plays a pivotal role in the recombination pathways of Tth, but is not completely essential. RecA, F and J would be near-essential, while RecR is dispensable and recO deletion selects for compensatory mutations, and NurA-HepA have been shown to be dispensable. Importantly, the RecO-like protein appears to be able to physically associate the replicative helicase DNAB with recombination complexes, suggesting that it may participate in recombinational repair in Tth together with some of the factors mentioned above. Understanding the characteristic recombinational repair network of Tth would probably help to shed light on the ability of this organism to live at high temperatures. This has implications for biotechnology as well as the transfer of antibiotic resistances in bacteria.

Abstract ID: 1615 / Poster Number: 041.15P

Establishing a Genomic Framework for Taxonomic Assignment: Insights from Williamsia Species

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Co-authors: *Utkarsh Sood, Gauri Garg, Rup Lal, Charu Dogra Rawat*

This study aims to resolve the taxonomic relationship between two closely related species of the genus *Williamsia*: *Williamsia muralis* and *Williamsia marianensis*. The classification of these species has been ambiguous, necessitating a comprehensive genomic analysis to clarify their taxonomic status.

Genomic data from 10 type strains were analyzed using a comprehensive analytical pipeline to ensure compliance with the latest genome classification guidelines. Comparative analyses included the 16S rRNA gene sequence similarity, average nucleotide identity (ANI), average amino acid identity (AAI), and digital DNA–DNA hybridization (dDDH). Phylogenomic analysis of core genomes was also conducted to assess evolutionary relationships.

The 16S rRNA gene sequences of *W. muralis* DSM 44343T and *W. marianensis* DSM 44944T exhibited 99% similarity. Whole-genome comparisons demonstrated high concordance, with ANI, AAI, and dDDH values of 98.07%, 98.29%, and 84.80%, respectively—exceeding established species delineation thresholds. Phylogenomic analysis revealed that the two strains form a monophyletic clade, further supporting their conspecific nature.

The results strongly suggest that *W. muralis* and *W. marianensis* represent the same species. The high concordance across multiple genomic metrics and phylogenomic evidence underscores the robustness of whole-genome approaches for resolving taxonomic ambiguities. By integrating multiple genomic tools, this study establishes a reliable framework for species assignment in the genus *Williamsia*.

Based on the genomic evidence, we propose reclassifying *Williamsia marianensis* Pathom-aree et al. 2006 as a later heterotypic synonym of *Williamsia muralis* Kämpfer et al. 1999. This work, published in ***The International Journal of Systematic and Evolutionary Microbiology (IJSEM)***, highlights the critical role of whole-genome data in microbial taxonomy and sets a precedent for future studies in bacterial classification.

Abstract ID: 2576 / Poster Number: 441.15P

Establishment and verification of multiplexed SNA Nanoflare-Based sensing for in vivo testing Paramylon Productivity in microalga Euglena gracilis

Presenting author: Mr. Yugeun Jung, Korea University, Seoul, South Korea

Co-authors: *Yoon-E Choi*

Euglena gracilis is a versatile microalga capable of both photosynthetic and heterotrophic growth, uniquely producing β-glucan in the form of paramylon. Paramylon, a bioactive compound with immunomodulatory, antioxidant, and anti-inflammatory properties, has significant potential in pharmaceuticals, nutraceuticals, and bioplastics. However, optimizing paramylon production is challenging due to difficulties in quantifying key transcripts or metabolites involved in its biosynthesis. Therefore, this study attempted *in vivo* sensing of paramylon biosynthetic transcripts.

To this end, knockdown strains of *E. gracilis* were generated with RNA interference (RNAi), targeting egGSL2 for paramylon synthesis, egP1 encoding beta-1,3-glucan phosphorylase responsible for paramylon degradation, and both genes simultaneously. These strains were used to validate the proposed *in vivo* detection method using SNA nanoflare technology for multiplex mRNA detection. The biosensor, designed with gold nanoparticles (AuNPs), enabled rapid and accurate identification of high paramylon-producing strains. RNAi-generated enzyme-deficient strains played a key role in ensuring sensing reliability and improving screening efficiency.

These results demonstrate the high efficacy of SNA nanoflare-based high-throughput screening method for identifying high-efficiency strains for bio-refinery applications, while providing new insights into the regulatory mechanisms of paramylon biosynthesis and degradation. This study introduces a novel strategy for optimizing paramylon production through RNA-level detection and high-throughput screening, providing foundational technology for enhancing production efficiency and advancing *Euglena gracilis*-based bio-refineries and innovations in microalgal metabolic engineering.

Abstract ID: 2501 / Poster Number: 193.16P

Establishment of microbial biomarkers for assessment of gastric cancer risk across populations

Presenting author: Jaekyung Yoon, PhD, Yonsei university, Seoul, South Korea

Co-authors: *SoonKyeong Kwon, Jun Chul Park, Yong Chan Lee, Jihyun F. Kim*

Gastric cancer (GC) is one of the leading causes of cancer-related mortality worldwide, and its prognosis is highly dependent on the stage at diagnosis. Early detection and effective identification of high-risk groups are thus crucial for improving patient outcomes. While *Helicobacter pylori* is a well-established microbial risk factor for GC; recent studies suggest other members of the gastric microbiota may also contribute to gastric carcinogenesis.

In this study, we analyzed four independent populations—one newly collected cohort from our research and three from published datasets—to identify microbial risk factors for GC. Each population included individuals in three different states: those with intestinal metaplasia or GC and control subjects (healthy or superficial gastritis). Using a random forest classification model, we identified twenty-one biomarker species that were most strongly associated with GC across the populations.

Building on these findings, we developed a Species Index for Risk Evaluation (SIREn) score, which integrates the presence of these biomarker species to quantify GC risk. The SIREn score was further validated in two additional independent cohorts, confirming its potential as a novel tool for stratifying GC risk. Future functional analyses of these key biomarker species will help clarify their mechanistic roles in gastric carcinogenesis and may ultimately guide new strategies for prevention and early detection of GC.

Abstract ID: 157

EU-JAMRAI-2_Europe united in the fight against antimicrobial resistance

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Co-authors: *Richard Vaux, Luis Lucena Baeza, Geoffrey Couraud, Marie-Cécile Ploy*

Introduction: Antimicrobial resistance (AMR), particularly antibiotic resistance, has arisen as a serious cross-border threat to health with the potential to undermine modern medicine. In the European Union (EU), antibiotic resistance accounts for at least 33,000 deaths, 2.5 million extra hospital days, and an estimated societal cost of about €1.5 billion each year. To mitigate AMR, a global approach is called for, combining awareness raising, surveillance, antimicrobial stewardship (AMS), and infection prevention/control (IPC) while ensuring access to antibiotics and diagnostics. Such interventions must be grounded in a One Health perspective, considering the interconnection between humans, animals and the environment.

Following the 2017 EU One Health Action Plan against AMR and the 2023 Council Recommendation on stepping up EU actions to combat AMR in a One Health approach, the European Commission has financed the 2nd Joint Action on Antimicrobial Resistance and Healthcare associated infections (EU-JAMRAI 2, https://eu-jamrai.eu/) to make Europe a best practice region and support European countries in their efforts to develop and update their National Action Plan (NAP) on AMR.

Objectives: EU-JAMRAI 2 aims to establish a European One Health community to combat AMR and assist participating countries in developing, improving, and implementing their AMR control policies.

Method: EU-JAMRAI 2 brings together 128 partners (ministries, public health agencies, research institution and hospitals…) from all 27 EU Member States, and Iceland, Norway, and Ukraine. It started in January 2024 and will last until December 2027.

Findings: Different intersectoral activities have been initiated and are currently in progress. Through its action, EU-JAMRAI 2 will contribute to:

- Develop European standards, requirements, training programs, and capacity-building initiatives for the implementation of antimicrobial stewardship (AMS) and infection prevention and control (IPC) programs in both human, animal health and the environment;

- Identify behavioural facilitators and lift barriers to the proper use of antimicrobials and IPC;
- Operate an integrated One Health surveillance of AMR in Europe, through the full-scale implementation of the “EARS-Vet” AMR surveillance network in diseased animals (initiated in EU-JAMRAI1) and the development of a European surveillance network for AMR in the environment: “EARS-Env”;
- Improve access to critical antibiotics and diagnostics across Europe in both human and animal health;
- Raise awareness about AMR among various audience and promote the AMR symbol developed during EU-JAMRAI-1;
- Build a network of policy officers across Europe to ensure the sharing of best practices and to support countries in the implementation of their "One Health" national action plans.

Conclusion: EU-JAMRAI-2 represents a unique opportunity to build a European One Health community on AMR and make Europe a region of excellence in the field to improve the health of European citizens.

Abstract ID: 4009 / Poster Number: 383.16P

Evaluating Bacillus mojavensis as a bioinoculant for improving green cabbage growth and advancing environmental sustainability

Presenting author: Mrs. Jovana Anđelković, Faculty of sciences and mathematics, Niš, Serbia

The application of plant growth-promoting bacteria (PGPB) as bioinoculants is a promising strategy for enhancing agricultural sustainability. These beneficial microbes stimulate crop growth, improve nutrient availability, and suppress phytopathogens, reducing reliance on chemical fertilizers and pesticides while promoting eco-friendly farming practices. This study evaluated the effects of *Bacillus safensis* and *Bacillus mojavensis* on the early growth of green cabbage (*Brassica oleracea* var. *capitata*) under greenhouse conditions. Cabbage seeds were inoculated with bacterial suspensions and sown in a sterile substrate. After germination in a growth chamber, seedlings were cultivated in greenhouse conditions for 35 days. Plant growth parameters, as well as pigment and element contents in leaves, were analyzed. Control plants were grown from seeds treated with a 0.9% sodium chloride solution. Both bacterial isolates enhanced plant growth, with *Bacillus mojavensis* showing the most pronounced effects. Compared to control plants (252.54 ± 4.32 mm height, 7.57 ± 0.39 g fresh weight, and 0.56 ± 0.04 g dry weight), *B. mojavensis* significantly increased plant height (273.75 ± 2.58 mm), fresh weight (11.26 ± 0.13 g), and dry weight (0.88 ± 0.07 g). Additionally, plants inoculated with *B. mojavensis* exhibited higher Cu, Mn, and Zn content in leaves. These findings highlight the potential of *B. mojavensis* as a bioinoculant that enhances plant growth, improves productivity, and contributes to environmentally sustainable farming practices.

Keywords: Plant growth-promoting bacteria; green cabbage; *Bacillus mojavensis*; greenhouse.

Abstract ID: 3462 / Poster Number: 392.17P

Evaluating biocontrol potential of Bacillus velezensis against Xanthomonas campestris pv. campestris on cabbage cv. Futoški under greenhouse conditions

Presenting author: Dr. Bojana Živanović, University of Belgrade – Institute for Multidisciplinary Research, Belgrade, Serbia

Co-authors: *Sonja Milić Komić, Sanja Marković, Petar Mitrović, Tatjana Popović Milovanović, Dragosav Mutavdžić, Ana Sedlarević Zorić, Aleksandra Jelušić*

Bacillus velezensis is recognized as a species with remarkable biocontrol potential against a wide range of plant pathogens, including the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of black rot of Brassicaceae crops.

This study aimed to establish the most suitable technique for application of four *B. velezensis* strains (BFC55, BFC88, BFC102, and BFC114) against *Xcc* on cabbage cv. Futoški.

The efficacy of three application techniques (seed-soaking, preventive and curative foliar spraying) was evaluated on cabbage plants under the controlled greenhouse conditions in two independent experiments. Simultaneously, the efficacy of whole cultures and cell-free supernatants of *B. velezensis* strains were tested. The disease intensity was scored 14 days after inoculation using the Horsfall–Barratt scale. Upon the end of the experiment, fresh and dry biomass, as well as chlorophyll and epidermal flavonoid content and nitrogen balance index were measured for all treatments.

Among the compared treatments, preventive application showed the greatest disease suppression (89.71–93.09%) ability, while the curative exhibited the lowest efficacy (55.00–75.68%). The highest efficacy was observed after preventive application of strain BFC88 in the form of whole culture (93.09%). Preventive treatment showed increased epidermal flavonoid accumulation compared to all other application treatments, along with a decrease in fresh/dry biomass ratio.

Preventive treatment has been highlighted as the most effective method for controlling black rot in cabbage cv. Futoški in comparison to other treatments. The demonstrated efficacy of tested strains in greenhouse conditions suggests their potential for successful application in the field.

Abstract ID: 1195 / Poster Number: 162.16P

Evaluating Engineered Endolysin PHICD111_20024_EAD for Controlling Clostridioides difficile in Combination with Probiotics

Presenting author: Youngjin Cho, PhD, Ajou university, Suwon, South Korea

Co-authors: *Hyunjin Yoon*

Clostridioides difficile is a Gram-positive, spore-forming anaerobe that causes toxin-mediated colitis. The primary factor causing *C. difficile* infection (CDI) is gut microbial dysbiosis, which is linked to the overuse of antibiotics such as clindamycin and ampicillin. This study explored the potential of endolysins as an alternative to antibiotics to treat CDI. Endolysins, phage-derived peptidoglycan hydrolases, offer narrow-spectrum bactericidal activity, providing an advantage over conventional antibiotics. PHICD111_20024 demonstrated specific bacteriolysis against *C. difficile* and was further engineered by removing its cell-wall binding domain, resulting in the variant PHICD111_20024_EAD. This modified endolysin exerted greater lytic activity against *C. difficile* and maintained its catalytic activity across a wide range of NaCl concentrations (0–200 mM). Probiotics such as *Bifidobacterium* spp. have been demonstrated to have the therapeutic effects against CDI. To examine the possibility of synergistic effects between endolysins and probiotics, PHICD111_20024_EAD was applied to a co-culture model of *C. difficile* and *Bifidobacterium adolescentis*. Co-culture with *B. adolescentis* retarded the growth of *C. difficile* and PHICD111_20024_EAD addition further decreased the number of *C. difficile*. Transcriptomic analysis revealed that PHICD111_20024_EAD stimulated *C. difficile* to alter the expression of peptidoglycan synthesis and lipid metabolism genes in the co-culture model. Transcriptomic analysis revealed that co-culture with *B. adolescentis* stimulated *C. difficile* to increase the expression of Stickland reaction-related genes and PHICD111_20024_EAD addition further altered the expression of peptidoglycan synthesis and lipid metabolism genes. These findings suggest that PHICD111_20024_EAD and probiotics can be employed in combination as CDI therapeutics

Abstract ID: 1724 / Poster Number: 081.15P

Evaluating molecular markers of pathogenicity in Candida albicans for strain-specific surveillance of river water posing health risks to vulnerable communities

Presenting author: Ms. Heidi Steffen, Stellenbosch University, Stellenbosch, South Africa

Co-authors: *Corné Van Deventer, Alfred Botha, Cleo Conacher*

Introduction: Microbial pollution in natural surface waters poses a risk to human health, particularly those dependent on alternative water sources in Africa[1]. Detection and surveillance of *Candida albicans*, a critical priority fungal pathogen with wide inter-strain virulence variability, currently lack strain-level specificity, which introduces a limitation in risk assessment[2]. **Objective:** This study investigated a variable region of *C. albicans*' ECE1 gene as a potential marker for distinguishing strains based on virulence and pathogenic potential—owing to the unique toxin peptide translated by this gene, candidalysin. **Methods:** We sequenced (Sanger technologies) a region of the ECE1 gene of 27 South African strains–clinical and environmental origin–followed by gene-specific phylogenetic analyses. Virulence-associated extracellular enzyme activity for each strain was determined through agar-based assays: phospholipase (Phz), esterase (Ez), and proteinase (Prz). Lastly, a haemoglobin release assay using human red blood cells was performed to determine the strains' haemolysis potential. **Findings:** Our study revealed a highly variable genetic and codon region that includes the sequence translating the second peptide (P2) of the ECE1 polypeptide. Furthermore, significant differences between strains and P2 variants were observed for Ez, Phz, Prz activity (Fig1 ABC; $p<0.05$) and haemolysis (Fig1 D; $p<0.05$). Since P2 is known to be involved in candidalysin folding and secretion[3], we suggest that this genetic region could be a potential molecular marker of strain-specific virulence. Our study also shows that the South African *C. albicans* strains are phenotypically and genetically different from the highly virulent Type strain (SC5314; $p<0.05$). **Conclusion:** The impact on human health of these particular strains is currently unknown. Thus, confirmation of their pathogenic potential would require studying virulence, and virulence factor expression, in host as well as environmental conditions—especially climate change driven stressors that affect the physiology and distribution of these yeasts in nature and in communities[4,5]. Our results are a step toward understanding strain-specific virulence of South African *C. albicans* strains, which will allow researchers to accurately detect *C. albicans* and denote the associated risk[6].

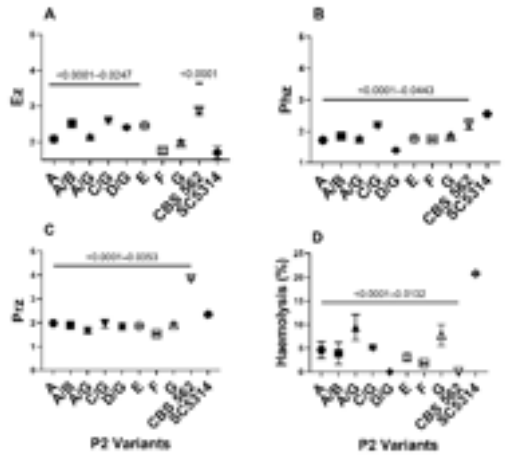


Figure 1: Virulence profiles of *Candida albicans* strains based on ECE1 peptide two (P2) variants. The activity of classic virulence-associated extracellular enzymes, namely esterase (Ez; A), phospholipase (Phz; B) and proteinase (Prz; C) is shown as the ratio between the hydrolysis zone and colony diameter over colony diameter (Z+C mm/C mm). Haemolysis activity (D) was investigated due to the peptide candidalysin's haemolytic properties—a critical virulence factor during systemic infections—and represents the percentage haemolysis relative to the full lysis control, Triton-X. Significance ($p<0.05$) is illustrated relative to the Type strain, SC5314.

Abstract ID: 3588 / Poster Number: 230.16P

Evaluating the Chemotactic Activity of Peripheral Blood Granulocytes in Lambs Supplemented with a Multi-Strain Probiotic Formulation (Lactobacillus, Bifidobacterium)

Presenting author: Prof. Roman Wójcik, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Phagocytosis is the key mechanisms of non-specific immunity which constitutes the first line of defence in the immune response. Phagocytosis consists of four main stages: chemotaxis and migration, adherence, absorp-tion, and intracellular digestion.

The chemotactic activity of peripheral blood granulocytes in lambs supplemented with a multi-strain probiotic formulation (containing three *Lactobacillus* strains: *Lactobacillus plantarum* AMT14, *Lactobacillus plantarum* AMT4, and *Lactobacillus rhamnosus* AMT15, and one *Bifidobacterium animalis* AMT30 strain) was evaluated with the use of the Migratest® kit.

The study was conducted on a breeding flock of Kamieniec sheep, on 16 young rams. At 10 days of age, the lambs were divided into two groups of 8 animals each: a control group (group C) and an experimental group (group E). The animals from both groups were slaughtered at the end of the experiment, at 40 days of age. The feed administered to experimental group lambs was supplemented with a multi-strain probiotic formulation with a viable count of 1.0×10^9 CFU/g, whereas control lambs were ad-ministered standard farm-made feed without supplementation.

Blood was sampled from the jugular vein immediately before the experiment (day 0) and on experimental days 15 and 30 to determine the chemotactic activity of peripheral blood granulocytes in lambs.

A significant in-crease in the chemotactic activity of peripheral blood granulocytes, expressed by an increase in the chemotactic index and a decrease in L-selectin expression, was observed in the supplemented lambs (group E) relative to group C lambs between experimental days 15 and 30.

Abstract ID: 3489 / Poster Number: 281.15P

Evaluating the potential of entomopathogenic fungi as endophytic symbionts in plants for enhanced pest resistance and improved plant-growth

Presenting author: Ms. Sneha Sabu, Erfurt University of Applied Sciences, Research Centre for Horticultural Crops, Erfurt, Germany; Friedrich-Schiller-Universität Jena, Jena, Germany

Co-authors: *Katja Burow, Philipp Franken*

The horticulture industry experiences severe losses globally because of insect attacks, which lower fruit quality and spread plant diseases. This project aims to explore endophytic entomopathogenic fungi (EEMPF) as environmentally biological alternatives to synthetic pesticides, particularly against the fungus gnat *Bradysia difformis*.

The fungal strains including *Beauveria bassiana, Metarhizium flavoviride, Mucor hiemalis and Niesslia tinuis* from the DSMZ were tested for entomopathogenic activity against *B. difformis* and for their ability to colonize *Petunia axillaris, Petunia exserta* and *Ocimum basilicum* plants. Also, several soil samples were analyzed for the presence of entomopathogenic fungi against *B. difformis*.

Preliminary and secondary screening and findings obtained suggest that *Metarhizium flavoviride* exhibits entomopathogenic potential against *Bradysia difformis*, effectively colonizing petunia and basil without significantly affecting plant growth. The reproducibility of its entomopathogenic activity was validated through experiments using varying spore concentrations. Enzyme assays further confirmed the strain's ability to produce cuticle-degrading enzymes such as chitinase and protease. Additionally, the screening of fungal isolates from soil samples identified *Trichoderma asperallum* as a potential entomopathogenic fungal candidate.

The project is ongoing, focusing on evaluating newly isolated fungi for their entomopathogenic activity against *B. difformis* and their ability to colonize plants along with their ability to act against abiotic stresses and plant pathogens.

Abstract ID: 3123 / Poster Number: 328.17P

Evaluation of acetate's influence on the growth physiology of Synechocystis sp. B12

Presenting author: Dielle Procopio, PhD, University of São Paulo, São Paulo, Brazil

Co-authors: *Louise Gracioso, Elen Perpetuo, Tomas Morosinotto, Cassius Stevani*

The cyanobacterium *Synechocystis sp.* B12 has emerged as a highly efficient producer of poly(3-hydroxybutyrate) (PHB), a derivative of polyhydroxyalkanoates (PHA) with significant industrial applications in bioenergy and biomaterials. To enhance PHB production, this research focused on explored the influence of acetate supplementation on growth physiology of the B12 cells. Given acetate's abundance, renewability, and low cost, understanding its influence on PHA production holds great promise for advancing biotechnological applications. The phenotypical growth response of *Synechocystis sp.* B12 to increasing acetate concentrations (0.2%, 0.4%, 0.5%, and 0.6% *w/v*) in BG11 medium supplemented with sodium bicarbonate (5 g L^{-1}) was evaluated. Under standard conditions without acetate, the wild-type strain reached the end of the exponential phase within 72 hours, achieving an OD_{750} of 4.05 ± 0.08 at 96 hours. With acetate supplementation, growth rates significantly improved, resulting in higher OD_{750} values of 6.68 ± 0.96, 6.42 ± 0.77, 5.6 ± 0.23, and 4.86 ± 0.53 for cultivations with acetate concentrations of 2, 4, 5, and 6 g L^{-1}, respectively. Under the evaluated conditions, acetate was completely consumed for the two lower initial concentrations. In contrast, when 5 or 6 g L^{-1} was used, 0.31 ± 0.26, and 1.59 ± 0.22, respectively, remained in the medium after 96h of cultivation. Further studies will focus on elucidating the cellular mechanisms behind acetate tolerance and its conversion into value-added products, which are essential for further genetic and metabolic engineering of *Synechocystis* for sustainable biotechnological applications.

Abstract ID: 1527 / Poster Number: 171.16P

Evaluation of Affinity assay based on a pseudotyped Virus for Screening Lassa Viruses mAbs

Presenting author: Dr. Jeongsu Lee, National Institute of Health, Cheongju-si, South Korea

Co-authors: *Soo Hyeon Kim, Jaehun Jeong, Da Sol Kim, Kyung-Chang Kim, Hee-Young Lim*

Introduction: Lassa virus (LASA) is the causative agent of Lassa fever, a human hemorrhagic disease transmitted by direct contact with infected Mastomys rats. LASA is the pathogens in the WHO R&D Blueprint list for emerging Infections. It is increasingly recognized as a major threat to global public health. There are current no approved vaccines and antiviral agents for treatment Lassa fever.

Objective: Neutralizing antibodies are important for the treatment and prevention of viral infections. In this study, we aimed to produce, evaluate and optimize the condition of pseudotyped LASV-GP expressing virions for LASA neutralization assay.

Method: The pseudotyped viruses of a lineage I, II, III, IV and V of LASA were constructed by the co-transfection into HEK 293T cells with a plasmid DNA mixture composed of packaging vector, expression vector and reporter vector including luciferase. To validate this pseudotype affinity assay for LASV, we performed the validation experiments using 37.7H and 8.9F antibodies against LASA in BSL-2.

Findings: Protective Efficacy of 37.7H and 8.9F antibodies against pseudotyped viruses of a lineage I to V was the highest neutralizing affinity for lineage II and III, respectively. On the other hand, lineage I and V were the low affinity, respectively.

Conclusion: In here, we have established a powerful platform using pseudotyped viruses for screening LASV-neutralizing mAbs under BSL-2 condition.

Abstract ID: 2536 / Poster Number: 128.17P

Evaluation of Cumulative Antimicrobial Susceptibility Data of Enterobacterales Isolates Obtained from Intensive Care Units of a University Hospital

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Co-authors: *Gökhan Kırbaş, Duygu Öcal, Zeynep Ceren Karahan*

Introduction: Antibiotic resistance is a critical public health issue. Cumulative data analysis is essential for understanding resistance patterns and optimizing infection control strategies.

Objective: This study aims to analyze recent antibiotic susceptibility data from ICU patients providing insights to guide infection management and empirical treatment.

Method: Enterobacterales isolates obtained from various samples collected from ICU patients at Ankara University Hospital Microbiology Laboratories between 2022-2024 were evaluated. Cumulative analysis was performed according to CLSI. Samples were collected from various clinical sources, including respiratory, urinary, blood, and tissue specimens. Isolates were identified using automated systems and susceptibility testing was performed according to EUCAST.

Findings: A total of 2,753 clinical isolates were included in the study three years. Among these isolates, 1,189 were *Klebsiella pneumoniae*, 711 *Escherichia coli*, 207 *Enterobacter spp.*, 196 *Proteus spp.*, 102 *Serratia spp.*, and 348 other Enterobacterales. The overall susceptibility rates in all ICUs were as follows: meropenem 66.8%, amikacin 71.7%, ciprofloxacin 43%, cefotaxime 39.3%. Neonatal ICU, rates were: meropenem 82.2%, amikacin 90.2%, ciprofloxacin 78.2%, cefotaxime 59.2%. Pediatric ICU, rates were: meropenem 60.8%, amikacin 73.7%, ciprofloxacin 52.5%, cefotaxime 37.1%. Anesthesia ICU, rates were: meropenem 56.1%, amikacin 56.7%, ciprofloxacin 30%, cefotaxime 28.8%. Internal Medicine ICU, rates were: meropenem 50.3%, amikacin 59.8%, ciprofloxacin 25%, cefotaxime 31.7%.

Conclusion: The data obtained serves as a valuable resource for updating empirical treatment approaches and improving infection control measures. Monitoring and regular reporting of cumulative data is essential for developing effective strategies against the

increase in antimicrobial resistance. Utilisation of these data may increase success rates in the fight against resistant infections.

Abstract ID: 3731 / Poster Number: 237.16P

Evaluation of Fourier transform-infrared spectroscopy (FT-IR) as a control measure for Stenotrophomonas maltophilia nosocomial outbreak investigations

Presenting author: Dr. Hanane Raqioui, University of Verona, Verona, Italy

Co-authors: *Francesca Deidda, Martina Bausani, Anna Bertoncelli, Marco Pane, Annarita Mazzariol*

Introduction and Objective: *Stenotrophomonas maltophilia* is an emerging pathogen that poses a significant threat, particularly as an opportunistic infection in immunocompromised patients. The study aims to evaluate and validate using Fourier transform-infrared spectroscopy (FT-IR) as a rapid and cost-effective method for controlling and investigating nosocomial outbreaks of *S. maltophilia*.

Methods: Fifty-seven *S. maltophilia* strains isolated during a multi-drug resistant screening program and from the infection site were analysed using FT-IR spectroscopy. The FT-IR analysis for each strain was performed in quadruplicate. Spectra acquisition and analysis were performed with IRBT spectrometers (Bruker) and OPUS software. Pulsed-field gel electrophoresis (PFGE) was used as the gold standard to evaluate the genetic correlation between the strains classified by the FT-IR.

Findings: The analysis of the 57 strains using FT-IR revealed a high variability among the strains and identified two distinct clusters. Subsequently, PFGE confirmed the clonal relationship of these groups, which was further validated by epidemiological data. Four strains in the first cluster were part of a mini outbreak between the intensive care unit and the burns unit during the same period. The second cluster is represented by two strains isolated from the same patient.

Conclusion: These results confirm that FT-IR spectroscopy was able to correctly identify the clonal relationship between the strain under study, suggesting its potential as a preliminary typing method in the investigation of *S. maltophilia* outbreakslease use this text box to insert your short abstract for Poster Display and Flash Talk submission.

Abstract ID: 1494 / Poster Number: 294.17P

Evaluation of Lactic Acid Bacteria Strains for Fermentation of Soy Milk Based on Alternatives: Sugar Utilisation and Proteolytic Activity

Presenting author: Jovana Nenadovic, PhD, University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

Co-authors: *Ivana Peric, Nikola Bajcetic, Milica Mirkovic, Zorica Radulovic, Nemanja Mirkovic*

The selection of lactic acid bacteria (LAB) strains is crucial for the successful fermentation of plant-based beverages such as soy beverages, as they influence both the taste and the nutritional content of the final product.

The ability of LAB to efficiently utilise sugars and break down proteins is crucial for improving the digestibility, taste and functional properties of soy beverages. This study investigates the sugar utilisation and proteolytic activity of different LAB strains during soy beverages fermentation with the aim of identifying the strains with the most efficient enzymatic activities to optimise the fermentation process.

Different strains from genera *Lactococcus, Lactobacillus* and *Leuconostoc*, isolated from fresh fruits and vegetables from Serbia were analysed for their ability to ferment soy milk based on alternative (SBMA). The sugar consumption pattern for glucose, lactose and sucrose was analysed in a synthetic medium. In addition, the proteolytic activity was analysed on the supernatant of the medium (cells and soy protein extract) after incubation at 30 °C for 4 hours on an SDS-polyacrylamide gel. In addition.

The results showed that almost all strains grew on sucrose and glucose. According to the results of the selection of 27 strains, 10 (1 *Lactococcus lactis*, 1 *Lactiplantibacillus plantarum*, 6 *Leuconostoc mesenteroides*, 1 *Lactobacillus pentosus*, 1 *Lactococcus lactis*) showed significant proteolytic activity in soy. Considering the results for all analysed parameters, these strains are considered particularly interesting candidates for the production of soy-based products.

Abstract ID: 3171 / Poster Number: 212.16P

Evaluation of MALDI-TOF and Genotype for the Identification of Non-Tuberculous Mycobacteria: A Comparative Approach

Presenting author: Dr. Maria Martins, Unidade Local de Trás-os-Montes e Alto Douro, Vila Real, Portugal

Co-authors: *Vera Ribeiro, Ana Silva, Ana Ferreira, Eliana Costa*

The non-tuberculous mycobacteria (NTM) are ubiquitous and environmental saprophytic organisms. There are over 170 NTM identities and their incidence has increased in recent years.

MALDI-TOF MS is an efficient and economical method for identifying microorganisms, including Mycobacterium spp. by mass spectrometry. Due to their capacity to provide rapid and reliable results, molecular genetic methods have emerged as the preferred choice for identification mycobacteria species. This study aims to compare the identification of mycobacteria from MGIT liquid cultures (BD) by MALDI-TOF MS (Bruker, Germany) to the identification obtained through the GenoType Mycobacterium kit (Hain Lifescience, Germany).

A total of 25 suspected NTM samples were processed. Out of the 16 samples with concordant results, MALDI identified 2 samples as M. peregrinum (Genotype - M. fortuitum group), 1 as M. intracellulare, and 3 as M. chimaera (Genotype - M. intracellulare). 6 samples were not identified by MALDI-TOF, as the viability of these cultures may have been compromised, as they were evaluated after 3 months of analysis by GenoType kit. In the case of the samples with discordant identifications, GenoType kit identified as M. lentiflavum and MALDI-TOF as M. nebraskense.

Regarding the cultures with discordant results, this can be explained by the fact that the species in question may have similar mass spectra, and since it is a method with lower resolution compared to genetic sequencing methods.

In this study we concluded that MALDI-TOF MS represents a reliable, cost-effective and rapid identification method which accelerates the diagnosis. However, it also has their limitations.

Abstract ID: 2633 / Poster Number: 133.17P

Evaluation of synergistic effects of fluconazole and ciannamon oil on nosocomial strains of Candida auris using the Galleria mellonella model

Presenting author: Mrs. Marina Ionela Nedea, Faculty of Pharmacy, "Carol Davila'' University of Medicine and Pharmacy, Bucharest, Romania

Co-authors: *Irina Gheorghe Barbu, Ioana Cristina Marinaș, Andreea Letiția Arsene, Bruno Ștefan Velescu*

Introduction: *Candida auris*, a recently identified etiologic agent, has piqued the scientific community's interest due to its high mortality rates and antifungal resistance. This study uses the *Galleria mellonella* infection model to investigate the impact of fluconazole and *Cinnamomum verum* essential oil (EO) treatments on the virulence factors of eight *C. auris* strains isolated in a Bucharest hospital unit.

Method: Fluconazole and cinnamon EO were administered at different doses to each group which contained seven larvae per strain. It was evaluated mortality rates and melanization responses post-treatment to investigate host-pathogen interactions and treatment efficacy in temporal dynamics (2h, 6h, 12h, 48h). Soluble virulence factors (caseinases, hemolysins, lecithinases, lipases, gelatinases, amylases, and esculin hydrolysis) were evaluated on larval homogenates after infection and treatment.

Findings: The optimal combination of fluconazole and cinnamon EO inhibited the *C. auris* proteases, which is an important virulence factor. The *in vivo* assay showed decreased mortality and an increase in the early melanization response, indicating a stronger immune response and a reduction in the microbial pathogenicity.

Conclusion: Combined therapy not only improves fluconazole's antifungal activity, but also affects the *C. auris* virulence. The *G. mellonella* model has demonstrated an effective tool for assessing the *in vivo* effects of antifungal treatments. Further research is required to evaluate the clinical efficacy of the optimized combination treatment for managing multidrug-resistant *C. auris* infections.

Abstract ID: 2251 / Poster Number: 203.17P

Evaluation of the Antibiotic Resistance Spread in Different Wastewater Treatment Processes for Reuse

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Climate change intensifies water scarcity in many regions worldwide. Although wastewater reuse is a key strategy to address this challenge, it carries health risks due to its microbiological content, including antibiotic resistance. Antibiotic resistance is a significant public health threat, and wastewater treatment plays a crucial role in the transmission of this phenomenon in the environment, with potential consequences for human health.

The project aimed to achieve the following **Objectives:** I) assess the spread of antibiotic resistance in the context of wastewater reuse in wastewater treatment plants (WWTPs); II) evaluate the effectiveness of specific treatments in reducing and/or promoting antibiotic resistance.

The WWTPs included were: WWTP1 (sampling points: influent, effluent, effluent after filtration, effluent after hydrogen peroxide treatment and effluent for reuse after the UV treatment); WWTP2 (sampling points: influent and effluent for reuse from membrane bioreactor treatment). Both WWTPs were sampled 6 times. Antibiotic-resistant bacteria (ARB) were counted on R2Agar supplemented with tetracycline (tetracycline-resistant bacteria-TRB) and sulfamethoxazole (sulfamethoxazole-resistant bacteria-SRB). Total heterotrophic count (HPC) was determined on media without antibiotics. Extended-spectrum β-lactamase *E. coli* (ESBL-Ec) were quantified according to the Tricycle method (WHO, 2021). Results are expressed as concentration (log CFU/ml, absolute abundance) and as percentage of ARB on total count (HPC or *E. coli*) (antibiotic resistance rate). Moreover, the samples were concentrated, and total DNA was extracted and used to quantify specific antibiotic resistance genes (ARGs) (*tet*W, *tet*A, $bla_{\text{CTX-M}}$, $bla_{\text{OXA-48}}$, *sul*I and *sul*II), *int*I1 (intended as antibiotic resistance indicator) and 16S rRNA (used to determine the relative ARG abundances) with droplet digital PCR (ddPCR). Statistical analysis was performed using SPSS software. Anova test (with Tukey's post-hoc) or T-test were applied to evaluate the difference among the steps in WWTP1 or WWTP2.

Significant decrease of almost all ARB monitored was observed between both the influents and effluents for reuse investigated ($p<0.05$), even with some fluctuations in the intermediate steps for WWTP1. In both WWTPs, SRB were the most abundant bacteria. Regarding the antibiotic resistance rate, a statistically significant increase was observed for WWTP1 between the effluent after hydrogen peroxide treatment and the other steps highlighting a prevalence of SRB and TRB. Regarding ARGs, the effluent values resulted significantly lower respect to the influent in both WWTPs ($p<0.05$) for all the genes monitored. In the WWTP1, the tertiary treatment steps reported no statistically significant differences indicating that these treatments did not contribute significantly to reduce the ARG load. Similarly to what observed for bacteria, the most widespread genes were *sul*I and *sul*II, beside *intI*1. Considering the ARG relative abundances in WWTP1, bla_{OXA-48} and *tet*W resulted decreased in all the steps respect to influent ($p<0.05$). The same trend was observed also in WWTP2 for *tet*W, *tet*A, bla_{CTX-M} and *int*I1 relative abundances ($p<0.05$). This indicates the ability of all the treatments to reduce the ARG contribute, irrespective to what observed for ARB.

Despite the overall decreasing trend between influent and effluent concentrations, the presence of both ARB and ARGs remains detectable, underscoring need to deep the potential risk of transmission to humans through wastewater reuse practices, such as agricultural irrigation.

Abstract ID: 3560 / Poster Number: 231.17F

Evaluation of the biofilm-forming abilities in Salmonella Typhimurium variants resistant to food preservatives

Presenting author: Jorge Andaluz Arbe, PhD, Instituto Agroalimentario de Aragón, Universidad de Zaragoza – CITA, Zaragoza, Spain

Co-authors: *Giacomo Di Giacinto, Alberto Fau Zamorano, Diego Gómez Lozano, Pierluigi Di Ciccio, Daniel Berdejo Martínez, Felice Panebianco*

The emergence of antimicrobial resistant bacteria (AMR) poses a serious threat to Food Safety. *Salmonella enterica* is one of the most concerning pathogens in the food industry and has been shown to develop resistance to antimicrobials in addition to antibiotics.

Food preservatives are commonly used to control *S. enterica* and help ensure Food Safety. However, prolonged exposure of bacteria to these substances can select for resistant variants.

Moreover, the biofilm-forming ability of *S. enterica* enhances its resistance, hindering cleaning and disinfection procedures in the food processing environment. It remains unclear whether the development of resistance to food preservatives influences the biofilm formation ability.

The objective of this work is to assess the biofilm-forming ability of *S*. Typhimurium resistant variants (RVs) to food preservatives and to compare this ability with their parental strain (PS).

After isolating 20 RVs (10 to sodium sulphite and 10 to sodium nitrite) through adaptive laboratory evolution assays (ALE), their biofilm-forming abilities were assessed employing a colorimetric micro-method assay. Based on optical density (OD) values, strains were classified as no, weak, moderate or strong biofilm producers.

This preliminary screening showed that, while the *S*. Typhimurium PS was classified as a moderate biofilm producer, some RVs seemed to slightly reduce their biofilm-forming abilities, becoming weak producers.

These results suggest that *S*. Typhimurium can develop resistance to food preservatives, which may reduce its ability to form biofilms. Nevertheless, RVs still retain the potential to colonize surfaces. Further evaluations will be conducted to assess their impact in food processing environments.

Abstract ID: 2479 / Poster Number: 020.15P

Evaluation of the cytotoxicity of the complex formed by the heparin-binding Hemagglutinin of Mycobacterium tuberculosis and oleic acid on A-549 cells and BCG

Presenting author: Mr. Oscar Fabian Porras Pinto, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

Co-authors: *Clara Inés Espitia Pinzón, Mayra Silva Miranda, María Cristina Parada Colin*

Heparin-binding hemagglutinin (HbhA) is a surface adhesin of *Mycobacterium tuberculosis* that is involved in the process of hematogenous dissemination of *M. tuberculosis* mainly through the interaction of its C-terminal domain with the proteoglycans of lung epithelial cells, in addition this protein can be also associated with lipid bodies of mycobacteria as the reservoirs of carbon sources in latent tuberculosis.

In our laboratory, we found that HbhA was able to bind free fatty acids such as oleic acid (OA). This important characteristic can be compared with the complex formed by alpha-lactalbumin, a human milk protein with OA, gives to the protein selective cytotoxic properties, againts tumor but not healthy cells. In addition, this complex has antimicrobial activity increasing the effectiveness of antibiotics for some infectious agents.

Our objective was to evaluate the cytotoxicity of the HbhA – oleic acid complex in cell lines and BCG. The protein was expressed in *Rhodococcus erythropolis*, obtaining a molecule with post-translational modifications such as the methylation of its C-terminal domain (rHbhA-met) as it occurs in *M. tuberculosis*. Furthermore, it was also expressed in *Escherichia coli*, obtaining an HbhA without the modifications described above (rHbhA-unmet). When the complex formed with methylated protein (HAO-met) or with the unmethylated protein (HAO-unmet), were exposed to cell lines such as epithelial cells A-549, citotoxicity effect was observed in those cells, toghether with mitochondrial changes detected by fluorescence microscopy. Even more, the complex was able also to inhibit the growth of *Mycobacterium bovis* BCG.

Abstract ID: 2214 / Poster Number: 398.15P

Evolution of intestinal Methanobacteriota and taxonomic revision of the genus Methanobrevibacter

Presenting author: Dr. Evgenii Protasov, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Co-authors: *Mayara Shiratori, Undine Mies, Katja Platt, Vincent Hervé, Sebastian Treitli, Cathrin Spröer, Boyke Bunk, Thomas Heimerl, Moriya Ohkuma, Stefan Spring, Andreas Brune*

Members of the phylum *Methanobacteriota* are common inhabitants of the digestive tract of various animals. With only few exceptions, all intestinal species belong to the genus *Methanobrevibacter*. However, phylogenomic analyses and the large relative evolutionary divergence of individual subclades indicated that the genus is severely underclassified. Therefore, we propose to reclassify members of the genus *Methanobrevibacter* sensu lato into *Methanobrevibacter* sensu stricto and eight novel genera that reflect coevolutionary patterns with major host groups among arthropods, ungulates, and primates (Fig. 1). Comparative genome analysis demonstrated a clear separation of the intestinal taxa from non-intestinal members of the phylum *Methanobacteriota*. They differ in size, GC content, and the coding density and number of pseudogenes in their genomes. Moreover, the intestinal lineages show adaptations to the copiotrophic gut environment and a decreased biosynthetic potential, which led to a dependence on other community members and limited their dispersal into other habitats. Members of several genera have colonized anaerobic intestinal protists and benefit from the H_2 produced by their host. Our results reveal that the transition of free-living *Methanobacteriota* to host-associated environments involves the same genome changes that were previously shown for intestinal and endosymbiotic bacteria. It illustrates a need for deeper understanding of archaeal lifestyles to unmask similarities between intestinal lineages that are caused by evolutionary convergence.

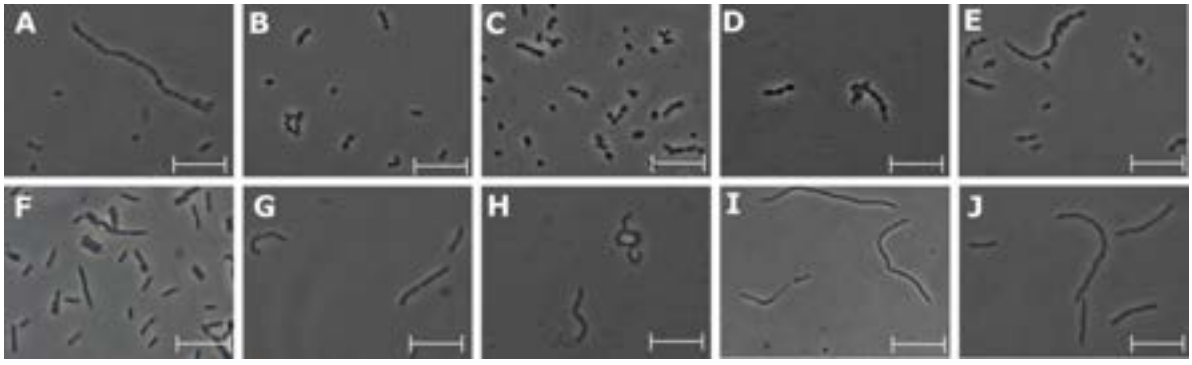


Figure 1: Phase-contrast photomicrographs of Methanobrevibacter ruminantium (A) and the type species of the proposed genera: Methanomonile shimae (B), Methanobotrum acididurans (C), Methanarmilla wolinii (D), Methanocatella smithii (E), Methanobinarius arboriphilus (F), Methanobaculum cuticularis (G), Methanoflexus curvatus (H), Methanacia filiformis (I). Methanobacterium formicicum (J) is shown for comparison. Scale bars represent 5 µm.

Abstract ID: 1455

Evolution of microbiomes and host-bacteria interactions in humans

Presenting author: Dr. Ana Paula Schaan, Kiel University, Kiel, Germany; The Global Microbiome Conservancy Consortium, Kiel, Germany

Co-authors: *Malte Rühlemann, Yue Ma, Lucas Moitinho, Eike Wacker, Frauke Degenhardt, David Ellinghaus, John Baines, Andre Franke, Mark Daly, Ramnik Xavier, Eric J. Alm, Mathieu Groussin, Mathilde Poyet, Global Microbiome Conservancy Consortium*

Introduction: The Global Microbiome Conservancy (GMbC) non-profit initiative was established to investigate the biodiversity and evolution of the human gut microbiome on a global scale. Current microbiome research is disproportionately focused on industrialized populations, which limits the generalizability of findings regarding how lifestyle, diet, and environmental changes influence the host-microbiome interactions. This comprehensive dataset addresses these knowledge gaps by integrating gut microbiome multi-omics data with host DNA and lifestyle information from foragers to fully industrialized groups, providing an unprecedented chance to investigate the global diversity of gut microbiomes and its implications for human health.

Objectives: The GMbC was founded to characterize the evolution of the human gut microbiome across populations living diverse lifestyles and understand its interactions with the human host. Our aim is to reveal global patterns of microbiome-host dynamics and identify factors driving these differences to promote more inclusive and impactful microbiome research.

Methods: The dataset currently includes over 1,500 human participants from 35 countries, representing a gradient of industrialized and non-industrialized lifestyles. Data integration consists of 16S rRNA gene sequencing, metagenomics, bacterial strain isolation and genome sequencing, host genotyping, dietary assessments, and lifestyle metadata. Host-microbiome interaction results were analysed using multivariate models, and focused on microbial diversity, community stability, and immune markers such as intestinal IgA.

Findings: Results show that industrialization significantly impacts the human gut microbiome. Microbial communities in industrialized populations reveal reduced alpha diversity, increased instability, and a higher prevalence of inflammation-associated taxa when compared to non-industrialized populations. These shifts align with elevated levels

of low-grade inflammation and disturbed immune responses, including altered IgA levels and IgA-bacteria interactions. Importantly, microbiome shifts associated with urbanization are like those observed in chronic diseases such as inflammatory bowel diseases, suggesting that lifestyle-driven changes may predispose populations to such conditions. Furthermore, models for disease risk evaluation, developed for industrialized settings, performed poorly when applied to non-industrialized groups, highlighting the need for more inclusive datasets to improve the applicability of microbiome-based clinical tools.

Conclusion: We highlight importance of broadening microbiome research to include underrepresented populations. Industrialization significantly influences host-microbiome dynamics, with implications for immune regulation and susceptibility to disease. Future expansion of the GMbC cohort to include clinical samples, such as individuals with inflammatory bowel diseases, will further elucidate the interplay between lifestyle, microbiome composition, and human health. These insights are critical for developing globally relevant microbiome-based diagnostic and therapeutic tools.

Abstract ID: 1575 / Poster Number: 209.15P

Evolution on a Microscale: Adaptation and Selection of Whole-Cells Through a Microfluidic Chip

Presenting author: Ms. Camilla Stolle, Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

Co-authors: *Christof M. Niemeyer, Kersten S. Rabe*

Background: Adaptive laboratory evolution (ALE) is a key tool for studying fundamental evolutionary processes and a widely used methodology for generating novel, optimized traits for bioproduction. Microfluidics have revolutionized microbial ALE as it can mimic naturally occurring microenvironments promoting the growth of biofilms alongside planktonic cells.

Objective: The aim was to develop a user-friendly and cost-effective microfluidic chip device to perform miniaturized, automated ALE experiments.

Method: The microfluidic evo.S (*evo*lution under *s*tress) chip was designed to create tuneable and spatially stable stressor concentration gradients across interconnected growth wells to adapt microbial cells to stressors such as antibiotics.

Results: The cultivation of microbial cells in the growth wells creates microenvironments within the chip, resulting in mixed populations, ranging from planktonic cells over to stiff biofilms. The chip was successfully used to adapt *Escherichia coli* to different antibiotics and revealed its ability to differentiate between persistence and resistance. Importantly, this approach led to the discovery of previously unknown mutations that confer resistance to nalidixic acid. Recently, a customized chip setup was used to successfully adapt the thermophilic organism *Thermus thermophilus* to kanamycin and to the utilization of an alternative carbon source.

Conclusion: The evo.S chip enhances the occurrence of mutations, resulting in the generation of stress-resistant strains. This kind of chip-based ALE can be applied to adapt virtually any microorganism to desired environmental conditions and offers crucial insights into the mechanism of antibiotic resistance and biofilm formation.

Abstract ID: 110 / Poster Number: 378.15P

Evolutionary Adaptations in Sphingopyxis sp. MC4 Enable Efficient Degradation of Persistent β- and δ-Hexachlorocyclohexane Isomers

Presenting author: Dr. Monika Sharma, Indian Institute of Technology, Delhi, Delhi, India; University of Delhi, Delhi, India

Co-authors: *Durgesh Narain Singh, Gunjan Uttam, Poonam Sharma, Shivam A. Meena, Akhilesh K Verma, Ram Krishan Negi*

Hexachlorocyclohexane (HCH), an organochlorine pesticide, exerts several harmful impacts on the ecosystem. The β- and δ-isomers, among the four major isomers of HCH, are highly toxic, persistent, and recalcitrant to biodegradation. Slow and incomplete degradation of these isomers has been reported in a few microbial strains. Despite the isolation of degradative microorganisms, a robust solution for the degradation of persistent β- and δ-HCH isomers remains lacking. As part of ongoing efforts toward the bioremediation of HCHs, we have isolated a strain, designated Sphingopyxis strain MC4, from HCH contaminated soil. This strain can tolerate and degrade high concentrations of α-, β-, γ- and δ-HCH isomers. To date, no other Sphingopyxis strain has been reported to degrade β- and δ-isomers.

To understand the genetic basis for the strain's adaptations, the whole genome of strain MC4 was sequenced. Comparative genome analysis revealed that strain MC4 harbors the complete set of lin genes required for HCH degradation. Genetic footprints, such as presence of lin genes on genomic islands, the proximity of IS6100 elements to these genes, and synteny in lin flanking regions with other strains, reflects that horizontal gene transfer has contributed to the acquisition of these genes in strain MC4. Positive selection and HGT drive the adaptive evolution of strain MC4 under the pressure imposed by HCH contamination experienced in its surrounding niche. In silico analyses showed efficient binding of β- and δ-isomers with enzymes, leading to rapid degradation, although further validation through cloning and biochemical experiments is needed.

Abstract ID: 1655 / Poster Number: 014.15P

Examining carbon cycle mechanisms in wheat-clover intercropping systems through the quantification of genes associated with cellobiose hydrolysis

Presenting author: Dr. Gemma Marchetti, University of Florence, Florence, Italy

Co-authors: *Riccardo Picone, Ahmed Sharaf, Paola Arfaioli, Giacomo Pietramellara, Shamina Imran Pathan*

Current modern agriculture practices like monocropping and high chemical use negatively impact soil health, biodiversity, and disease vulnerability. Sustainable practices such as organic fertilizers and crop diversification should replace these. Intercropping, an ancient technique of growing multiple plant species simultaneously in one field, offers a promising alternative. Legume-cereal intercropping maximizes agroecosystem productivity and efficiency, improves resource use, and reduces external inputs. However, the ecological processes regulating this system are not fully understood.

This study evaluates the effect of legume-based intercropping on edaphic factors and microbial biodiversity in rhizosphere and bulk soils. A one-year field trial assessed the short-term impact of wheat-clover intercropping under two nitrogen fertilizer levels on soil physicochemical properties, carbon cycle-related enzymatic activities, and abundance of functional genes linked to cellobiose hydrolysis.

Soil physicochemical properties showed no significant impact from soil depth, management practices, or fertilization treatments, except for soil moisture, which increased in cereal monoculture with reduced fertilizer rates. Monocultures and intercropped plots had similar enzyme activity potential. However, cultivation strategy and N fertilizer dosages significantly affected fungal and bacterial β-glucosidase gene abundance. Intercropping increased bacterial GH1 family genes in bulk soil, especially under reduced N fertilization. Fungal β-glucosidase genes were more affected by cultivation strategy and N fertilization than bacterial genes, particularly in rhizosphere soil. Intercropping significantly increased fungal GH1 and GH3 gene abundance, especially with reduced N fertilization. The findings indicate that intercropping systems involving legumes can enhance soil carbon accumulation, underscoring the role of intercropping in global carbon storage.

Abstract ID: 2225 / Poster Number: 194.16P

Examining the in vitro effectiveness and collateral damage of a synthetic antimicrobial peptide (M33) against pig respiratory pathogens

Presenting author: Ms. Ana Laura Pereira Lourenço, Centre de Recerca en Sanitat Animal (CReSA), Barcelona, Spain

Co-authors: *Oussama Rouam-el Khatab, Chiara Falciani, Alessandro Pini, Virginia Aragon, Marta Cerdà Cuéllar, Karl Kochanowski*

Facing the era of the emerging lack of effective antibiotics against resistant bacteria, antimicrobial peptides (AMPs) are a promising alternative. Current efforts focus on generating or discovering new AMPs and testing their efficacy against human pathogens. However, how well these AMPs work against animal pathogens is often unclear. Moreover, for most AMPs we know little about their impact on the commensal microbiota. To address these questions, we here used a synthetic AMP (M33) (1) as our test case, and examined its efficacy against pig respiratory pathogens. We found that M33 is highly effective (MICs in low micromolar range) against clinical isolates of *Glaesserella parasuis*, but ineffective against *Streptococcus suis* or *Actinobacillus pleuropneumoniae*. Next, we examined the impact of M33 against a collection of commensal pig nasal microbiota members (2). We found that while these strains differed widely in their sensitivity to M33, several important members of the commensal microbiota (e.g. *Rothia nasimurium* and *Streptococcus pluranimalium*) were highly resistant to this AMP. Therefore, we hypothesized that M33 could selectively inhibit *G. parasuis* growth while preserving the commensal microbiota. To test this hypothesis *in vitro*, we performed commensal-pathogen co-cultures with *R. nasimurium* and *G. parasuis* in presence or absence of M33. We found that M33 was indeed able to selectively kill the pathogenic *G. parasuis* strain, without affecting the commensal *R. nasimuruim* strain. Taken together, this study provides a template for identifying new therapeutic use cases for AMPs that minimize collateral damage on the commensal microbiota.

Abstract ID: 2148

ExcludonFinder: Mapping Transcriptional Overlaps Between Neighbouring Genes

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Bacterial genes can coordinate their expression through overlapping transcription, forming structures called excludons (Fig. 1A). In these paired arrangements, genes exhibit inverse expression patterns through two distinct mechanisms: transcriptional interference from RNA polymerase activity and RNase III degradation of overlapping transcript duplexes. This regulatory system has been identified across diverse bacterial species, playing crucial roles in adaptation to environmental stresses, including antibiotic exposure and intracellular survival, where rapid and coordinated changes in gene expression are essential. Despite their biological significance, no systematic method existed to identify excludons in bacterial genomes. We developed ExcludonFinder, a web-based tool that identifies transcriptional overlaps in neighboring genes using RNA-seq coverage analysis (Fig. 1B). We validated the tool by mapping excludons in *Escherichia coli* and *Staphylococcus aureus* genomes, revealing that *E. coli* contains significantly more excludons, with convergent overlaps (3' UTR) being more prevalent than divergent ones (5' UTR). Multiple complementary approaches validated the presence of transcriptional overlap in excludons. In S. aureus, analysis of short RNAs (20 nt) revealed a balanced sense/antisense strand ratio in excludon regions compared to non-excludon intergenic regions, indicating RNase III-mediated degradation of overlapping transcripts. In *E. coli*, validation was performed by comparing the excludon map against double-stranded RNA sequences captured by the *Tombusvirus* p19 protein, which confirmed an enrichment of predicted excludons. Single-cell transcriptomics validated the excludon concept by revealing that paired genes within excludons exhibit minimal co-expression relative to neighboring non-excludon genes. Finally, analysis of antibiotic response transcriptomes revealed consistent expression modulation of excludons, notably the *relBE-ydfV* pair. Under various antibiotic treatments, *ydfV* upregulation consistently accompanied *relB* downregulation, uncovering a novel regulatory component of the *RelEB* toxin-antitoxin system influencing bacterial persistence. Our findings demonstrate ExcludonFinder's effectiveness in identifying coordinated gene pairs, providing valuable insights into bacterial adaptation mechanisms and establishing a foundation for investigating transcriptional overlap in stress response.

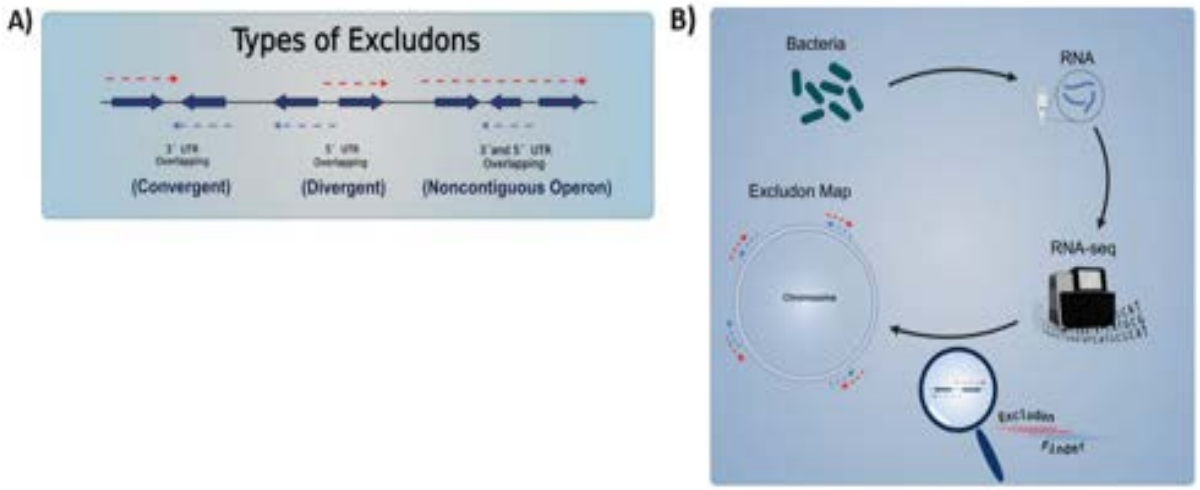


Figure 1. Overview of excludon types: convergent (3' UTR overlap), divergent (5' UTR overlap), and noncontiguous operon (both overlaps). (B) Schematic representation of the ExcludonFinder pipeline for identification and mapping of excludons across bacterial chromosomes.

Abstract ID: 206 / Poster Number: 176.15P

Expanding the collection of methane-oxidizing bacterial isolates from rice roots: cultivation, isolation, and genomic characterization

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Introduction: Methane emission from rice fields is determined by the balance of methane production by methanogens and methane consumption by methane-oxidizing bacteria (MOB). To date, many MOB strains have been isolated from rice fields. However, most of them have not been well characterized.

Objective: We aimed to expand the collection of MOB isolates from rice roots, which are one of the most important hotspots for methane oxidation in rice fields, by examining phenotypic and genotypic characteristics of the isolates.

Method: We used the roots of three rice cultivars and nitrate mineral salt (NMS) liquid and agar media for the enrichment of MOB. The cultures showing methane-oxidizing activity were further purified by colony isolation and/or extinction dilution methods. We characterized the isolates by morphological, phylogenetic, and genomic analyses.

Findings: We obtained one hundred MOB cultures and isolated twelve strains of type I (*Gammaproteobacteria*) and type II (*Alphaproteobacteria*) MOB. They differed by the phylogenetic analyses of 16S rRNA and particulate methane monooxygenase (*pmoA*) genes from already known cultivable MOB isolates from rice roots. Two type I and two type II strains were closely related to *Methylomonas* aurea SURF-1^{T} and *Methylocystis echinoides* IMET10491^{T}, representing novel MOB species based on genome analysis. The other eight strains were related to *M. echinoides* IMET10491^{T} or *Methylosinus sporium* 5^{T}.

Conclusion: This study largely expanded the collection of MOB isolates inhabiting rice roots. In addition, four of the twelve strains belonging to the genus *Methylomonas* or *Methylocystis* represented putative novel species based on phenotypic and genotypic characteristics.

Abstract ID: 2847 / Poster Number: 129.15F

Exploiting a Caenorhabditis elegans model to develop probiotics to improve bee resistance to climate change

Presenting author: Ms. Charlotte Sprason, Lancaster University, Lancaster, United Kingdom

Co-authors: *Philip Donkersley, Jason Chin, Alexandre Benedetto*

Bees are essential insect pollinators on which the reproduction of 87.5% global plant species depends. Worldwide, bee species are in decline, a problem majorly attributed to global climate changes impacting on the bees themselves and their environment. The bee microbiome plays essential roles in bee health and some microbial supplements have been shown to improve bee fitness against stressors. However, developing bee prebiotics and probiotics at scale is limited by the expense and low throughput of bee phenotypic screens.

To circumvent this, we leverage an intermediate screening platform: the nematode model *Caenorhabditis elegans*, to develop candidate bee probiotic cocktails for improved bee resilience to environmental pressures brought by climate change.

Culturable microbes are isolated from bee and other invertebrate guts and identified by MALDI-biotyping and 16s sequencing. Microbes are then tested in gnotobiotic *C. elegans* for their ability to improve heat tolerance, lifespan and fecundity. Microbe f interests are then fluorescently tagged for *in-vivo* study, before honeybee testing.

In our first year, hundreds of bacterial isolates were collected with a tenth identified to species level. We successfully tagged several and expanded our fluorescent tagging system, to enable individual *in-vivo* tracking of multiple isolates simultaneously. Phenotypic and thermotolerance assays in *C. elegans* so far show that core bee gut microbiotas can sustain and differentially impact worm physiology.

Our work to date demonstrates the practicality of using *C. elegans* for bee gut microbiota screening and its potential to assist in the development of probiotics for bees.

Abstract ID: 915

Exploiting bacteriophage-encoded depolymerase to potentiate antibiotic activity against multidrug-resistant Acinetobacter baumannii in low-serum conditions

Presenting author: Ms. Honglan Wang, The Chinese University of Hong Kong, Hong Kong, Hong Kong

Co-authors: *Sharon Shui Yee Leung*

Introduction: The emergence of multidrug-resistant Acinetobacter baumannii (MDR-AB) has posed a significant clinical challenge, necessitating the development of novel treatment strategies. One promising strategy involves the use of bacteriophage-encoded depolymerases, which target and degrade the polysaccharide components of bacterial capsules and biofilm matrix, thereby exposing bacteria to antibiotics and host immune attacks. Nevertheless, depolymerases do not kill bacteria directly and are currently limited to treating bloodstream infections due to their dependency on serum-mediated bacterial killing.

Objectives: To extend the treatment paradigm of depolymerases under low-serum conditions, combining them with antibiotics exhibiting bactericidal synergy might be a feasible option.

Methods: The combined effects of a model depolymerase (Dpo71) with interested antibiotics against the susceptible MDR-AB2 strain were evaluated both in the serum-free and 5% serum-containing conditions by determining the changes in minimum inhibitory concentration values (MICs) of antibiotics. A promising combination, Dpo71 and ceftazidime (CFZ), was selected for further evaluation into the in vitro antibacterial and antibiofilm synergism. To elucidate the underlying synergistic mechanisms, the roles of Dpo71 and 5% serum in impairing the penetration barriers, including biofilm matrix, bacterial capsules, outer membrane (OM), and peptidoglycan (PG) layers, were evaluated using confocal laser microscopy imaging and transmission electronic microscopy observation. Finally, the binding efficiency of CFZ to Dpo71-treated MDR-AB2 in 5% serum was determined using high-performance liquid chromatography.

Findings: We observed that Dpo71 could effectively potentiate antibiotic activity against MDR-AB2 bacteria in 5% serum but showed no adjuvant effect in serum-free conditions. Dpo71 could synergize with CFZ against both planktonic bacteria and biofilms in a 5% serum environment. Mechanistic studies showed that Dpo71 improved the penetration of drug combination through carbohydrate barriers by degrading biofilm

exopolysaccharides and bacterial capsular polysaccharides, and sensitized bacteria to serum-induced permeabilization of OM and PG layer. Resultantly, the binding of CFZ to the bacterial cells was significantly improved by Dpo71 in 5% serum. Collectively, these results indicated that the collaborative actions of Dpo71 and serum effectively impaired the permeation barriers, thereby facilitating the accessibility of CFZ to its intended targets, ultimately exerting enhanced bactericidal effects (Figure 1).

Conclusion: The present work has identified a promising Dpo71-CFZ combination that demonstrated a synergistic antibacterial effect against MDR-AB2 in a low-serum condition. This study unraveled the low-serum-dependent mechanisms by which depolymerase potentiated antibiotic activity, highlighting the potential of depolymerase-antibiotic combination therapy as a novel therapeutic strategy in controlling low-serum local infections.

Figure 1: Graphical abstract of Dpo71-mediated antibiotic potentiation in 5% serum and synergistic mechanisms of Dpo71-CFZ combination.

Abstract ID: 3229 / Poster Number: 401.16P

Exploration of exopolysaccharide derived from Lacticaseibacillus rhamnosus IR06 as a postbiotics for preventing UVB-induced skin barrier dysfunction

Presenting author: Mr. Jin Hwan Kim, Korea University, Sejong, South Korea

Co-authors: *Nam Su Oh*

Exopolysaccharides (EPSs) are extensively utilized in the food industry as stabilizing agents, viscosity modifiers, and thickening agents due to their ability to enhance rheological properties. Beyond their role in food applications, EPSs have attracted significant interest for their various biological activities, including antioxidative, anti-inflammatory, and anticancer properties.

Ultraviolet B (UVB) radiation triggers the generation of intracellular reactive oxygen species (ROS), which contribute to skin damage such as disruption of the epithelial barrier, wrinkle development, and inflammatory responses. In this study, we examined the protective potential of an EPS derived from a promising probiotic lactic acid bacterium. The EPS extracted from *Lacticaseibacillus rhamnosus* IR06 (EPS-IR06) demonstrated notable *in vitro* efficacy in safeguarding against photoaging and inflammation.

Structural analysis revealed that EPS-IR06 is a heteropolysaccharide with a glucose-branched galactomannan composition, possessing a molecular weight ranging between 3.9×10^4 and 5.8×10^5 Da. Application of EPS-IR06 in a UVB-exposed 3D human skin model mitigated epidermal damage and enhanced biomarkers related to epidermal barrier integrity. Additionally, EPS-IR06 effectively neutralized intracellular ROS and restored homeostasis in the Nrf2-mediated antioxidant defense system within UVB-treated human epidermal keratinocytes.

EPS-IR06 also significantly suppressed the expression of matrix metalloproteinases and pro-inflammatory mediators by modulating the MAPK and NF-κB signaling cascades. These results indicate that EPS-IR06 has potential as a novel protective compound against UVB-induced skin damage, with promising applications in both cosmetic and food industries.

Abstract ID: 978 / Poster Number: 181.16P

Exploration of Novel Inhibitors Targeting the Mrp Antiporter of Pseudomonas aeruginosa

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Co-authors: *Masahiro ITO, Mami YAMAMOTO*

The Mrp antiporter functions as a critical enzyme in maintaining cellular cation homeostasis by expelling cations while facilitating proton uptake into the cell. Observed in both bacteria and archaea, the Mrp antiporter is composed of multiple subunits (1). Notably, MrpA shows homology with NuoL of the Escherichia coli respiratory chain complex I, and MrpD with NuoM and NuoN, suggesting that these subunits play key roles in cation transport (2). Pathogenic bacteria, such as Staphylococcus aureus and Pseudomonas aeruginosa, which are responsible for opportunistic infections, also harbour the Mrp antiporter (3,4). Mutants lacking Mrp in these strains exhibit reduced infection rates and pathogenicity in mice, highlighting this enzyme as a promising drug target.

In the E. coli KNabc strain, which lacks major Na^+/H^+ antiporters, expression of the mrp gene from P. aeruginosa or S. aureus enables growth in LBK+0.3 M NaCl medium. However, growth is inhibited when Mrp is blocked. Leveraging this property, a growth assay was conducted using candidate compounds identified in previous studies from our laboratory that inhibit Mrp activity derived from S. aureus. Additionally, Na^+/H^+ antiport activity was measured using fluorescence quenching in the presence or absence of these candidate compounds. As a result, potential inhibitor compounds targeting Mrp activity from P. aeruginosa were identified. Our findings show that candidate compounds effectively target Mrp activity, offering the potential for novel antimicrobial agents. Future studies will refine these inhibitors and assess their in vivo efficacy to address antimicrobial resistance.

Abstract ID: 1861 / Poster Number: 012.16P

Exploring Antimicrobial Resistance Through Honeybee Microbiota in a One Health Context

Presenting author: Mrs. Silvia Rosa, Free University of Bozen/Bolzano, Bolzano, Italy

Co-authors: *Lorenzo Brusetti, Maria Ludovica Saccà, Federico Martignago, Franco Multinelli, Giovanni Cilia, Salvatore Barone*

Antimicrobial resistance (AMR) represents a significant global health threat, and understanding the factors driving the spread of resistance among environmental microorganisms within a One Health framework is crucial. Honeybees (*Apis mellifera*) forage over large areas, with their hairy bodies trapping environmental particles, including bacteria and contaminants. We hypothesized that bees may act as trapping vectors of AMR, behaving as bioindicators of the environmental resistome diversity within a One Health framework. This study aimed to characterize and assess the presence of AMRs in the microbiome of honeybees collected from various environments in South Tyrol, Italy. Sampling was conducted across different seasons (spring, summer, autumn) and habitats, including urban, agricultural, natural, and livestock-dominated areas. Microorganisms isolated from honeybees' gut and surface were identified using MALDI-TOF mass spectrometry. The presence of AMRs was assessed phenotypically by MIC tests and genotypically by qPCR, for each isolate. The results revealed a noteworthy microbial diversity between the different samples. From the 99 species identified, 53% were isolated only once. Among these species encompassed various plant and animal pathogens, like *Pantoea agglomerans, Bacillus cereus, Pseudomonas aeruginosa*. Most of the isolates were resistant to at least one antimicrobial class, and many were multidrug-resistant bacteria, including ampicillin resistance and *tetM* and *tetC* genes. We will show how geographic and seasonal factors may influence the composition of the honeybee microbiome and the prevalence of antimicrobial resistances. We will also aim to explore on antibiotic resistance genes transmission from honeybees' microbiome to better understand the ecological impact of pollinator-mediated resistance spread.

Abstract ID: 3680 / Poster Number: 067.15P

Exploring Antioxidant-Mediated Changes in Roseburia intestinalis and Eubacterium ramulus: Growth Patterns, Metabolic Profiles and Oxygen Adaptation

Presenting author: Nurainina Binti Ayob, PhD, University of Ljubljana, Ljubljana, Slovenia

Co-authors: *Maša Vodovnik, Katja Palovšnik, Ema Rezar*

Introduction & Objective: An increasing number of studies have highlighted the critical role of gut microbiome in maintaining host health. Recently, antioxidants have been shown to modify gut microbiome structure and diversity. However, functional and mechanistic insights into these interactions remain limited. In this study, we evaluated the response of two important gut commensals, *R.intestinalis* and *E.rectale* to selected antioxidants in terms of growth, metabolic profiles, oxygen sensitivity and membrane adaptations.

Methods: Six antioxidants of different concentrations (from 0.5mg/mL to 20mg/mL) including ascorbic acid, astaxanthin, ferulic acid, curcumin, propyl gallate, and α-tocopherol were added to anaerobic cultures of selected strains. Following OD650 measurements, the fermentation and LCFA profiles were analyzed. The survival during short-term oxygen exposure was also assessed.

Results: Propyl gallate, astaxanthin and (±)-α-tocopherol significantly enhanced *R. intestinalis* growth compared to the control group, but *E. ramulus* showed no significant increase. On the other hand, astaxanthin, and curcumin increased the amounts of selected metabolites, specifically acetic acid, butyric, lactate, and succinate during fermentation in both strains. Further, the survival of *E. ramulus* in the presence of oxygen was improved in the presence of ascorbic acid, curcumin, astaxanthin, and ferulic acid.

Conclusion(s): The responses to selected antioxidants on all levels were strain- and molecule-specific. All of the antioxidants modulated the metabolic pathways, leading to substantial strain-specific changes in their fermentation profiles. Further, the improved survival of the target strain in microaerobic conditions suggested the protective role of antioxidants during short-term increases in redox potential within the gut environment.

Abstract ID: 1222

Exploring aptamer-based biosensors for the detection of foodborne bacterial toxins

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Co-authors: *Maria Margarida Barros, Nuno Filipe Azevedo, Carina Almeida*

Bacterial toxins are the second leading causative agent (15.7%) of foodborne outbreaks in Europe. Among them, *Staphylococcus aureus* toxins are the second most reported agent and the one that has caused the highest number of hospitalisations. The detection of staphylococcal enterotoxins (SEs) in food products is based on an Enzyme Linked Fluorescent Assay (ELFA) technique, which is time-consuming and requires specialised equipment. Aptamers, short single-stranded nucleic acids, have been seen as promising alternatives to antibodies in diagnostic applications.

The aim of this work was to evaluate the use of aptamers in lateral flow assay (LFA) biosensors for a simple, rapid and cost-effective point-of-need (PoN) detection of SEs.

In this work, DNA aptamers previously selected by our group (Apt1, Apt2, Apt3, Apt4 and Apt5) for staphylococcal enterotoxin A (SEA) were applied as recognition molecules in the development of an LFA biosensor. For this, lateral flow strips consisting of a sample pad with glass fibre, test zone with nitrocellulose membrane and absorbent pad with cellulose membrane were assembled. Different assay formats were established using biotinylated aptamers (140 pmol of Apt1, Apt2, Apt3 or Apt4) immobilised in the test zone using streptavidin as an anchor. A DNA probe (140 pmol) complementary to Apt5 was also immobilized as a test control. To visualise the results, Apt5 covalently attached gold nanoparticles (AuNPs) were synthesised.

Then, SEA solutions (1 ng/µL) as well as negative samples (1 mg/mL BSA) were prepared and incubated with Apt5-AuNPs (OD3) in 1x binding buffer (BB) in total volume of 100 µL for 10 min at RT. The samples were applied to the sample pad, allowing the solution to flow on the strip until the test lines were visualized by the accumulation of AuNPs. The different assay combinations (Apt5-AuNPs + Apt1/Apt2/Apt3/Apt4) were tested.

All the SEA samples were positively detected, but some of them with a signal that was more visible to the naked eye (Figure 1.3 and 1.4). The combination of Apt5-AuNPs with Apt3 (Figure 1.3) provided the most promising result, followed by Apt4, Apt2 and Apt1 (Figure 1.4, 1.2 and 1.1, respectively). Negative controls were validated by the control line (Figure 1.5, 1.6, 1.7 and 1.8). Further tests to determine the detection limit and improve the noise ratio are being carried out.

These results show that aptamer-based LFA biosensors can be a simple and rapid alternative for the PoN detection of SEA. More important, this approach can be adapted to any other toxin, including other relevant foodborne contaminants, demonstrating a promising alternative for improving food security in the future.

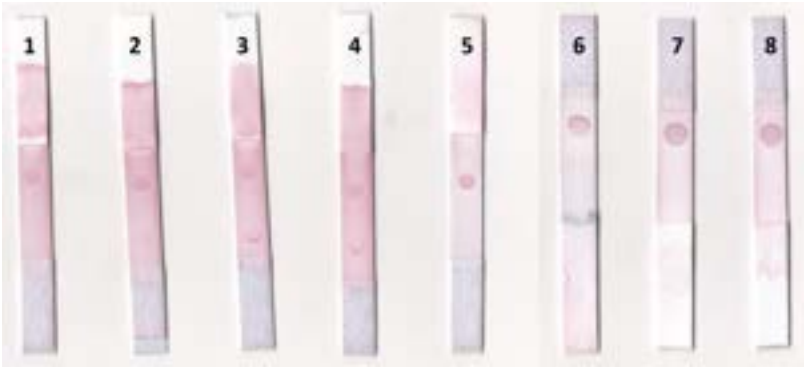


Figure 1: Lateral flow tests of SEA samples (1 ng/µL) as well as BSA (1 mg/mL) and BB samples. In order from 1 to 4, the strips correspond to the test formats Apt5-AuNPs + Apt1/Apt2/Apt3/Apt4. Strip 5 corresponds to the BB-only solution while strips 6-8 correspond to the BB samples.

Abstract ID: 3513

Exploring Bacterial Chemoreceptors in Rare and Uncultured Microbial Communities

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Chemoreception, the ability of microbes to detect and respond to environmental signals, is fundamental to microbial adaptability, survival, and ecological interactions[1]. Chemosensory pathways allow microbes to sense changes in their environment and modulate behaviors such as nutrient acquisition, predator evasion, and host interactions[2]. Central to these processes are chemoreceptor proteins (CRs), which initiate signal transduction pathways by recognizing signals through their ligand-binding domains (LBDs) for subsequent regulation of cellular responses[3]. However, despite the importance of CRs, much of their diversity and ecological roles remain uncharacterized, especially in uncultured and low-abundance microorganisms. This work aims to fill these gaps by studying CRs at the genomic and metagenomic scale, providing a comprehensive view about their natural biodiversity, ecological links and evolutionary significance.

This study aims to address these knowledge gaps by (1) exploring the diversity and ecological patterns of CRs across genomic and metagenomic datasets and (2) developing novel methodologies to characterize the CR repertoire in low-abundance microbial taxa.

We constructed the GlobalCR catalog, the most extensive database of CRs to date, comprising over 1.4 million sequences from 800,000 metagenome-assembled genomes (MAGs)[4] and 229,000 sequences from 41,000 representative reference genomes[5]. Using a combination of sequence homology-based clustering, ecological annotation, and phylogenetic analysis, we investigated the diversity, habitat-specificity, and evolutionary trends of CRs. Additionally, a targeted capture sequencing method was developed to enhance the detection of CRs in rare microbial taxa, enabling the study of low-abundance microorganisms diverse environmental samples.

Analysis of the GlobalCR catalog revealed an immense diversity of sensing domains, particularly extracellular domains, which are strongly linked to specific ecological

niches. Taxonomic profiling uncovered numerous CR-rich lineages, many of which represent previously uncharacterized taxa, highlighting their potential adaptive roles in diverse habitats. Phylogenetic reconstruction of 400,000 unique CR sequences provided insights into their evolutionary history, revealing co-evolutionary patterns with other components of the chemosensory pathways and evidence of horizontal gene transfer events.

The novel capture sequencing approach significantly enhanced the detection of CRs in rare microbial taxa, identifying up to 200 times more CR genes per sample compared to conventional metagenomic sequencing. Application of this method to plant rhizosphere samples uncovered a hidden diversity of chemosensory microorganisms, including latent pathogens and symbionts, with important implications for plant-microbiome interactions.

This work advances our understanding of microbial chemosensory systems by providing an extensive resource for CR diversity and linking their ecological and evolutionary patterns to environmental adaptation. The novel methodologies developed here open new avenues for exploring the chemosensory capacity of rare and uncultured microbial taxa, offering valuable insights into their roles in ecosystem dynamics.

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Abstract ID: 2912 / Poster Number: 166.15P

Exploring Bacterial communities and antibiotic resistance in pastoral farmland ecosystems

Presenting author: Dr. Juliana Lukša-Žebelovič, Nature Research Centre, Vilnius, Lithuania; Vilnius Tech, Vilnius, Lithuania

Co-authors: *Alper Melih Ucar, Živilė Strazdaitė-Žielienė, Elena Servienė*

Introduction: Bacteria are among the most abundant microorganisms in nature, playing a pivotal role in diverse ecosystems. In farmland environments, they are essential for soil fertility, plant growth, and the sustainability of agricultural practices. However, pastoral systems pose potential public health risks due to close interactions between livestock, humans, and the environment, with the prevalence of antibiotic-resistant bacteria (ARBs) adding to these challenges.

Objective: This study aims to investigate the distribution and antibiotic resistance of bacteria in farmland ecosystems, with a focus on soil, feed, and freshwater in pastoral farmlands.

Methods: Samples were collected from four pastoral farmland sites in Lithuania. Bacterial identification was conducted using cultural methods and 16S rRNA metagenomic sequencing to analyze community composition. Antibiotic resistance profiles were determined by testing bacterial strains against ampicillin, streptomycin, tetracycline, and chloramphenicol.

Findings: The 16S metagenomic analysis revealed distinct bacterial communities in each environment. Soil samples were dominated by *Pseudomonas*, *Nocardioides*, *Rhizobium*, and *Streptomyces*. Grass samples featured *Pseudomonas*, *Sphingomonas*, *Rhizobium*, and *Massilia*, while water samples exhibited *Acidobacteria*, *Nocardioides*, and *Pseudomonas*. Antibiotic resistance testing identified 61 resistant strains, predominantly in soil (31 strains), with high resistance to streptomycin and ampicillin. Opportunistic genera such as *Solibacillus, Enterobacter*, and *Acinetobacter* were detected in soil, water, and feed, while potentially pathogenic genera like *Bacillus* were found in soil.

Conclusion: This study highlights the ecological importance of bacterial diversity in farmland ecosystems and underscores the public health implications of antibiotic-resistant and pathogenic bacteria.

Abstract ID: 1385 / Poster Number: 241.17P

Exploring Bacterial Resistance Mechanisms to Strontium for Environmental Applications

Presenting author: Mr. Masaharu Furuyama, Toyo University, Asaka, Saitama, Japan

Co-authors: *Ichigo Takemoto, Masahiro Ito*

Strontium (Sr) is an alkaline earth metal widely distributed in nature, with radioactive isotopes such as ^{90}Sr contributing significantly to environmental pollution. The physical half-life of ^{90}Sr is 29 years, and its biological half-life is 50 years, underscoring its persistence as a contaminant. Sr behaves similarly to calcium (Ca) in biological systems, often substituting for Ca in cellular processes, which may exacerbate its environmental and biological impacts. Similarly, ^{137}Cs, a radioactive cesium isotope, is a well-documented pollutant. While previous studies in our laboratory have focused on isolating cesium-tolerant bacteria and unraveling their resistance mechanisms (1-4), investigations into Sr-tolerant bacteria and their mechanisms remain scarce. In this study, Sr-tolerant bacterial strains were successfully isolated from natural environments using screening methods optimized for high-concentration Sr^{2+} conditions. Resistance testing identified strains exhibiting robust growth in Sr^{2+} concentrations exceeding 500 mM, significantly higher than those observed in common bacteria. Notably, a model microorganism was identified that could thrive in the presence of 700 mM Sr^{2+}, providing a valuable system for further studies. Comparative analyses revealed variability in Sr tolerance among bacterial species, with certain strains displaying exceptional adaptability to Sr-rich environments. These findings offer valuable insights into the molecular and physiological strategies underlying bacterial Sr^{2+} tolerance. This research enhances our understanding of Sr adaptation mechanisms and provides a foundation for the development of microbial bioremediation technologies targeting Sr-contaminated environments. Future studies will focus on validating identified genes and pathways to advance the practical application of these microbial systems.

Abstract ID: 2483 / Poster Number: 214.17P

Exploring common and unique mechanisms of antimicrobial resistance across diverse bacterial species

Presenting author: Dr. Tomoya Maeda, Hokkaido University, Sapporo, Japan; RIKEN, Suita, Japan

Co-authors: *Chikara Furusawa*

Introduction: Antimicrobial resistance (AMR) poses a critical threat to global public health. Leveraging bacterial evolutionary constraints offers a promising strategy to combat AMR, but understanding resistance mechanisms in pathogenic bacteria is crucial. Current knowledge primarily focuses on a few species, such as *Escherichia coli* and *Pseudomonas aeruginosa*, making it unclear if these insights apply broadly across bacterial species.

Objective: This study investigates commonalities and species-specific resistance mechanisms across diverse bacteria.

Method: Using a laboratory automation system, we conducted high-throughput laboratory evolution experiments on nine bacterial species, including both Gram-negative (*E. coli*, *Klebsiella variicola*, *Enterobacter kobei*, *Citrobacter amalonaticus*, *Acinetobacter bouvetii*, and *P. putida*) and Gram-positive bacteria (*Bacillus subtilis*, *Lactobacillus plantarum*, and *Staphylococcus auricularis*), against three antibiotics (doxycycline, meropenem, and ciprofloxacin). We sequenced 144 evolved strains to identify resistance-conferring mutations, confirming their effects through reconstructed mutants by analysing resistance profiles and drug uptake/efflux rates (Figure 1).

Findings: Mutations in drug target genes (*rpsJ*, *pbp*, *gyrAB*) and stringent response-related genes were detected in both Gram-negative and Gram-positive bacteria. Gram-negative bacteria shared mutations in RNase E, while mutations in Lon protease were exclusive to *Enterobacteriaceae*. Functional analyses revealed that many mutations altered drug uptake or efflux, but only a few displayed a combined effect of reducing uptake and enhancing efflux. Species-specific variations included mutations with differing effects despite homologous gene presence.

Conclusion: This study systematically identified underexplored resistance-related mutations that affect drug uptake and efflux, revealing universal and species-specific bacterial strategies to reduce intracellular drug concentrations. These findings broaden our understanding of resistance mechanisms and their evolutionary dynamics.

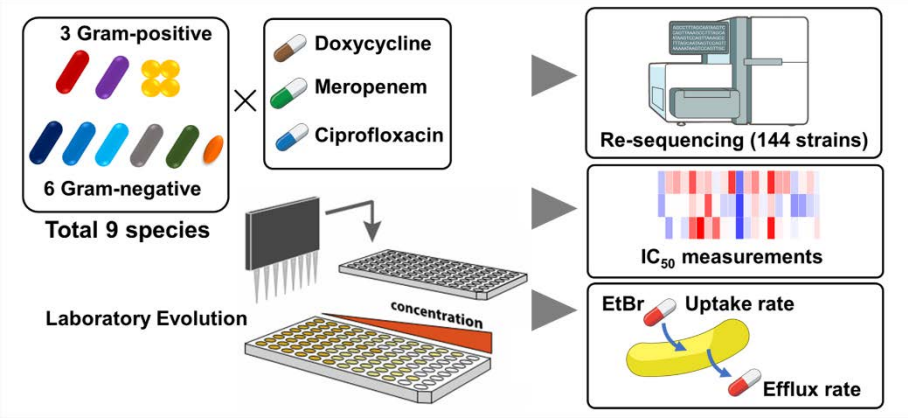


Figure 1: Schematic of the experimental setup

Abstract ID: 4039 / Poster Number: 372.17P

Exploring increased concentration of capture agent and methanogenic activity in Bio-Integrated Carbon Capture and Utilization (BICCU) reactors

Presenting author: Madhuni Madhushika Wijesooriya, PhD, Aarhus University, Aarhus, Denmark; The Novo Nordisk Foundation CO2 Research Center, Aarhus, Denmark

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Carbon capture and utilization is a promising approach to address the dual crises of increasing CO_2 emissions and growing demand for renewable fuels. However, CO_2 capture is energy-intensive, posing economic challenges. The bio-integrated carbon capture and utilization (BICCU) concept replaces conventional energy-intensive CO_2-desorption with biological methanation, where methanogens desorb CO_2 and converts it into biomethane directly from a capture agent mixture. However, biocidal properties of capture agents like methyl diethanolamine (MDEA) inhibit microbial activity, limiting biological efficiency. This study explores the performance of biomethanation in a mixed community under increasing CO_2-fed MDEA concentrations, hypothesizing that microbial adaptation to increasing MDEA concentrations improves system performance. A continuous stirred-tank reactor of 1.0 L at thermophilic (55 ℃) was operated using synthetic flue gas (N_2:CO_2; 80%:20%), 100% hydrogen, and anaerobic digestate as inoculum (3340 mg/L VSS). The reactor was supplied with CO_2-loaded MDEA ($1mol_{CO2}$:$1mol_{MDEA}$) starting at 30 mmol/L and reaching a maximum concentration of 150 mmol/L, with 10 mmol/L increases in MDEA after a minimum of three hydraulic retention times. The average volumetric methane production rate gradually increased to 965 ± 28 NmL/L/day at 150 mmol/L MDEA with a stable methane yield, followed by a sudden drop to zero methane at 175 mmol/L MDEA. With shifting MDEA concentration, total volatile fatty acids accumulated, while heptanoic acid spiked (594 ± 86 mg/L) at 150 mmol/L MDEA, resulting in decreased performance. In conclusion, while microbial adaptation improved performance at lower MDEA concentrations, high MDEA levels inhibited performance, necessitating sequencing and bioinformatics to identify key microbes.

Abstract ID: 1758 / Poster Number: 214.15P

Exploring lignocellulolytic microorganisms in forest soil by stable isotope probing

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Lignocellulosic biomass can be used for production of sustainable, eco-friendly energy, chemicals and materials. Efficient conversion of the biomass is required but not achieved. In natural environments, a variety of microorganisms can degrade the biomass effectively and synergistically. However, there is only limited information on the diversity and physiology of the lignocellulolytic microorganisms.

The objective of this study is to identify the soil microorganisms involved in degradation of lignocellulosic biomass, i.e., cellulose and phenol (a component of lignin), by high-sensitivity stable isotope probing [(1)].

Soils were collected from a valley area of the Takayama deciduous broadleaf forest site. The soil and water were mixed, and the resulting slurries were incubated aerobically at 25°C with ^{13}C-labeled phenol and cellulose. The slurry incubations with non-labeled substrates were prepared as control. The substrate degradation and/or gaseous CO_2 increase were observed during the incubation. The incubated soils were collected and subjected to RNA extraction. The V4 region of 16S rRNA was amplified by RT-PCR. The high-throughput sequencing indicated that the presence of *Paraburkholderia* sp., *Burkholderia* sp. was characteristic for phenol-amended soils, while a *Verrucomicrobiia* bacterium was predominant in cellulose-amended soils. Furthermore, the total RNA was separated and fractionated by density gradient ultracentrifugation. The sequencing of density resolved RNA will be performed to determine the ^{13}C-incorporating microorganisms responsible for the lignocellulosic biomass degradation in the forest soils.

Abstract ID: 3822 / Poster Number: 119.15F

Exploring Microbiome Interventions to Combat Climate Stress in Deep-Sea Corals

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Deep-sea corals play a crucial role in marine ecosystems, relying on symbiotic relationships with microorganisms. However, rising global temperatures pose a significant threat to their survival, and little is known about their response to thermal stress or the potential for microbiome-based interventions to mitigate these impacts. This study evaluated the effects of increased temperature and microbiome manipulation on the health of *Desmophyllum pertusum*. Beneficial microorganisms were isolated from healthy corals and applied as a microbial consortium during a 30-day mesocosm experiment simulating deep-sea conditions with gradual temperature increases up to 12.5°C. Coral health was assessed phenotypically through tissue loss and mucus release scores, with fragments collected for microbiome, metabolome, and transmission electron microscopy analyses. Results showed significant declines in coral health under thermal stress. Corals inoculated with the microbial consortium displayed quicker recovery after high-temperature exposure, suggesting a protective effect. Microbiome analysis revealed significant shifts in microbial composition across treatments (PERMANOVA, $p < 0.05$), and differential abundance analyses confirmed successful microbial manipulation. Integrative analysis with metabolome and microscopy data is ongoing to further elucidate the role of the consortium in maintaining coral health under stress. These findings demonstrate the potential of microbiome-based interventions to enhance the resilience of deep-sea corals to thermal stress, offering promising avenues for conservation strategies in the face of climate change.

Abstract ID: 1779 / Poster Number: 042.17P

Exploring of defence strategies against sodium hypochlorite in a tolerant strain of Salmonella enterica

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Introduction: Sodium hypochlorite (NaClO) is widely used in food industry to reduce microbial contamination. Recently, more studies reported the chlorine-adaptive strains of *Salmonella enterica* (*S. enterica*), posing significant food safety concerns. The defence mechanisms of tolerant strains remain poorly understood.

Objective: This study aimed to elucidate the defense strategies of a chlorine-tolerant strain of *S. enterica*.

Method: A tolerant strain (MT) was developed by continuously exposing the standard strain (*S. enterica* CVCC 1806) to sublethal concentrations of NaClO. Phenotypic characteristics, including morphology, motility, biofilm formation, and chlorine tolerance, were analyzed using transmission electron microscopy, puncture assays, laser confocal scanning microscopy, and microbial challenge tests. Comparative transcriptomics and metabolomics identified key defence mechanisms.

Findings: The MIC of MT for NaClO increased fourfold. MT exhibited enhanced flagella production, thickened cell walls, increased motility, elevated biofilm formation, and improved growth. After treatment with 100 ppm NaClO, efflux pump-related genes such as *arcA* (2.76-fold), *arcB* (1.4-fold), *tolC* (1.4-fold), and *mdtJ* (3.75-fold) were upregulated in MT compared to CVCC 1806. Bacterial chemotaxis and flagellar assembly were significantly activated, with genes such as *cheY*, *cheA*, *fliA*, and *motA* upregulated 5.7-, 4.5-, 6.2-, and 5.1-fold, respectively. Genes involved in lipopolysaccharide synthesis and antioxidant activity were also upregulated. Metabolomic analysis revealed elevated levels of antioxidant-related metabolites, including gamma-L-glutamyl-L-cysteine (0.76-fold), glutathione (0.69-fold), and N-acetyl-L-cysteine (4.6-fold).

Conclusion: The chlorine-tolerant strain of *S. enterica* employs multifaceted defence strategies, including efflux mechanisms, enhanced motility, oxidative stress mitigation, and improved energy metabolism, to survive chlorine stress, offering insights into its adaptive resilience.

Abstract ID: 181

Exploring Quorum Sensing Lactonases as Modulators of Fungal Pathogenesis in plants

Presenting author: Dr. Livnat Afriat Jurnou, Tel Aviv University, Tel Aviv, Israel

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Quorum sensing (QS) is a critical, density-dependent communication mechanism that enables bacterial populations to coordinate gene expression in response to environmental cues. In Gram-negative bacteria, QS is largely mediated by N-acyl homoserine lactones (AHLs), whose levels are regulated by enzymatic degradation via AHL lactonases. Interestingly, fungi also produce lactone ring-containing molecules, such as the mycotoxin patulin, which is synthesized by plant pathogens such as Penicillium expansum.

This study explored the dynamic interactions between bacteria, fungi, and microbial enzymes that can degrade microbial secondary metabolites within the apple microbiome. We found that patulin inhibits the fruit tree phytopathogen Erwinia amylovora and triggers the expression of its AHL lactonase gene, eaaiia.

We then demonstrated that E. amylovora expressing the EaAiiA lactonase could reduce patulin both in vitro and in vivo. Notably, this degradation reduced apple lesion development and inhibited patulin production by P. expansum. In contrast, the inactivation of EaAiiA or using an E. amylovora Δeaaiia knockout strain failed to protect apples from P. expansum infection, underscoring the functional importance of bacterial lactonases in this interplay.

Our findings provide novel insights into how bacterial AHL lactonases can influence fungal pathogenicity in apples, suggesting that bacterial-fungal interactions mediated by secondary metabolites and QS signaling molecules are key drivers in microbial competition. This work also supports the hypothesis that both fungal lactones and bacterial lactonases play pivotal roles in shaping the outcomes of microbial interactions, potentially offering new strategies for managing plant diseases.

Abstract ID: 3332 / Poster Number: 003.17S

Exploring Spatial Heterogeneity in Methane Biogeochemistry Through Microbial Community Structure and Geomorphology in Sub-Arctic Freshwater Ecosystems

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Co-authors: *Ludwig Jardillier, Maialen Barret, Léa Cabrol, Laure Gandois, Antoine Sejourné, Frédéric Bouchard, Urania Christaki*

Climate warming is particularly pronounced in high latitudes, where permafrost thaw impacts many freshwater ecosystems. This leads to the release of large amounts of organic matter available to methanogens that enhance their contribution to methane (CH_4) production. Although methanotrophic prokaryotes oxidize a significant portion of this CH_4, a substantial fraction reaches the atmosphere, further amplifying the positive feedback loop of climate warming. In this study, prokaryotic communities were studied in various freshwater ecosystems in a Yedoma (ice-rich Pleistocene permafrost) area in Yukon Territory, Canada. Although Yedoma permafrost accounts for only 7% of the total permafrost area, it contains approximately 30% of its carbon stock.

Our objective was to investigate the structure of microbial communities, particularly those involved in the methane cycle, at different spatial scales in aquatic ecosystems differing in depth and size, in a dynamic and rapidly changing landscape. Water samples were collected in August 2022 at different depths from 16 aquatic ecosystems (lakes, ponds, and water sources).

To assess the structure and function of the prokaryotic communities, 16S rRNA sequencing (metabarcoding) quantification of functional genes involved in methanogenesis (*mcrA*) and methanotrophy (*pmoA*) with qPCR were combined with in-situ measurements of physicochemical parameters and CH_4 concentrations and emissions. Carbon and deuterium isotopes of CH_4 were also analyzed to evaluate the processes driving these fluxes.

All sites exhibited high alpha diversity (median Simpson 1-D >0.8). However, even though they belonged to the same watershed, community structures differed significantly between sites, highlighting the influence of specific geomorphological and physicochemical conditions. Microbial communities also differed across the water column, with distinct communities in deeper anoxic waters. Methanotrophs were primarily found in the oxic layer, notably from the family *Methylacidiphilaceae*, but were

also present in anoxic layers, represented by the genera *Crenothrix* and *Methylobacter*. Although methanogens were generally scarce, the oxic layer of at least one site showed an increased CH_4 concentration (reaching 4×10^{-6} $mol.L^{-1}$) associated with *Methanobacterium* presence. Finally, while methane emissions measured at most sites were comparable to those reported previously in similar ecosystems (from 1.6×10^{-3} to 15×10^{-5} $mol.m^2.s^{-1}$), one water source – fed by permafrost thaw – exhibited methane emissions of nearly 1,000 times higher than any other site (1.7×10^{-1} $mol.m^2.s^{-1}$). This exceptional emission underscores the potential for localized "hotspots" of methane emissions driven by unique environmental conditions.

Overall, this study emphasized the importance of integrating geomorphological and microbiological perspectives to understand CH_4 heterogeneous spatial distribution in permafrost-influenced ecosystems.

Abstract ID: 1072

Exploring the antibiotic adjuvant potential of a new membrane-perturbing dispirotripiperazine compound against Pseudomonas aeruginosa

Presenting author: Gabriele Trespidi, PhD, University of Pavia, Pavia, Italy

Co-authors: *Sara Costa, Arooba Arshad, Viola Camilla Scoffone, Giulia Barbieri, Vadim Makarov, Maria Rosalia Pasca, Silvia Buroni*

Introduction: Among the bacterial species composing the ESKAPE group, *Pseudomonas aeruginosa* has a leading role as one of the most dangerous pathogens worldwide. Indeed, the increase of multidrug-resistant isolates is now accompanied by the appearance of colistin and carbapenem-resistant strains, leaving no therapeutic options available for patients suffering from these infections, especially people with immunodeficiency and cystic fibrosis (CF). For this reason, the development of alternative antibacterial strategies has become essential and the research on new antibiotic adjuvants (AA) is considered a promising approach. To this regard, we recently repurposed the dispirotripiperazine derivative PDSTP, an already characterized antiviral, as an antivirulence and AA compound against *P. aeruginosa* (Bonacorsi *et al.*, 2024).

Objectives: This research had the aim of unveiling the antimicrobial potential and safety of PDSTP as an AA in combination with antibiotics of different classes against *P. aeruginosa*, focusing the study also on its molecular target and mechanism of action (MoA). Also, PDSTP spectrum of activity against relevant ESKAPE bacteria was tested.

Methods: Checkerboard assays were used to test the synergy of different antibiotics with PDSTP, while the cytotoxicity was quantified by MTT assay. Dansyl-polymyxin B displacement was used to assess PDSTP binding affinity for *P. aeruginosa* lipopolysaccharide (LPS) and the outer membrane perturbation potential of the compound was clarified by nitrocefin degradation assay and scanning electron microscopy.

Findings: PDSTP showed strong synergy (FICI<0.5), at concentrations as low as 16 µg/mL, in combination with antibiotics having disparate MoA, such as ceftazidime, rifampicin and nalidixic acid against a panel of *P. aeruginosa* strains including MDR and colistin-resistant CF clinical isolates. Moreover, the compound had a highly safe profile, with a calculated 50% cytotoxic concentration >800 µg/mL on wild type and CF epithelial cell lines. Regarding the MoA, PDSTP was demonstrated to bind to LPS with an affinity

higher than colistin, leading to significant perturbation of the outer membrane integrity. The synergistic behaviour of PDSTP with antibiotics was also tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Staphylococcus aureus*, confirming its AA effect on Gram-negatives, but being ineffective on the Gram-positive *S. aureus*.

Conclusions: In this work, we demonstrated the broad-spectrum AA potential of PDSTP against some of the major Gram-negative pathogens threatening global health, focusing the research on *P. aeruginosa*. Given the promising results obtained, we plan to better characterize the MoA of the compound, by studying PDSTP-LPS interaction, and to test its *in vivo* efficacy, using pre-clinical mouse models of lung infection. Altogether, this will be essential to validate the translational potential of this new therapy, establishing the best combinations to test in the future clinical development.

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Abstract ID: 535 / Poster Number: 018.17F

Exploring the antimicrobial potential of the Siwa hotsprings microbiome

Presenting author: Dr. Basma Abdoullateef, The American university in Cairo, Cairo, Egypt

Co-authors: *Shimaa Ahmed, Muhammad Ajagbe, Amged Ouf, Rehab Abdallah, Rania Siam*

Introduction: There is an urgent need to discover novel antimicrobial agents to overcome antimicrobial resistance. A promising strategy is to explore previously untapped microbial diversity for bioactive compounds. The hot springs of Siwa in Egypt's Western Desert—a popular tourist destination—is largely unexplored in this context. Metagenomics offers a powerful tool to access the vast microbial potential of such environments, enabling the discovery of new antimicrobial compounds. One effective metagenomic technique is fosmid library construction, which allows for the functional screening of microbial genes.

Objective: Screening Siwa lakes' microbiome for antimicrobial activity.

Method: Environmental DNA was extracted from two hot springs, Cleopatra and Fatnas, located in Siwa Oasis, Egypt, to construct a metagenomic fosmid library. Water samples were filtered then DNA was extracted and used to construct a fosmid library with the EpiFOS Fosmid Library Production Kit. In total, 6,151 clones were generated.The fosmid library was subjected to functional screening using an agar-based assay to identify potential antimicrobial compounds. This screening tested the clones against a range of bacterial strains.

Findings: Two clones were identified that exhibited zones of inhibition against Acinetobacter and a Methicillin-Resistant Staphylococcus aureus (MRSA) strain. High-Performance Liquid Chromatography (HPLC) were performed for both clones and the characterization of the antimicrobial compounds will be presented.

Conclusion: This study will enable the identification of antimicrobial compounds capable of targeting multidrug-resistant bacteria, with the potential for development into pharmaceutical treatments to address emerging infectious threats.

Abstract ID: 1791 / Poster Number: 302.17P

Exploring the antioxidant properties of Deinococcus-derived peptides against ionizing radiation

Presenting author: Dr. Sangyong Lim, Korea Atomic Energy Research Institute, Jeongeup, South Korea

Co-authors: *Ha-Yeon Song, Jong-Hyun Jung*

Reactive oxygen species (ROS), byproducts of cellular metabolism and environmental factors, are associated with conditions like cancer and aging. Antioxidant peptides (AOPs) have been recognized as effective shields against ROS-induced harm. The Deinococcus genus, known for its exceptional resistance to ionizing radiation (IR), harbors intricate antioxidant systems aimed at countering ROS produced by IR. In our research, we synthesized four peptides, each consisting of 9 to 11 amino acids, from the leaderless mRNA (lmRNA) sequences of D. deserti. Lacking a 5′ untranslated region, these lmRNAs start protein synthesis directly, potentially leading to the production of small peptides such as AOPs. Among these peptides, Ddes-P3 demonstrated substantial antioxidant properties in vitro, efficiently neutralizing ABTS radicals. Ddes-P3 offered significant protection against IR-induced oxidative stress in CHO-K1 cells, showing a marked decrease in ROS levels and lipid peroxidation. The peptide also enhanced cell survival and preserved mitochondrial membrane potential under irradiative conditions, underscoring its potential as a safe and effective radioprotector for alleviating radiation-induced cellular damage. This investigation into the potential of lmRNA for synthesizing AOPs in Deinococcus could expand our knowledge of their cellular defenses against oxidative stress and contribute to the development of novel biotechnological and therapeutic AOPs.

Abstract ID: 3054 / Poster Number: 316.16F

Exploring the Biotechnological Potential of the Non-conventional Yeast Pichia kudriavzevii strain ZMUM_K002 from a Natural Habitat

Presenting author: Dr. Tadeja Vajdic, University of Maribor, Faculty of Natural Sciences and Mathematics, Maribor, Slovenia

Co-authors: *Marjanca Starcic Erjavec*

The natural environment harbors a variety of microorganisms with significant biotechnological potential. The natural non-conventional yeast *Pichia kudriavzevii* is a promising candidate for bioethanol, organic acids, enzymes and single-cell protein production and thus suitable for applications requiring high biomass yields. This study focused on the *P. kudriavzevii* strain ZMUM_K002 isolated from grape pomace compost to evaluate its industrial applicability and safety.

The biotechnological potential of *P. kudriavzevii* strain ZMUM_K002 using low-cost carbon sources, including spent coffee grounds (SCG), was investigated in comparison to *Saccharomyces cerevisiae* strain ZMUM_K003, which was also isolated from the environment. To ensure safety and address concerns about potential antifungal resistance, susceptibility to fluconazole was assessed using the E-test and agar diffusion method.

In YP medium containing 20 g/L glucose, *P. kudriavzevii* ZMUM_K002 reached a wet cell weight (wcw) of 49.6 ± 4.2 g/L within 48 hours, which is more than 30% higher than *S. cerevisiae* ZMUM_K003. When glucose was replaced by 40 g/L SCG, the strains of *P. kudriavzevii* and *S. cerevisiae* reached 41.1 ± 2.6 g/L and 34.3 ± 4.8 g/L of wcw, respectively. *P. kudriavzevii* strain ZMUM_K002 exhibited low fluconazole resistance (MIC = 16 mg/L), which was confirmed by both used methods.

These results indicate that *P. kudriavzevii* ZMUM_K002 has a potential for biotechnological utilization, including the production of biomass on alternative substrates. This underlines the potential of environmental yeast isolates to improve biotechnological processes by utilizing waste and supports the principles of circular economy.

Abstract ID: 1262 / Poster Number: 105.15F

Exploring the effects of low antibiotic concentrations on bacteriophage and bacteriocin induction using Escherichia coli strains isolated from the healthy gut microbiome

Presenting author: Miryam Bonvegna, PhD, APC Microbiome Institute, Biosciences, University College Cork, Cork, Ireland

Co-authors: *Pauline Scanlan*

The use and misuse of antibiotics can have myriad consequences for the ecology and evolution of complex microbial communities such as the human gut microbiome. These effects are known to include the horizontal transfer of antibiotic resistance genes, but collateral events such as lysogenic bacteriophages and bacteriocins induction are also likely[1,2,3].

To test the effects of low antibiotic exposure (1µg/mL) on the induction of prophages and bacteriocins we exposed isolates of the clinically relevant bacteria *E. coli* (n =15), sampled from healthy adult and infant gut, to 15 commonly used antibiotics. Variation in prophage and bacteriocin induction was assessed using soft-agar overlay assays against two *E. coli* indicator hosts. We complemented this analysis with whole genome sequence (WGS) analysis to characterize genetic variation and the diversity of phages and bacteriocins associated with each isolate.

This analysis highlighted that prophages are generally stable, and that induction due to antibiotic exposure is strain and antibiotic specific. Whilst bacteriocins were also stable there was two notable exceptions where the fluoroquinolones Ciprofloxacin and Norfloxacin were identified as potent inducers for all strains. WGS revealed the presence of diverse virulence biomarkers, some of which were associated with specific *E. coli* pathotypes e.g. ExPEC[4]. A number of these genes were associated with prophage and bacteriocin regions (e.g. the enterotoxin gene *senB* on the colicin-associated plasmid Col156) which highlights the potential for clinically relevant genes to be mobilized in the human gut after exposure to low antibiotic concentrations.

Abstract ID: 2604 / Poster Number: 315.15P

Exploring the epigenetic mechanisms regulating stress response and bioconversions in Rhodococcus bacterial strains

Presenting author: Prof. Martina Cappelletti, University of Bologna, Bologna, Italy

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Introduction: DNA methylation is the main form of epigenetic regulation in bacteria and is catalyzed by methyltransferases (MTases), which transfer methyl groups to adenine or cytosine bases. DNA methylation in prokaryotes plays an important role in regulating not only the host defence system, but also the cell cycle, gene expression, and virulence. However, these studies mostly focused on model bacterial strains and pathogenic bacteria. Only a few works have investigated MTase functions in bacteria with biotechnological relevance.

Among bacteria with biotechnology applications, members of *Rhodococcus* genus have outstanding metabolic diversity and flexibility in association to their capacity to use different organic contaminants, to convert them into high value-added products (e.g. lipids in the form of triacylglycerols), and to withstand various stress conditions including high concentrations of solvents and metal(lloid)s. Several metabolic engineering approaches have been applied to improve the production and accumulation of lipids in *Rhodococcus opacus* PD630. These approaches have mainly focused on functional genes involved in bioconversion pathways, while nothing is known about the possible use of epigenetic regulation components as targets for process optimization.

Objectives: This work is aimed at unravelling the role of DNA methylation in the growth and stress response of *Rhodococcus* bacterial strains to explore the connection between epigenetic regulation and biotechnological relevant processes like lipid production, metal stress resistance, aromatic hydrocarbon biodegradation.

Method: Genes encoding methyltransferases were identified in *Rhodococcus opacus* PD630 genome and mutants were constructed deleted in three of these MTases with chromosomal localization. Phenotypic analyses of the PD630 wild-type and mutant strains were compared by analysing their growth rate and their methylomes (using PacBio and Oxford Nanopore sequencing). RT-qPCR gene expression analyses were performed to correlate DNA methylation level with gene expression. The construction of gene

reporter systems is ongoing by cloning lux genes downstream of methylated promoters to assess the promoter activity in the presence or absence of active MTases.

Findings: PD630 WT and mutant strains showed different phenotypes in terms of sensitivity to stresses and growth rate under culture conditions that promote lipid production and accumulation. Methylomic analyses identified methylation motifs and pointed out different levels of methylation in the different MTase mutants at the level of some promoters involved in stress response and lipid biosynthesis. Gene expression analyses supported the role of DNA methylation in the regulation of some genes in *Rhodococcus*.

Conclusion: This study provides for the first time indications on the involvement of DNA methylation on *Rhodococcus* phenotypic characteristics for biotechnological applications.

Abstract ID: 2578 / Poster Number: 317.17P

Exploring the Evolutionary Trade-Offs Between Antimicrobial Resistance and Virulence in the Opportunistic Human Pathogen Pseudomonas aeruginosa

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Co-authors: *Barbara Pees, Michael Habig, Hinrich Schulenburg*

The global rise of antimicrobial resistance (AMR) poses a critical challenge to public health. Resistance evolution often incurs strong fitness costs, such as reduced growth rates, altered colony morphology, and hypersensitivity to unrelated antibiotics, reflecting diverse evolutionary paths. We hypothesized that such constraints might extend to virulence, a resource-intensive and multifaceted trait. To investigate this, we employed a panel of *Pseudomonas aeruginosa* PA14 clones derived from experimental evolution under three distinct antibiotic environments: Ciprofloxacin (C), Piperacillin/Tazobactam (P), and Streptomycin (S). We assessed antibiotic susceptibility and fitness in drug-free conditions, and further characterized virulence using the nematode *Caenorhabditis elegans* as a host model for both acute (toxin-mediated fast-killing) and chronic (colonization-dependent slow-killing) infection. Evolved resistance in the clones was linked by genome analysis to mutations in known AMR genes, including *gyrA, mexS, mpl,* and *rpsL*. Our experiments demonstrated distinct resistance-virulence relationships. C-evolved clones generally exhibited reduced virulence, with some retaining ancestor-like pathogenicity. P-evolved clones displayed a consistent trade-off, where increased resistance correlated with decreased virulence; notably, three clones were rendered avirulent due to large genomic deletions encompassing essential virulence and AMR genes. In contrast, S-evolved clones showed heterogeneous outcomes, ranging from ancestor-like to markedly reduced virulence. This study provides novel insights into the evolutionary interplay between AMR and virulence, emphasizing how antibiotic-specific selective pressures shape resource allocation and pathogen fitness, likely affecting. infection dynamics.

Abstract ID: 1735 / Poster Number: 353.16P

Exploring the impact of organic and inorganic additives released by bioplastics on bacterial growth

Presenting author: Carola Parolin, PhD, University of Bologna, Bologna, Italy

Co-authors: *Cristina Pellegrino, Aurora Botti, Serena Scala, Stefano Fedi, Beatrice Vitali, Elena Fabbri, Chiara Samorì*

Introduction: Bioplastics are bio-based and/or biodegradable materials to which additives are added to improve properties and performance. Inorganic and organic additives can migrate from bioplastics analogously to what happens for traditional plastics, but their impact on aquatic and terrestrial ecosystems has been neglected so far.

Objective: The impact of organic and inorganic additives released by bioplastics on the growth of model (*Escherichia coli* and *Staphylococcus aureus*) and environmental bacteria was evaluated.

Methods: Starting from objects made of Mater-Bi® and polylactic acid (PLA), leachates were prepared in deionized water and organic extracts were prepared in methanol. Model and environmental bacterial strains were cultured in the presence of different concentrations of leachates or organic extracts and growth was evaluated.

Findings: Organic and inorganic additives that can be released from bioplastics display different effects depending on the type of biopolymer and the test bacteria. Overall, leachates of Mater-Bi® bioplastics stimulate microbial growth and can even act as a carbon source in a minimal medium; organic extracts tend to inhibit bacterial growth, especially of gram-positive bacteria. The impact of additives was also correlated with COD measurements and the chemical composition of leachates and organic extracts.

Conclusion: Herein, we demonstrated that the leaching of organic and inorganic additives is negligible and does not negatively impact the tested species' growth, while the organic additives present in starch-based and PLA bioplastics can be potentially hazardous. Although bioplastics are claimed to be more sustainable than traditional plastics, a deeper evaluation from an ecotoxicological perspective is needed.

Abstract ID: 851 / Poster Number: 388.16P

Exploring the Impact of Soil Treatments on Fungal Communities Diversity in Vineyards

Presenting author: Dr. Pablo Villarreal, Millennium Institute for Integrative Biology (iBio), Santiago, Chile; Nucleus of Patagonian Limit of Life (LiLi), Valdivia, Chile

Co-authors: *Francisco A. Cubillos, Amanda Cerda-Carrasco*

Soil industrial practices are critical determinants of microbial diversity, shaping microbial communities and influencing agricultural productivity and ecosystem health. However, how soil treatments affect fungal diversity and vineyard soil health remains unclear, demanding further investigation. This study, conducted at Santa Ema Vineyard in Chile's Central Valley, evaluated the impact of three vineyard soil management strategies on fungal diversity and yeast populations: (1) conventional soil treatment with standard agricultural inputs, (2) excessive herbicide application, and (3) regenerative management with organic matter to enhance plant growth and soil health. We assessed alpha and beta diversity across these soils using metabarcoding approaches targeting fungal communities. We observed marked differences in fungal community composition and richness depending on the soil treatment. Regenerative soils consistently exhibited the highest levels of fungal diversity, suggesting a strong positive relationship between organic matter enrichment and microbial ecosystem complexity. This diversity was further explored by isolating yeast colonies from the regenerative soil. We were able to identify six different genera. The yeast isolates were subjected to an extensive phenotypic characterization to determine their ecological roles, stress tolerances, and potential applications as plant growth promoters. Our results emphasize that regenerative practices enhance soil health and contribute to sustainable agricultural systems by stimulating a diverse and functional fungal community. This study underscores the need for further investigation into the functional implications of microbial diversity in managed soils and highlights the potential utility of microbial populations as resources for sustainable viticulture.

Abstract ID: 3594 / Poster Number: 032.15P

Exploring the impact of sugar content on the microbial composition of traditional Lebanese boiled wine

Presenting author: Pamela Bechara, PhD, Lebanese University, Beirut, Lebanon; SPO, Université Montpellier, INRAE, Institut Agro, Montpellier, France, Montpellier, France

Co-authors: *Olivier Rué, Lucie Arnould, Pierre Abi Nakhoul, Rabih El Rammouz, Delphine Sicard, Jean-Luc Legras, Marie-José Ayoub*

Fermenting high-sugar grape musts is an increasing challenge caused by rising temperatures due to climate change. Boiled wine, a traditional Lebanese wine, is produced through a process involving concentration of grape must by heating to concentrate sugars before allowing spontaneous fermentation to occur, which results in a sweet wine with a distinctive flavor profile. Despite its importance in the Lebanese culture, to our knowledge, this product has never been explored before. This study aims to analyze its microbial diversity and how it can be impacted by high sugar must concentrations. Sugars were measured by HPLC on samples collected from seven fermentations followed over a course of one year from four monasteries in different Lebanese locations. Sugar levels ranged between 400-640g/L at the start of fermentation and stopped around 150-250g/L or higher depending on each fermentation. In parallel, metabarcoding analysis was performed on these samples using V3-V4 and ITS2 for bacterial (16S) and fungal (ITS) diversity respectively. Fructilactobacillus sanfranciscensis was the most frequently encountered bacterial species in our samples, detected throughout the whole fermentation (20.5% of the total reads) followed by Tatumella ptyseos (7.4%). For fungi, Hanseniaspora uvarum (15.8%) and Starmerella bacillaris (6.2%) were the most frequently encountered yeast species through all stages as well. Sugar concentrations significantly influenced the microbial composition of wine where higher sugar concentrations showed higher fungal and bacterial diversity. Several hypotheses to explain this finding will be discussed.

Abstract ID: 731 / Poster Number: 020.17F

Exploring the Influence of Arabinofuranoside Patterns on the Colonic Fermentation of Wheat Arabinoxylan

Presenting author: Ms. Yu-Wen Chiao, National Taiwan University, Taipei, Taiwan

Co-authors: *Su-Mei Lu, Ming-Hsu Chen*

Arabinoxylan, a dietary fiber derived from cereal-based foods, comprises a xylan backbone substituted with arabinose residues at the C(O)-2 or C(O)-3 positions of xylose units. The structural features of arabinoxylan govern its physicochemical properties and fermentability in the human colon. However, systematic research linking arabinoxylan branching structures with gut microbiota responses remains limited despite wheat and rye being staple foods. In this study, we aim to depict the influence of arabinofuranoside patterns on human gut microbiota responses. Three types of wheat arabinoxylan (WAX) with varying molecular sizes and rheological properties were treated with α-L-arabinofuranosidase to selectively remove monosubstituted arabinose residues. Both native and debranched WAX substrates were subjected to an *in vitro* fermentation system inoculated with human fecal microbiota. Our results demonstrated that the arabinofuranosidase treatment effectively reduced A/X ratios among WAX substrates, leading to significant shifts in microbial abundances, particularly among genera *Bacteroides*, *Bifidobacterium*, and *Blautia*. Compared to native WAX, the debranched substrates were associated with decreased fermentation gas yields and reduced medium acidification. Furthermore, we observed significant alterations in short-chain fatty acid profiles, including lower concentrations of acetate and butyrate and an increase in propionate. These findings confirm that the removal of monosubstituted arabinofuranosides alters the fermentation outcomes of WAX, offering insights into modulating intestinal microbial communities through the structural design of dietary fibers.

Abstract ID: 2964 / Poster Number: 144.15P

Exploring the potential for exopolysaccharide production in flower- and insect-associated lactic acid bacteria

Presenting author: Ms. Marion Gallus, Technical University of Munich, Munich, Germany

Co-authors: *Matthias Ehrmann, Julia Bechtner*

Lactic acid bacteria (LAB) are a large group of bacteria found in a variety of different habitats. This study focused on LAB isolated from flowers and insects. Since the microbiota found in these two habitats partially overlap, it is plausible that bacteria are transferred between them. Exopolysaccharides (EPS) are high molecular substances, which are known to help bacteria cope with environmental stressors. Certain species frequently found in flower- or insect-related samples are known to be EPS-producers. In our study we hypothesised, that flower- and insect- associated LAB species could be a rich source of EPS-producing LAB strains. LAB were cultivated and collected using selective enrichment. Flower- and insect-associated strains were identified to species level by MALDI ToF-MS and assigned to 31 different species. 21,4 % of the 145 tested isolates showed EPS production when grown on sucrose, mostly isolates assigned to different Leuconostoc species and the species Apilactobacillus kunkeei. The genomes of 13 selected Leuconostoc strains were screened for potential genes encoding enzymes of the GH70-family. A total of 43 different genes was found and compared to genes previously characterized in the literature. The results of this study prove that the LAB microbiota of flowers and insects is indeed a perfect source of EPS-producing microorganisms.

Abstract ID: 2708 / Poster Number: 376.17P

Exploring the potential of functional probiotic strains for the development of functional sourdough bread

Presenting author: Mr. Bilal Sajid Mushtaq Mushtaq, Free University of Bozen-Bolzano, Bolzano, Italy

Co-authors: *Olga Nikoloudaki, Raffaella Di Cagno, Marco Gobbetti, Kashika Arora*

, *in vitro* protein digestibility (IVPD), predictive glycemic index (pGI), amino acids, and texture parameters. Results showed that Type IV sourdough, fermented with functional probiotic strains with or without enzyme had lower gluten content compared to Type II sourdough and baker's yeast bread. Additionally, it exhibited higher IVPD and lower pGI than baker's yeast bread and comparable to Type II sourdough bread. These findings indicated that the functional probiotic starter and Type IV sourdough preparation, results to sourdough breads with comparable digestibility, pGI, biochemical and textural properties to Type II sourdough breads but with reduced preparation time.

Abstract ID: 2853 / Poster Number: 259.15P

Exploring the Soil Microbiota in Organic Agroforestry Rice Paddies: Implications for Soil Health and Grain Quality

Presenting author: Prof. Elisa Bona, Università del Piemonte Orientale, DiSSTE, Vercelli, Italy

Co-authors: *Chiara Bazzano, Alice Caramaschi, Annalisa Givonetti, Maria Cavaletto, Fulvia Tambone, Martina Nasuelli*

The importance of rice as a staple food worldwide is of common knowledge, as so the environmental impact of its cultivation. More sustainable approaches to rice production, such as agroforestry practises, are proved to be beneficial for the overall resilience of the agroecosystems, especially through the improvement of the microbiota biodiversity. In this study, we utilised a metabarcoding approach targeting the 16S rRNA gene to investigate the microbiota inhabit the soil deriving from a rice paddy field cultivated in an agroforestry setting, thoughtout the entire production cycle. Besides, chemical-physical properties and the nutritional profile of the caryopsis were estimated and correlated to the microbiota.

The results showed an significant differentiation in the soil microbiota along the growing cycle. The general diversity of organisms increase considerably reaching its highest values in the vegetative and flowering phase of the crop. In addition, a conspicuous species turnover has been found, with distinct communities and signature species between each phase. A functional analyses on the bacteria highlighted an increased metabolic activity as the rice growth and metabolism proceeded, especially in the aerobic/anaerobic chemoheterotrophy and nitrogen cycle, supported by the RDA analyses which evidenced the TKN as the main environmental driver for the differentiation in the active rice phases.

In conclusion, the characterised cropping system supports soil biodiversity allowing for a more sustainable cultivation of rice, also taking into account the total elimination of chemical inputs, both fertilisers and pesticides.

Abstract ID: 836 / Poster Number: 274.17P

Exploring the Tradicional Serbian Cheeses as a Probiotic Goldmine: Promising Insights of Novel Lactobacillus Strains for Gut Health

Presenting author: Mr. Nikola Bajcetic, University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

Co-authors: *Ivana Peric, Jovana Nenadovic, Nemanja Mirkovic, Zorica Radulovic, Nikola Popovic, Milica Mirkovic*

Traditional cheeses from specific ecological regions are a reservoir of microbial diversity and often contain unique lactic acid bacteria (LAB) with probiotic potential. This study investigates lactobacilli isolates from five different semi-hard and white brined cheeses produced in a traditional way from raw milk from rural mountainous areas of Eastern Serbia.

Based on preliminary probiotic tests for survival under simulated gastrointestinal (GI) tract conditions and antimicrobial potential against pathogenic and spoilage microorganisms, four strains were selected to test their effects on a model of differentiated intestinal epithelial Caco-2 cells. *Lactiplantibacillus plantarum* 237, *Lactiplantibacillus plantarum* 337, *Lacticaseibacillus paracasei* KB and *Limosilactobacillus fermentum* KT showed high survival rates under harsh GIT conditions. In addition, the selected strains have a broad spectrum of antimicrobial activity against Gram-negative and Gram-positive pathogens. None of the strains tested exhibited cytotoxicity in the differentiated Caco-2 cell model, with approximately 5% adhering to the cell line. Gene expression analysis revealed a reduction in oxidative stress markers (*NRF-2* and *NQO-1*), enhanced autophagy (*p62* and *MAP1LC3B*) and upregulation of tight junction-related genes (*CLDN4* and *CDH1*).

This study highlights the importance of the discovery of novel autochthonous isolates with exceptional probiotic potential that can be further used as probiotics or probiotic starter cultures in the production of functional foods.

Abstract ID: 3144

Exploring yeast biodiversity and process conditions for optimizing ethylene glycol conversion into glycolic acid

Presenting author: Dr. Immacolata Serra, University of Milano-Bicocca, Milano, Italy

Co-authors: *Vittorio Senatore, Riccardo Milanesi, Fiorella Masotti, Letizia Maestroni, Luca Campone, Paola Branduardi*

Plastics have become an indispensable material in many fields of human activities, with production increasing every year; however, most of the plastic waste is still incinerated or landfilled, and only 10% of the new plastic is recycled even once. Among all plastics, polyethylene terephthalate (PET) is the most produced polyester worldwide; ethylene glycol (EG) is one of the two monomers released by the bio-recycling of PET. While most research focuses on bacterial EG metabolism, this work reports the ability of *Saccharomyces cerevisiae* and nine other common laboratory yeast species not only to consume EG, but also to produce glycolic acid (GA) as the main by-product. A two-step bioconversion of EG to GA by *S. cerevisiae* was optimized by a design of experiment approach, obtaining 4.51 ± 0.12 g L^{-1} of GA with a conversion of 94.25 ± 1.74% from 6.21 ± 0.04 g L^{-1} EG. To improve the titer, screening of yeast biodiversity identified *Scheffersomyces stipitis* as the best GA producer, obtaining 23.79 ± 1.19 g L^{-1} of GA (yield 76.68%) in bioreactor fermentation, with a single-step bioprocess. Our findings contribute in laying the ground for EG upcycling strategies with yeasts.

Abstract ID: 201 / Poster Number: 095.15S

Explosive legacies: Revealing wartime TNT pollution in the North Sea through Bacterial Indicators

Presenting author: Ms. Wyona Schütte, Flanders Marine Institute, Ostend, Belgium

Co-authors: *Josefien Van Landuyt, Matthias Brenner, Andrey Vedenin, Maren Gundlach, Tobias Bünning, Jennifer Strehse, Edmund Maser, Nico Boon, Maarten De Rijcke*

Millions of tons of munitions from the First and Second World War are littering the North Sea. They were lost during battles, still on board of ships at the time of their sinking, or dumped after the Wars. One of the most important substances in this context is 2,4,6-trinitrotoluene (TNT). Used commonly as an explosive for military applications, it is likewise known for its toxicity and to be potentially mutagenic and carcinogenic [1,2]. In the marine environment, corrosion ultimately results in the leakage of TNT from underwater munitions, affecting the surrounding sediments and biota, and potentially accumulating in the food chain [3, 4]. By analysing changes in marine microbial communities in the presence of TNT and its metabolites, we aim to identify bacterial taxa that can serve as indicators for pollution of dissolved explosives.

We collected sediments from the munition dumping area Minsener Oog in the German Bight, which is known to contain different munition types (mines, artillery grenades). In a 4-week laboratory experiment, sediments from the dumping area and a reference site were divided for experimental treatments. These consisted of seawater spiked with different concentrations of TNT (0, 10, or 1000µg/l). After mixing with seawater, the sediments were kept statically in the dark at 16°C, aiming to mimic natural scenarios of TNT leaking from corroding underwater munitions. Autoclaved sediments served as a control to measure the adhesion of TNT to sediment particles. Water and sediment samples are analysed for TNT and its metabolites with gas chromatography combined with mass spectrometry (GC-MS). Using full-length 16S rRNA Nanopore gene sequencing, the microbial community structure is being determined.

Initial measurements show a rapid decline of TNT in water from low and high treatments, with the explosive being longer detectable in high treatments (1 day and 7 days, respectively). Metabolites of TNT (2-ADNT and 4-ADNT) increased during the first 24 hours of the experiment. Relative to the parent compound, their concentration decreased after a time delay. Overall, the metabolites were present longer than TNT (up to 18 days). This trend was less pronounced in treatments with autoclaved sediments. Here, all compounds showed a higher concentration relative to non-autoclaved samples

taken at the same time (e.g., after 7 days, 14.7x higher TNT concentration than in dumping area sediments), but were not detectable anymore after 11 days. These findings give a strong indication for the importance of microbial activity for the degradation of TNT. Currently ongoing measurements of the explosive and its metabolites in sediments are expected to confirm this observation. In our previous work, where we linked concentrations of TNT to the microbial fingerprint in sediments collected next to a World War II destroyer, we observed a shift towards several species of Rhodobacteraceae in TNT-rich sediments [5]. Thus, we anticipate sensitive shifts in the bacterial community structures in this study depending on the different experimental treatments.

Our results highlight the influence of explosives leaking from lost munitions on their surrounding water and sediments. Changes in the microbial composition related to TNT and its metabolites will help to identify taxa potentially serving as indicators for dissolved explosives and their degradation potential.

Abstract ID: 3175 / Poster Number: 330.17P

Expression, purification, and characterization of a recombinant cryptococcal cellulase

Presenting author: Mr. Dylan Moodley, University of the Witwatersrand, Johannebsurg, South Africa

Co-authors: *Angela Botes*

Introduction: The yeast *Cryptococcus gattii* is of medical significance due to its drug resistance, pathogenesis, and virulence. However, its ecological persistence in woody environments, a primary niche, remains largely unexplored despite links between environmental populations and human infections. An open reading frame from *C. gattii* (WM276) has been annotated as a putative cellulase. Cellulases catalyze the hydrolysis of 1,4-β-D-glycosidic bonds in cellulose, a major component of wood. Investigating this enzyme provides insight into the pathogen's environmental physiology and potential biotechnological applications.

Objective: To elucidate the structural and functional properties of the putative cellulase CGB_E4160W from *Cryptococcus gattii*.

Method: The CGB_E4160W gene was codon-optimized, synthesized, and cloned into the pMAL-c5x vector for recombinant overexpression in *Escherichia coli*. Recombinant protein expression was induced with 0.2 mM IPTG at 16°C for 24 hours, followed by purification using amylose-affinity chromatography. Far-UV circular dichroism assessed secondary structure, intrinsic tryptophan fluorescence evaluated substrate interactions, and DNS assays determined optimal activity conditions (pH, temperature, and salt concentration).

Findings: The cellulase was successfully expressed as a soluble enzyme and purified to homogeneity. Circular dichroism revealed secondary structural features with minima at 208 and 218 nm. Tryptophan fluorescence exhibited an emission maximum at 340 nm, blue-shifting with 0.5% (w/v) carboxymethyl cellulose, indicating substrate binding. Functional DNS assays established optimal activity at pH 6.0, 50°C, and 500 mM NaCl.

Conclusion: The thermotolerant and halotolerant properties of this cellulase underscore its potential for industrial biomass conversion into biofuels and enhance our understanding of *C. gattii*'s survival on lignocellulosic substrates.

Abstract ID: 3043 / Poster Number: 373.16P

Extending the Shelf Life of Fresh-Cut Pineapple Using Protective Cultures and Modified Atmosphere Packaging

Presenting author: Dr. Natalia Merino Almalé, Instituto Agroalimentario de Aragón-IA2, Universidad de Zaragoza-CITA, Zaragoza, Spain

Co-authors: *Nerea Garin-Murguial, Laura Espina, Rafael Pagán Tomás, Raquel Virto, Esther Arias*

Introduction: The demand for fresh-cut fruits is rising due to consumer preferences for healthy and convenient foods. However, their shelf life is limited by high moisture content, perishability, and susceptibility to microbial contamination and enzymatic browning. Combining treatments like protective cultures and MAP packaging can effectively extend shelf life.

Objective: This study aimed to evaluate the efficacy of cell-free supernatants (CFS) of *Bacillus subtilis* CNTA 517, *Pediococcus acidilactici* CNTA 1059, *Levilactobacillus brevis* CNTA 1374, and *Lactiplantibacillus plantarum* CNTA 600 as protective cultures to extend the shelf-life of fresh-cut pineapple sticks.

Method: Pineapple sticks were treated with 5% of CFS of the selected bacteria and stored under passive modified atmosphere packaging (MAP) (15% O2 + 5% CO2). Physicochemical parameters, microbial growth, and sensory attributes were monitored over 12 days at refrigeration temperature.

Findings: The CFS of *P. acidilactici* and *L. brevis* were the most effective in inhibiting yeast growth, extending the shelf life of pineapple sticks by four days. Among these, the CFS of *P. acidilactici* better preserved the physicochemical properties of the sticks. When combined with optimized MAP packaging, the shelf life was extended to 12 days, achieving a 150% increase compared to the control batch. Sensory analysis revealed that treated samples exhibited better colour, odour, and flavour compared to the controls.

Conclusion: The combination of the CFS of *P. acidilactici* CNTA 1059 with MAP extends the shelf-life of fresh-cut pineapple, while preserving their quality for up to 12 days. This approach aligns with the clean-label concept and offers a viable alternative to traditional preservation methods.

Abstract ID: 1126

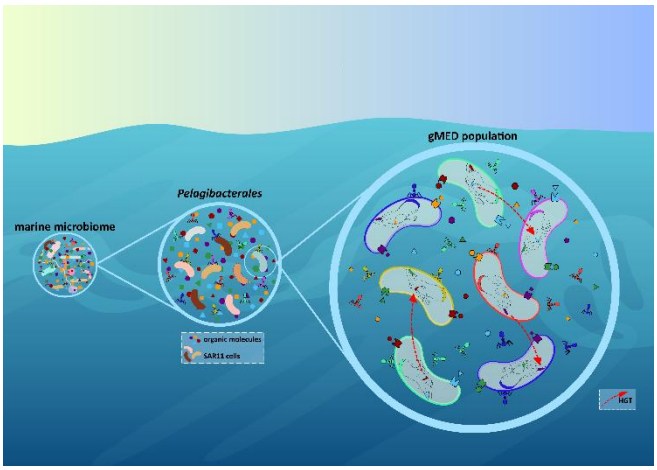
Extensive Paralogism in the Environmental Pangenome: A Key Factor in the Ecological Success of Natural SAR11 Populations

Presenting author: Assoc. Prof. Mario López-Pérez, University Miguel Hernández, Elche, Spain

Background: The oceanic microbiome is dominated by members of the SAR11 clade. Despite their abundance, challenges in recovering the full genetic diversity of natural populations have hindered our understanding of the eco-evolutionary mechanisms driving intra-species variation. In this study, we employed a combination of single-amplified genomes and long-read metagenomics to recover the genomic diversity of natural populations within the SAR11 genomospecies Ia.3/VII, the dominant group in the Mediterranean Sea.

Results: The reconstruction of the first complete genome within this genomospecies has revealed that the core genome represents a significant proportion of the genome (~81%), with highly divergent areas that allow for greater strain-dependent metabolic flexibility. The flexible genome was concentrated in small regions containing mostly a single gene and located in equivalent regions within the genomospecies. Each variable region was associated with a specific set of genes that, despite exhibiting some divergence, maintained equivalent biological functionality within the population. The environmental pangenome is large and enriched in genes involved in nutrient transport and cell wall synthesis and modification, showing an extremely high degree of functional redundancy in the flexible genome (i.e. paralogisms).

Conclusions: These results suggest a new model of eco-evolutionary dynamics (Figure 1), in which the SAR11 population acts cooperatively, using its extensive gene pool as a public good to exploit a wide range of substrates and adapt rapidly to fluctuations in nutrient availability. Moreover, this study demonstrates the power of long-read metagenomics for capturing the full genetic diversity of environmental SAR11 populations, overcoming the limitations of second-generation sequencing technologies in genome assembly.



SAR11 population evolution model. The population acts as a large reservoir of all genetic diversity, creating numerous subpopulations that coexist and exchange public goods (flexilome), maintaining this genetic richness within the natural population. This mechanism not only optimises the use of available resources, but also promotes a form of evolutionary cooperation that could be analogous to the Black Queen hypothesis, in which populations retain essential functions at the community level, allowing individuals to specialise or even lose certain individual capabilities for the benefit of collective efficiency. The high prevalence of genes involved in cell wall modification represents an evolutionary mechanism related to phage recognition evasion. This mechanism promotes polyclonality and maintains a constant balance of genetic diversity within the population.

Abstract ID: 3384 / Poster Number: 147.15P

Extracellular Vesicles isolated from Streptomyces violaceoruber: an interesting natural tool for plant growth and health

Presenting author: Flavia Cannizzaro, PhD, STEBICEF, Palermo, Italy

Co-authors: *Teresa Faddetta, Claudia Bonafede, Gugliemo Puccio, Ciro Caldiero, Giorgio Nasillo, Loredana Abbate, Francesco Mercati, Giuseppe Gallo*

Climate change and growing food demand necessitate eco-sustainable solutions to optimize resources use and reduce chemical inputs, aligning with the *Farm to Fork* strategy. Biofertilizers, based on Plant Growth Promoting Bacteria (PGPB), offer a promising approach. Among these, *Streptomyces* species show significant potential. One key mechanism may involve extracellular vesicles (EVs), nanosized structures carrying proteins, nucleic acids, and bioactive compounds.

This research focuses on the isolation and characterization of EVs from *Streptomyces violaceoruber*, a PGPB that is a prolific source of bioactive compounds, evaluating their plant-growth promotion and stress-mitigation capabilities on *Solanum lycopersicum* tomato plants.

EVs were isolated using sequential filtration, centrifugation, and ultracentrifugation from *S. violaceoruber* six-days cultures performed in liquid minimal medium. Morphological properties were analysed using transmission electron microscopy and dynamic light scattering. Molecular profiling has involved omics-based approaches (i.e. next-generation sequencing, mass spectrometry analyses), while functional assays evaluated antimicrobial activity and plant growth promotion (PGP).

EVs, ranging from 100–200 nm in size, contain the whole *S. violaceoruber* genomic DNA. Additionally, they contain proteins as the metabolic cargo has to be characterized. Interestingly, EVs exhibited antibacterial properties and showed PGP effects on tomato seedlings. Experiments are underway to evaluate their capability to mitigate plant abiotic and biotic stresses.

Therefore, this research will be able to provide further evidences on *Streptomyces* EVs in trans-kingdom communication (microbe-microbe and plant-microbe interactions) and to pose the basis for the development of new EV-based biofertilizers.

Abstract ID: 4030 / Poster Number: 331.16F

Extremophile Bacillus exopolysaccharides enhance soil water retention and plant drought resilience

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Co-authors: *Carla Belén Jorquera Pulgar, Johanna Alejandra Cortés Guzman, Mariam Charifeh Fernández, Cristóbal Gajardo Alcayaga, Martha Hengst López, Carolina Yáñez Prieto*

Climate change aggravates water scarcity, threatening global food security and demanding eco-friendly strategies to optimize water use in agriculture. This study explores the potential of hygroscopic exopolysaccharides (EPS) produced by thermophilic *Bacillus* spp. strains isolated from the Lirima hydrothermal system (Chile) to mitigate drought stress in plants and soils. *Bacillus* LB7 and NP42 were characterized for EPS production under varying conditions of temperature (25–58°C), pH (4–12), NaCl (0.6–3 M), and glucose (0.005–0.2 M). Optimal conditions yielded up to 404 mg L^{-1} (LB7) and 187 mg L^{-1} (NP42) of EPS. Genomic annotation and metabolic reconstruction revealed key EPS biosynthesis pathways, including glucose phosphorylation, NDP-sugar formation, polymerization, and transport. Proteomic analysis (LC-MS/MS) identified 613 and 615 proteins in LB7 and NP42, respectively, highlighting variations in NDP-sugar metabolism that may influence EPS composition. Bacterial inoculation (10^9 CFU/mL) increased soil water-holding capacity by 41% (LB7) and 24% (NP42) in sandy soils, with EPS production reaching 115 and 231 mg g^{-1}, respectively. In plants trials, lettuce seedlings under drought stress exhibited increased catalase activity (9.56 and 7.14 U mg $protein^{-1}$) and superoxide dismutase levels, reducing oxidative stress and improving plant health. Greenhouse tomato trials revealed enhanced fruit yield and maturation in inoculated plants under drought. These findings suggest that EPS-producing *Bacillus* spp. from extreme environments offer a sustainable strategy to enhance soil moisture retention and plant drought resilience, promoting water-efficient agriculture.

Abstract ID: 1801 / Poster Number: 213.17P

Fabrication of a lipopolysaccharide-targeting peptide-based colloid for alleviating carbapenem-resistant Enterobacteriaceae-induced cutaneous infection

Presenting author: Ms. Honglan Wang, The Chinese University of Hong Kong, Hong Kong, Hong Kong

Co-authors: *Shui Yee Leung, Ping Zeng,*

Introduction: Dissemination of carbapenem-resistant Enterobacteriaceae usually lead to high mortality rate since rational treatment options are limited. In May 2024, carbapenem-resistant Enterobacteriaceae was listed as a critical priority level pathogen by the World Health Organization, highlighting its threats to human health. The carbapenem antibiotics family, represented by meropenem and imipenem, is gradually losing grounds in the protracted warfare against “superbugs”. To address this issue, discovering novel antibacterial agents with different mode of action is urgently required.

Objectives: A panel of clinical Enterobacteriaceae strains were isolated and further identified as carbapenem-resistant superbugs, harbouring several crucial β-lactamase determinants. In this work, exploiting the idea of tandem-repeat sequence design, two novel hexadecapeptides, namely IW and WI, composed of isoleucine, tryptophan, and arginine residues were engineered to kill these notorious pathogens.

Methods: Peptides were obtained via solid phase peptide synthesis. Their properties were then predicted by several bioinformatic tools. After confirming *in vitro* activity, the antibacterial mechanism was investigated by multiple fluorescent staining methods. Adopting the analytic strategies of molecular dynamics simulation in combination with experimental verification, a potential target of the peptide was proposed. For better administration, the lead peptide, IW, was formulated with a commercial pharmaceutical excipient poloxamer 407 to fabricate an antibacterial colloid. Its *in vivo* efficacy was verified using mouse skin infection model.

Findings: This study conceptually demonstrated the potential of a synthetic peptide denoted as IW to combat carbapenem-resistant Enterobacteriaceae strains. It possessed a typical helical structure and was able to inhibit the growth of multiple clinical-isolated “superbugs” at the micromole level. Molecular dynamics simulation suggested that IW could bind to lipopolysaccharides located outside the cell membrane of Gram-negative bacteria and form a stable complex. Subsequently, IW led to membrane potential dissipation and triggered reactive oxygen species accumulation

intercellularly. IW was then formulated with P407 to form IW-P407 colloids and this simple formulation was proved to alleviate cutaneous infection in a mouse skin model.

Conclusion: IW could be regarded as a potential candidate against increasingly rampant carbapenem-resistant Enterobacteriaceae bacteria. In summary, this work expanded the potential arsenal against carbapenem-resistant strains by introducing a new engineered peptide molecule delivered using a cheap, concise formulation strategy.

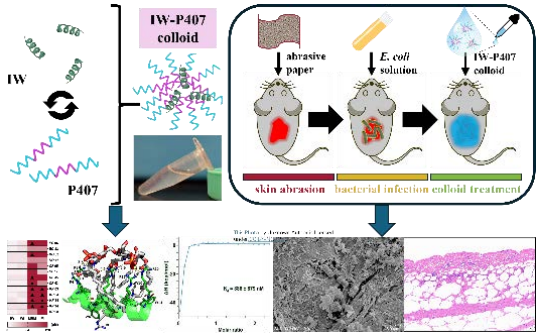


Figure 1: Lipopolysaccharide-targeting IW formulated colloid alleviate skin bacterial infection.

Abstract ID: 2886 / Poster Number: 261.15P

Facility-Specific Distribution and Cytotoxic Effects of Airborne Fungi in Indoor Environments

Presenting author: Prof. Wonsuck Yoon, Korea university, Seoul, South Korea

Co-authors: *Yongsung Park, Chanhee Chung, Iel-Soo Bang*

Background: Airborne fungi are ubiquitous in indoor environments and pose significant risks to public health, particularly through respiratory and dermatological conditions. The identification of fungal species across different facility types and their cytotoxic effects is critical for developing targeted strategies to improve indoor air quality globally.

Objectives: This study investigates the distribution and characteristics of airborne fungi in multi-use facilities, focusing on facility types and environmental factors that influence fungal prevalence and their cytotoxic effects on human skin cells.

Methods: Airborne fungi were collected from 563 indoor facilities categorized into public transport hubs, residential buildings, underground parking lots, retail stores, general restaurants, and public restrooms. Species were identified through ITS region sequencing, and cytotoxicity was assessed by testing 20 dominant fungal species on HaCaT human keratinocyte cells.

Results: Facility-specific fungal distribution revealed that Cladosporiumsp. was predominant in public transport hubs, Penicilliumspp. in residential and retail spaces, Bjerkanderasp. in underground parking lots and public restrooms, and Fusariumsp. in general restaurants. Environmental factors such as ventilation, humidity, and human activity were identified as key contributors to fungal presence. Cytotoxicity tests demonstrated that most identified fungi inhibited HaCaT cell proliferation, with 90% showing significant cytotoxic effects. Notably, Rhodotorulasp. and Moesziomycessp. were non-toxic to skin cells.

Conclusions: The prevalence and toxicity of airborne fungi vary significantly by facility type and indoor environmental conditions, highlighting the importance of tailored indoor air quality management strategies. These findings underscore the global relevance of identifying high-risk facilities and developing targeted interventions to mitigate health risks associated with airborne fungi.

Abstract ID: 1324 / Poster Number: 112.16P

Factors impacting hospital-associated microbial communities composition and stability

Presenting author: Iván Linares Ambohades, PhD, Ramón y Cajal Institute for Health Research (IRYCIS), Madrid, Spain

Co-authors: *Natalia Guerra Pinto, Francisco Amaro Torres, Silvia Serrano, Cruz Soriano, Raul De Pablo, Ana Elena Pérez-Cobas, Teresa M. Coque*

Introduction: Microbial communities inhabit the hospital-built environment and could be reservoirs of pathogens that impact nosocomial infections. Those communities remain latent as resistant forms or surface biofilms. Moreover, the factors that determine their composition and diversity remain unknown.

Objectives: To evaluate the factors influencing microbial diversity, composition, and interactions in hospital sink communities across spatial and temporal scales.

Methods: Sinks from 14 rooms belonging to 3 distinct areas (A, B, and C) of a hospital ward were sampled (n=93). The samples were collected from the sink p-traps every three months (2021-2023). The ward was previously an ICU, then remained empty for around two months ("empty"), and since then, it has been used for blood extraction and other functions ("multifunction"). The sink microbiome characterization was done by Illumina sequencing of the 16S/18S rRNA genes and ITS. The bioinformatics analysis was based on Kraken2 and R. The alfa/beta diversity metrics and the composition of the different areas between the two periods were compared. A co-occurrence network was performed to identify putative microbial interactions that explain the microbial community's stability.

Results: The most prevalent genera across the different kingdoms were *Magnetospirillum* among bacteria, *Philodinavus* among protozoa, and *Exophiala* among fungi. Significant differences in bacterial diversity, richness, and composition were observed between the farthest areas (A-C and A-B) (**Figure 1A-B**), highlighting the influence of spatial distribution and associated activities (e.g., hospital staff sink usage). Diversity and composition also shifted between the "empty" and "multifunction" periods, with higher alpha diversity observed during the "empty" period, showing the importance of changes in medical activities in hospital service (**Figure 1C-D**). Network analyses revealed that the most abundant genera also served as highly connected nodes, suggesting their potential role in maintaining community stability.

Conclusions: The spatial location and the nature of hospital activities, including changes in ward services over time, are critical factors shaping the diversity, composition, and persistence of hospital-associated microbial communities. Factors shaping hospital-built environment-associated microbial communities should be considered in preventive strategies to control hospital nosocomial outbreaks.

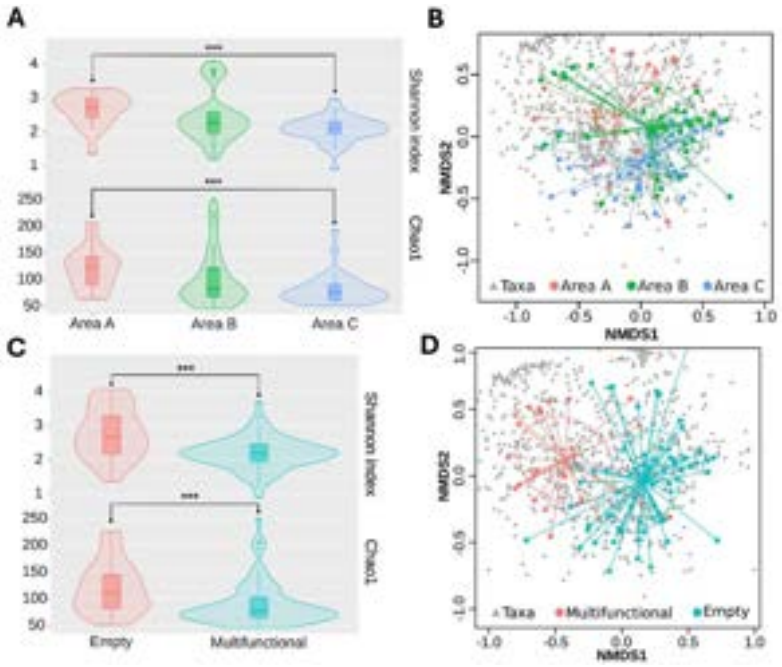


Figure 1:

A) Bacterial α-diversity between areas represented by Shannon index and Chao-1. B) Bacterial β-diversity between areas represented by Non-Metric Multidimensional Scaling (NMDS). C) Bacterial α-diversity between periods represented by Shannon index and Chao-1. D) Bacterial β-diversity between periods represented by Non-Metric Multidimensional Scaling (NMDS).

Abstract ID: 3948

Faecal microbiota transplantation is more effective than fecal filtrate transplantation to improve gut health in weaned piglets

Presenting author: Carla Tort-Miró, PhD, Institute of Agrifood Research and Technology (IRTA), Barcelona, Spain; Centre De Recerca En Sanitat Animal (CReSA), Barcelona, Spain

Co-authors: *Àlex Cobos, Jordi Argilaguet, Luca Guardabassi, Mattia Pirolo, Noemí Giler-Baquerizo, Judith Guitart-Matas, Laura Montoro-Dasí, Andrea Aria, Climent Casals, Clara Marin, Lourdes Migura-Garcia*

Weaning is considered a critical period in swine conventional farming. During weaning, there is an increasing risk of gut dysbiosis, which can promote colonisation by enterotoxigenic *Escherichia coli* (ETEC) causing post-weaning diarrhoea. Approaches such the use of antibiotics, contribute to the development of antimicrobial resistance. To address this issue, faecal microbiota transplantation (FMT) has been considered as a modulator of piglet's microbiome. Alternatively, faecal filtrate transplantation (FFT), containing mainly viral particles, has also shown promising results. This study is aiming to determine the effect of encapsulated FMT and FFT on the gut bacterial community, immune response, and gut barrier function.

Faecal content was collected from a negative donor farm regarding the major swine pathogens and was processed to obtained two different transplant materials, FMT and FFT, which were lyophilized and encapsulated. A total of 36 28-day-old weaned piglets were transported to the experimental farm, afterwards, they were divided in three treatment groups, control, FMT and FFT. Treatments were administered at arrival to the farm and four days post-arrival. During a three weeks trial, faecal samples were collected at several timepoints and microbiota analysis was carried out with MinION device. Finally, intestinal samples were taken for morphometric and immunology studies.

Animals from the FMT group exhibited a significantly greater villus height:crypt depth ratio in the jejunum, ileum and duodenum organs, together with higher concentration of immunoglobulin A in the ileum content. Additionally, both FMT and FTT treatments showed differences in microbial dynamics measure as beta-diversity, being statistically significant after 7- and 21-days post-treatment. Interestingly, abundance of those genera associated with a probiotic role, such as *Roseburia* or *Faecalibacterium,* tended to increase earlier in time only for the FMT group while potential pathogenic genera such as

Escherichia-Shigella decreased. Finally, genera associated with intestinal inflammation such as *Clostridium sensu stricto* 1 were found in significantly higher abundance only in the control group.

The results show that both treatments are safe and modulate the intestinal microbiome composition. However, only FMT contribute to increase IgA, enhancing barrier function and mucosal immunity, thus indicating a better gastrointestinal health. Moreover, FMT is associated with a greater proportion of functional compartment versus the proliferative ones throughout the small intestine, indicating a better gut performance.

Abstract ID: 1591 / Poster Number: 297.17P

FalconSyn: a hydroponic growth system for high-throughput phenotyping of Arabidopsis thaliana with synthetic microbial communities

Presenting author: Dr. Xinming Xu, Leiden University, Leiden, Netherlands

Co-authors: *María Negre Rodrígue, Ákos T. Kovács*

Soil and plants harbor an extensive, taxonomically diverse reservoir of microbes that are essential for plant development, fitness, and overall health. The vast diversity and complex interactions within plant-associated microbes pose challenges in deciphering the beneficial microbiota functions. Synthetic microbial communities (SynComs), that are designed to represent naturally occurring plant microbiomes, provide a powerful tool to reduce the complexity and establish causalities in plant-microbe interactions. Emerging research on SynComs has showcased its potential to translate mechanistic and ecological knowledge of plant-microbe interactions into sustainable crop management strategies. However, to effectively optimize SynComs in terms of size, composition, plant specificity, and multifunctionality, a high-throughput SynCom phenotyping system is essential. Such a phenotyping system should generate tractable data, including colonization efficiency, root morphology, plant biomass, stress resilience effects, and changes in root exudates, following the inoculation of diverse SynComs.

Here, we introduce FalconSyn, a novel hydroponic growth system for cultivating *Arabidopsis thaliana* with SynComs. FalconSyn employs standard 15ml Falcon tubes as growth vessels, ensuring cost-efficiency and adaptability. Using FalconSyn, we successfully cultivated *Arabidopsis* under sterile hydroponic conditions and demonstrated its versatility in applying various treatments, including stress assays, nutrient variation, and SynCom inoculation. Overall, this innovative platform provides a scalable and tractable solution for optimizing SynComs, with potential to advance research on plant-microbe interactions.

Abstract ID: 1079

Fecal microbiome in Anorexia Nervosa: shotgun genomics sequencing reveals major differences with controls and longitudinal effects of inpatient treatment

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Introduction: Anorexia Nervosa (AN) is a severe psychiatric disorder characterized by extreme dietary restriction, profound weight loss, psychological distress, and multisystemic dysfunction. Primarily affecting adolescent and young adult females, AN has one of the highest mortality rates among psychiatric disorders. Emerging evidence suggests gut dysbiosis plays a role in the pathophysiology of AN, yet shotgun sequencing has been underutilized, particularly in longitudinal studies.

Objectives: This study aimed to: i) Characterize the compositional and functional differences in the gut microbiome between individuals with Anorexia Nervosa (AN) and age-matched healthy controls; ii) Investigate the longitudinal dynamics of the gut microbiome during and after treatment for AN. Iii) Identify microbial taxa (in a broader sense, including bacteria, archaea, viruses and fungi), gene families, and metabolic pathways associated with clinical outcomes, such as BMI and treatment duration, to uncover potential biomarkers and therapeutic targets.

Methods: We conducted a comprehensive investigation of the fecal microbiome in 151 patients with AN at treatment admission and 74 age-matched controls from three European sites using shotgun metagenomics. Additionally, we longitudinally analyzed the microbiome of 53 adolescent patients at three time points: admission, discharge from inpatient treatment, and one year post-admission. Associations between microbiome composition (including bacterial, archaeal, fungal and viral members of the community), functional potential, metabolic fluxes and clinical characteristics were examined.

Findings: At admission, both geographic origin and health status significantly influenced the microbiome composition and functional profiles. These changes correlated with key clinical parameters. Longitudinal analyses revealed that while weight gain partially

restores microbiome composition, notable differences persist one year post-admission. Several microbial taxa and gene families were associated with treatment duration and BMI one year after admission, suggesting potential biomarkers or therapeutic targets.

Conclusions: Our study underscores profound compositional and functional alterations in the gut microbiome of individuals with AN, with potential implications for its etiology and treatment. The identification of specific taxa and functions linked to recovery highlights opportunities for probiotic-based interventions to complement traditional treatments and support long-term recovery.

Abstract ID: 3357 / Poster Number: 219.16P

Fenticonazole: evaluation of an alternative strategy to treat Candida spp. infections

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Co-authors: *Damiano Squitieri, Silvia Rizzo, Debora Talamonti, Benedetta Falasca, Maura Di Vito, Francesca Bugli, Brunella Posteraro, Maurizio Sanguinetti, Riccardo Torelli*

Introduction and objective: Approximately 7% of Candida strains are resistant to Fluconazole (FLZ), a widely used antifungal for treating Candida infections. Fenticonazole nitrate (FTZ), an imidazole derivative approved for topical candidiasis, was evaluated in this study for its in vitro effects on both planktonic and biofilm forms of Candida spp.

Method: The study included isolates collected from blood cultures: FLZ-resistant *C. auris* (n=10), FLZ-susceptible or dose-dependent (n=10), and FLZ-resistant *C. albicans* and *C. glabrata* isolates (n=10). Minimal inhibitory concentrations (MICs) for FTZ were determined using CLSI methods. MIC ranges, MIC50, and MIC90 values were calculated for each group. Growth and time-kill curves at various FTZ concentrations (8×MIC, 4×MIC, 2×MIC, and MIC) demonstrated a fungistatic effect, inhibiting fungal growth without complete eradication. Biofilm analysis via Crystal Violet, XTT, and fluorescence staining assays showed mild inhibition of biofilm formation by FTZ.

Findings: FTZ MICs ranged from 1 to 8 µg/mL across all isolates, regardless of their FLZ resistance status. Additionally, qPCR analysis revealed that FTZ downregulated secreted aspartyl protease (SAP) gene expression in a dose-dependent manner in treated *C. glabrata* strains. Preliminary data suggest that FTZ remains effective against FLZ-resistant strains, demonstrating a secondary mechanism of action through SAP gene inhibition.

Conclusion: The study highlights FTZ's potential as an alternative for treating FLZ-resistant Candida bloodstream infections. Ongoing research includes systemic mouse model evaluations and SAP expression analysis in other Candida species to further explore FTZ's effectiveness and mechanisms of action.

Abstract ID: 1588 / Poster Number: 296.17P

Fermentation of barbecue waste sauce: study of a new industrial recycling approach for target product reincorporation

Presenting author: Mrs. Ana Catarina Costa, LEAF – Landscape, Environment, Agriculture and Food Research Center, Lisbon, Portugal

Co-authors: *Joana Braga, Maria Ramos, Ana Tasso, Anabela Raymundo, Catarina Prista*

Industrial barbecue sauce waste are rich in carbon and nitrogen sources and are potential candidates for valorisation through fermentation. This study aims to ferment the barbecue sauce waste stream (BBQ), contributing to circularity through its incorporation into the target product. Samples diluted 1:2 (50%) and 1:4 (25%) with demineralised water were inoculated with a consortium of lactic acid bacteria and yeast (*Lactiplantibacillus plantarum*, *Lactobacillus rhamnosus*, *Weisella confusa*, and *Saccharomyces boulardii*), and let to ferment at room temperature for 12 days. Fermentation performance was monitored by measuring pH, TSS and titratable acidity, sugar consumption, and metabolites produced (analysed by HPLC) until stable and safe values were reached. The product pH dropped, reaching 3.34 on day 12 for BBQ50 and BBQ25. Regarding TSS values, the observed decrease was coherent with the significative consumption of glucose/fructose, from 20.9 to 12.7 ºBrix in BBQ50 and from 10.2 to 5.67 ºBrix in BBQ25. Accordingly, to the fermentative metabolism of the consortium, titratable acidity values (1.795/100g for BBQ50 and 1.341/100g fresh biomass for BBQ25) and organic acid concentration increased throughout the fermentation, reaching values of lactic concentration of 1.08g/100g and 2.17g/100g fresh biomass, respectively. The final products were evaluated for shelf-life and preservation activity against major food industry contaminants. Both fermented BBQ kept their characteristics after 6 months of preservation at room temperature. The antimicrobial tests demonstrated a reduction of most contaminants (yeasts) and a complete inhibition of *Bacillus cereus*, revealing the potential to substitute food preservatives in the target sauces.

Abstract ID: 3545 / Poster Number: 393.17P

Fermentation of fruit preparations by LAB: an approach for clean-label yoghurt preservation and nutritional improvement

Presenting author: Prof. Catarina Prista, LEAF, ISA-ULisboa, Lisbon, Portugal

Co-authors: *Cristiana Santos, Rita Barracosa, Daniela Correia, Luís Paulico, Cristina Rodrigues, Anabela Raymundo, Juliana Botelho Moreira*

Synthetic preservatives like sorbate are widely used to ensure food safety. However, the growing demand for healthier, natural and sustainable foods led the food industry to develop clean-label products using natural preservatives. Lactic acid bacteria (LAB) fermentation is a promising strategy since LAB produce weak acids, bacteriocins, and other metabolites with antimicrobial activity while enhancing these food products' nutritional value and bioactivity.

This study assessed the impact of LAB fermentation on the antimicrobial and antioxidant properties of strawberry and red fruit preparations for yoghurt incorporation. Fruit preparations were fermented for four days with two consortia: one typical from Bifidobacterium (BIF) and the other from Kefir (KL) fermented milk. Fermentation was monitored daily for pH, Total Soluble Solids, and titrable acidity (TA). The antioxidant activity was analysed using ABTS, DPPH, and FRAP methodologies. The preservative potential against spoilage yeast and bacteria was studied by drop tests.

During fermentation, pH decreased, and TA increased, reflecting sugar conversion into lactic and acetic acids. Antioxidant activity significantly improved in fermented strawberry preparations by BIF and KL consortia (28% and 13% for ABTS and 32% and 33% for FRAP, respectively). No significant changes were displayed for fermented red fruits.

Candida intermedia was entirely inhibited by KL red fruit preparation, and no growth of *Escherichia coli* and *Lactiplantibacillus plantarum* was observed in any fermented preparations.

LAB fermentation can be used as a clean-label approach to replace chemical additives used to preserve food products and add additional nutritional/health advantages to these products.

Abstract ID: 2176 / Poster Number: 247.15P

Feverish interactions between fungi, bacteria and rocks in the dark of a volcanic cellar

Presenting author: Lorenzo Pin, PhD, CNR, Istituto per i Sistemi Biologici, Montelibretti, Italy

Co-authors: *Ilaria Mazzini, Laura Chelazzi, Francesca Trapasso, Emanuela Tempesta, Luca Bindi, Sandro Conticelli, Flavia Pinzari*

Volcanic hypogean environments can host peculiar fungal communities that develop separately from the open-air ones. Factors like substrate chemistry, moisture, temperature, and light can help select species and shape communities. A model environment was chosen to study fungal diversity and geomicrobiological activity associated with tuff rocks. It was intended to analyse by culturing and culture-independent methods which fungal species were dominant and by scanning electron microscopy (SEM), microanalysis (EDS) and X-ray diffraction (XRD) their relationship with the volcanic substrate. The study site was a hypogean cellar near Pitigliano (Central Italy), carved in red tuffs with black scoria of the Sovana ignimbrite, rich in metals and with stable microenvironmental conditions. The cellar is used to produce wine by Sassotondo organic farm. Fungal efflorescences are constantly present on the cellar's walls and ceiling. Shotgun metagenomic analysis of the fungal and bacterial communities documented the presence of some dominant fungal rhizomorphs formed by species of the genera *Piloderma, Fibularhizoctonia* and *Athelia*. Several bacterial species appeared strongly associated with fungal mycelia, particularly from the genus *Sphingomonas*. SEM revealed abundant biogenic crystals embedded in the fungal biofilm covering the tuff surface, which, according to EDS and XRD observations, consisted of calcium oxalates and sulphates. Despite the presumed environmental uniformity of the hypogeum, a fair amount of variability at the microscale level was observed, with different fungal species dominating slightly distant spots on the walls. Intense interactions between fungi and bacteria emerged, as did mycelia's ability to leach and translocate mineral components from the substrate.

Abstract ID: 1314 / Poster Number: 211.17P

Fighting Staphylococcus aureus infection: a virtual screen-based approach to identify FtsZ inhibitors

Presenting author: Dr. Viola Camilla Scoffone, University of Pavia, Pavia, Italy

Co-authors: *Gabriele Trespidi, Sara Costa, Giulia Barbieri, Arooba Arshad, Antonio Coluccia, Silvia Buroni*

Introduction: Multi-drug resistant (MDR) *Staphylococcus aureus* are well-known pathogens in nosocomial, community-associated, and cystic fibrosis (CF) infections, characterized by high levels of morbidity and mortality. In particular, *S. aureus* and methicillin-resistant *S. aureus* (MRSA) strains can persist and adapt to hostile airway niches. In the search for new compounds able to tackle MDR strains, FtsZ inhibitors represent a valuable option.

Objectives: To identify new drugs active against MDR *S. aureus*, the essential divisome machinery protein FtsZ was targeted by Computer Aided Drug Desing (CADD) methods.

Methods: A structure-based virtual screening was applied starting from the *S. aureus* FtsZ crystal structure. Libraries of natural and non-natural compounds were docked to the co-factor binding sites, and the identified molecules were tested for their inhibitory effect on FtsZ GTPase and polymerization activity, using a coupled assay and sedimentation and 90° light scattering assays, respectively. The antibacterial activity was tested in Muller-Hinton II (MH-II) and Synthetic CF sputum medium (SCFM) using the microdilution method and timekilling assays. Cryo-electron microscopy explored the most active compound's mechanism of action. Time-lapse fluorescent microscopy was performed to localize FtsZ during the treatments, and the inhibition and eradication activity on biofilm was determined. Checkerboard assays were used to evaluate the interaction between the investigated compound and commonly used antibiotics. The cytotoxicity and in vivo activity were evaluated using tubulin polymerization and MTT assays and a *Galleria mellonella* infection model, respectively.

Findings: A list of 24 molecules was obtained from the virtual screening, and three of them were found to block FtsZ GTPase activity without affecting its polymerization. However, compound C11 alters FtsZ polymerization in vitro. C11 is bacteriostatic with a minimum inhibitory concentration (MIC) of 2 µg/ml against *S. aureus* and CF MRSA clinical isolates. Biochemical assays and cryo-electron microscopy indicated that compound C11 targets FtsZ by altering its polymerization and bundling processes.

Single-cell time-lapse fluorescent microscopy performed in the presence of C11 showed FtsZ forming different foci and larger cells (Figure 1). The combination of C11 with methicillin disclosed a synergistic effect restoring methicillin susceptibility to MRSA strains. Confocal laser scanning microscopy showed that C11 can inhibit and eradicate *S. aureus* biofilms. The safety of C11 was also assessed by testing tubulin polymerization and human cell survival rate. Moreover, C11 was shown to increase larval survival in the *Galleria mellonella* infection model, compared to the untreated control.

Conclusions: Taken together, the reported results indicate that C11 is a promising drug candidate with great potential to be further structurally optimized to improve its efficacy.

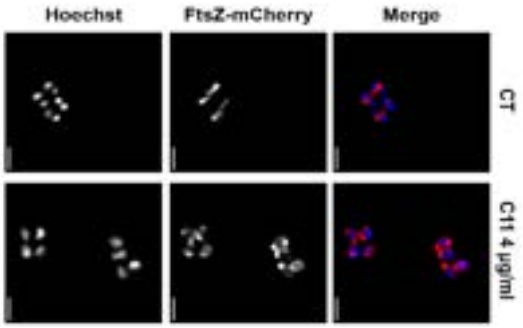


Figure 1: *Fluorescence microscopy images of S. aureus TD276 carrying the FtsZ-mCherry.*

Abstract ID: 1206 / Poster Number: 320.16F

Finding Good Rootmates: Can Resistant Neighbors Protect Susceptible Pines?

Presenting author: Frederico Leitão, PhD, University of Coimbra, Coimbra, Portugal; University of Aveiro, Aveiro, Portugal

Co-authors: *Glória Pinto, Isabel Henriques*

Pine pitch canker (PPC), caused by *Fusarium circinatum*, affects species within the *Pinus* genus, with varying degrees of susceptibility. *Pinus pinea* exhibits resistance to PPC. *Pinus radiata* is highly susceptible. Previous studies indicate that the rhizobiome of *Pinus pinea* contains more defense-associated bacteria than *P. radiata*, potentially contributing to its resistance. Our goal is to investigate whether co-planting *P. pinea* and *P. radiata* can modulate the rhizobiome of *P. radiata*, enhancing PPC tolerance.

Five-month-old seedlings of *P. pinea* and *P. radiata* were planted in 40cm diameter pots as follows: 1 *P. radiata* surrounded by 4 *P. pinea* (25 pots) and 1 *P. radiata* surrounded by 4 *P. radiata* (25 pots). After one-year, central *P. radiata* was inoculated with *F. circinatum* (15 pots), maintaining a non-inoculated Control group (10 pots). The experiment ended when 50% of infected *P. radiata* seedlings (single-species setup) displayed photosynthetic rates less than half those in control group. At sampling, gas-exchange parameters were measured. Needles, exudates and rhizospheric soil were flash-frozen for further processing.

Inoculated *P. radiata* seedlings from mixed-species pots displayed significantly lower needle electrolyte leakage, malondialdehyde levels and higher photosynthetic rate and sugar content compared to *P. radiata* in single-species pots. Additionally, *P. radiata's* rhizobiome (mixed-species pots) showed increased ASV abundance, including bacteria from plant-growth-promoting families such as *Rhizobiaceae*, *Chitinophagaceae* and *Xanthomonadaceae*. These changes correlated with significant delay in PPC symptom development.

Our findings show that co-planting differently resistant *Pinus* species can enhance *P. radiata* resilience to PPC, highlighting a promising strategy to mitigate it.

Abstract ID: 1838

Fire severity impacts DNA and RNA viral communities in fire-adapted shrubland soil wildfire pyrocosms

Presenting author: Dr. Luke Hillary, University of California, Davis, Davis, United States

Co-authors: *Maria Ordonez, Ken Czapla, Kaela Amundson, Mike Wilkins, Peter Homyak, Sydney Glassman, Joanne Emerson*

Extreme wildfires are predicted to increase by 30% globally by 2050, posing a growing threat to both fragile and fire-adapted ecosystems, food security, and human health. While pyrophilous or “fire-loving” bacteria and fungi are known to facilitate post-fire soil microbial community recovery, the response of soil viral communities remains poorly understood. Viruses, especially bacteriophages, can modulate their hosts through cell lysis and altered metabolism, with consequences to the biogeochemical cycling of elements like carbon and nitrogen. Therefore, elucidating viral community dynamics in fire-impacted soils is essential for predicting ecosystem recovery under future wildfire scenarios. We aimed to characterize both DNA and RNA viral community responses to fire under controlled conditions within fire-adapted shrubland (chaparral) soil, which is globally abundant in Mediterranean habitats. We hypothesized that (i) higher burn severities would lead to greater disruption of viral community structure and delayed recovery, and (ii) RNA viral communities would show different recovery patterns to their DNA viral counterparts. We constructed wildfire-simulating mesocosms (pyrocosms) with southern California chaparral soil under two burn severities: high (peak surface temperature 554 ± 52.2 °C) and low (273 ± 58.0 °C). Soil samples were collected from two depths (0–5 cm and 5–10 cm) at seven time points ranging from 1 to 17 weeks post-fire. We adapted our viromics protocols to capture DNA and RNA viruses from the same sample, achieving an eightfold increase in RNA viral read recovery compared to standard approaches.

Our results show that, while DNA viral community richness in high- and low-severity burns returned to levels comparable to control pyrocosm after 5–7 weeks, community structure remained altered throughout. Predicted Bacillota-infecting bacteriophages increased in relative abundance during the first 10 weeks, especially at higher burn severities. In contrast, Nitrospirota-infecting bacteriophages showed a negative response to more severe burns. RNA virome yields revealed a slower recovery of RNA viral communities, likely due to a slower rebound of eukaryotic hosts. Total-soil metatranscriptomes from weeks 7-17 showed a weak effect of fire severity, with

Duplornaviricota (dsRNA viruses) enriched in high-severity pyrocosms. Our findings indicate that fire severity has a persistent effect on soil viral community structure and may disrupt microbial nutrient cycling. Improved understanding of virus–host dynamics following fire can help to (i) refine ecological models predicting post-disturbance effects on biogeochemical cycles that affect regional air quality and Earth's climate, and (ii) identify habitats at risk of delayed recovery due to disruption to the complex cross-kingdom interactions among viruses and their hosts within soil ecosystems.

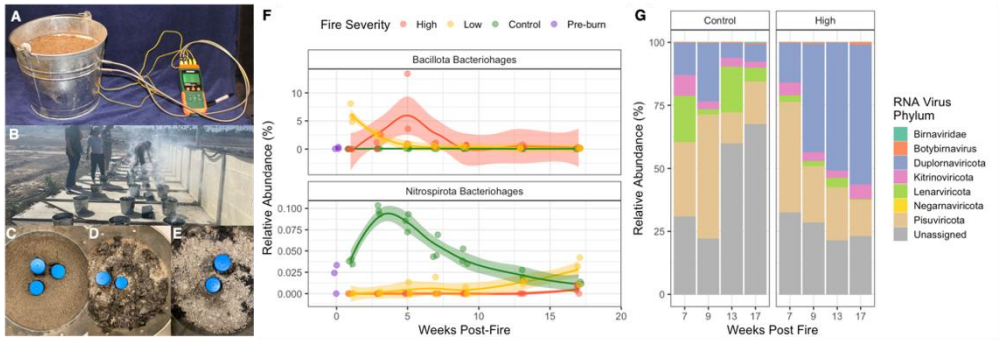


Figure 1: (A) Pyrocosm setup (adapted from Bruns et al. 2020, PLoS One), (B) simulated wildfire and (C-E) pyrocosm sampling. (F) Temporal changes in relative abundances of bacteriophages predicted to infect Bacillota (potential spore formers/ denitrifiers) and Nitrospirota (potential nitrifiers). (G) Changes in relative abundance of RNA viral phyla 7-17 weeks post-fire.

Abstract ID: 4103 / Poster Number: 307.15P

First insights into bacterial and endosymbiont microbiome communities of various coral morphotypes from Maldives

Presenting author: Mrs. Alessia Avesani, University of Genoa, Genova, Italy

Co-authors: *Manon Auguste, Lapo Doni, Caterina Oliveri, Annalisa Azzola, Emanuele Bosi, Monica Montefalcone, Luigi Vezzulli*

The Maldives archipelago hosts extensive coral reefs, which have been the focus of annual monitoring since 1997. Corals are holobionts which are intimately affected by microbiome composition alterations caused by different stress types. Their microbiome includes different groups of microalgae, bacteria, fungi, archaea, viruses, and protists, all playing essential roles in host metabolism and health. For this reason, this long-term project aims at characterizing the microbiome diversity in various coral morphotypes, and how this is affected by natural and anthropic perturbations.

The samples collected in spring 2022 consisted in corals specimen collected across several atolls in the Maldives (Ari, North Malé, South Malé and Rasdhoo) (Fig 1), which have been analysed to gain insights into their microbiome composition. A total of 26 samples, including *Acropora*, *Pocillopora*, *Porites*, *Fungia*, and gorgonian corals, were picked by scuba diving from both ocean-exposed reefs and lagoon reef. Additionally, surface seawaters were sampled utilizing the eDNA pump (citizen scientist) developed by Smith-Root.

Metabarcoding analyses were performed on the extracted DNA, targeting the 16S rRNA gene and the Internal Transcribed Spacer 2 (ITS2) rRNA region. These analyses were conducted to characterize bacterial composition and the microalgal endosymbiont clade diversity.

The results revealed a significant diversity pattern primarily influenced by coral morphotype rather than depth or atoll geography. In Acropora, the dominant bacterial genera included Pseudomonas, Bradyrhizobium, and Synechococcus_CC9902, resembling the microbiota of Pocillopora morphotypes, with additional abundance of Epulopiscium and Romboutsia. Porites, Fungia, and gorgonian corals show distinct compositions compared to other morphotypes.

The distribution of *Symbiodinium* showed analogous trends, predominantly belonging to the clade C; nevertheless, a noteworthy presence of clade D was discerned within

Acropora corals. At the subclade level, clear patterns between morphotypes were distinguished.

In contrast, seawater samples showed heightened diversity relative to coral, without showing differences between sites, and bacterial SAR groups were observed among the most dominant bacteria genera in surface water.

Overall, this study offers foundational insights crucial for monitoring local coral health and predicting potential impacts of environmental changes.

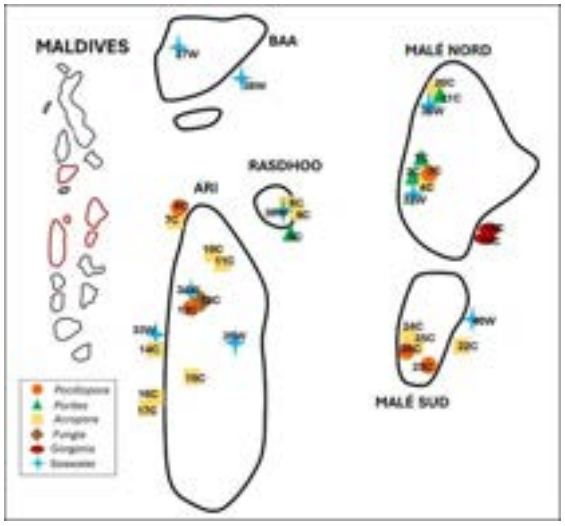


Figure 2: Collection sites of the different corals morphotypes and waters

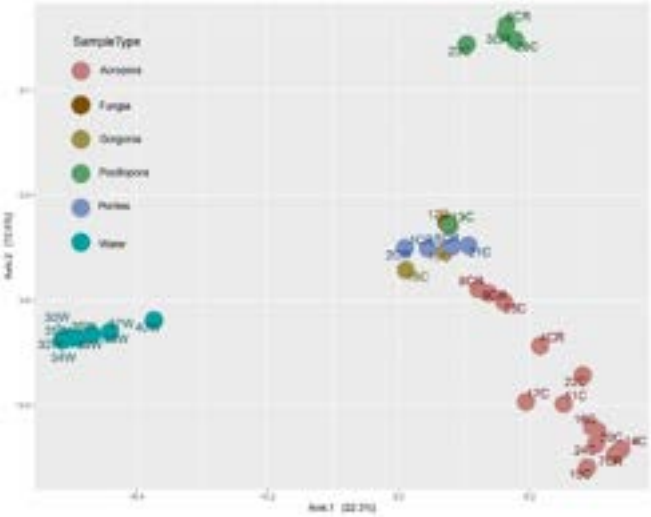


Figure 2: PCoA plots of beta diversity including all samples corals and seawater for their bacterial community composition.

Abstract ID: 2492

Fisheries management measures select for oligotrophic seawater microbes across the Great Barrier Reef

Presenting author: Mr. Marko Terzin, Australian Institute of Marine Science, Townsville, Australia

Introduction: The Great Barrier Reef (GBR) is critical for reef biodiversity and ecosystem services such as carbon sequestration and coastal protection, yet it faces threats from climate change and localised human impact, requiring effective conservation and management action. Rezoning of the Great Barrier Reef Marine Park (GBRMP) in 2004 expanded No-Take (green) zones to prohibit extractive activities like fishing and collecting in 33% of the GBRMP, which created the world's largest network of marine reserves. This has shown benefits such as increased biomass of fisheries-targeted species and improved coral community health metrics, although the potential effects of zoning measures on water chemistry and seawater microbiomes have not been explored.

Objective(s): To investigate the bacterioplankton communities across 48 offshore reefs in the Great Barrier Reef (GBR), including 24 green zones closed to fishing and 24 reefs open to fishing, and to explore the potential effects of reef zoning on seawater microbiomes.

Methods: Seawater samples were collected from 48 sites across the GBR for metagenomic DNA extraction and water quality analysis, alongside field benthic surveys as part of the Long-Term Monitoring Program by the Australian Institute of Marine Science. The mixOmics R package was employed to integrate 5,283 metagenome-assembled genomes (MAGs) of surface seawater bacteria and archaea with in-situ benthic cover data (including coral species, algae, abiotic components, and fish densities/biomass) and physico-chemical parameters (such as temperature, salinity, and nutrient levels), in order to identify relationships between microbial communities and environmental variability.

Findings: Supervised classification method identified 260 species-level indicator microbes that predict reef zoning with ~72% accuracy. Oligotrophic Pelagibacter and SAR86 MAGs with streamlined genomes were indicative of green zones, while opportunistic taxa like Flavobacteriaceae and Rhodobacteraceae were associated with reefs open to fishing. Patterns in green zones were partially explained by increased crustose coralline algae cover, encrusting and tabulate Acroporid corals, higher numbers of herbivorous and detritivorous fish, elevated phosphate, and lower particulate nutrients and dissolved inorganic nitrogen. In contrast, extractive zones were

characterised by elevated nitrite, nitrate, and turf algae cover. A metric of microbial networks (community 'cohesion') showed a prevalence of likely competitive microbe-to-microbe interactions in reefs open to fishing, where indicator microbes are potentially outcompeting other microbes for nutrients.

Conclusion: These indicator microbes contribute to the broader understanding of the relationships between seawater microbial communities and reef health, zoning, and water chemistry, which could support future monitoring efforts and help build a knowledge base for more targeted reef health assessments.

Abstract ID: 2023 / Poster Number: 422.17P

Fitness screening of the lithoautotrophic bacterium Cupriavidus necator reveals importance of selected energy generating pathways

Presenting author: Dr. Michael Jahn, Max-Planck-Unit for the Science of Pathogens, Berlin, Germany

Co-authors: *Nick Crang, Arvid Gynnå, Deria Kabova, Stefan Frielingsdorf, Oliver Lenz, Emmanuelle Charpentier, Elton Paul Hudson*

The 'knallgas' bacterium *Cupriavidus necator* is attracting interest due to its extremely versatile metabolism. *C. necator* can use hydrogen or formic acid as an energy source, fixes CO_2 *via* the Calvin-Benson-Bassham (CBB) cycle, and grows on organic acids and sugars. Many of the relevant energy-related genes are, however, present in multiple copies (CBB cycle, hydrogenases, nitrate reductases).

We investigated the energy metabolism of *C. necator* by growing a transposon knockout library on succinate, fructose, hydrogen (H_2/CO_2) and formic acid. The fitness contribution of each gene was determined from enrichment or depletion of the corresponding mutants (Figure 1).

Our fitness screening revealed that some genes/operons are preferred over others, depending on the growth substrate and utilized pathway. For example, in order to metabolize formate, soluble formate dehydrogenase (FDH) was preferred over membrane-bound FDH. For hydrogen consumption, both soluble and membrane-bound hydrogenases were utilized. Of the six terminal respiratory complexes in *C. necator*, only three were utilized, and utilization depends on the growth substrate. In conclusion, our study gauges the contribution of each of *C. necator*'s genes to fitness in biotechnologically relevant conditions, and highlights origin and consequences of genomic redundancy.

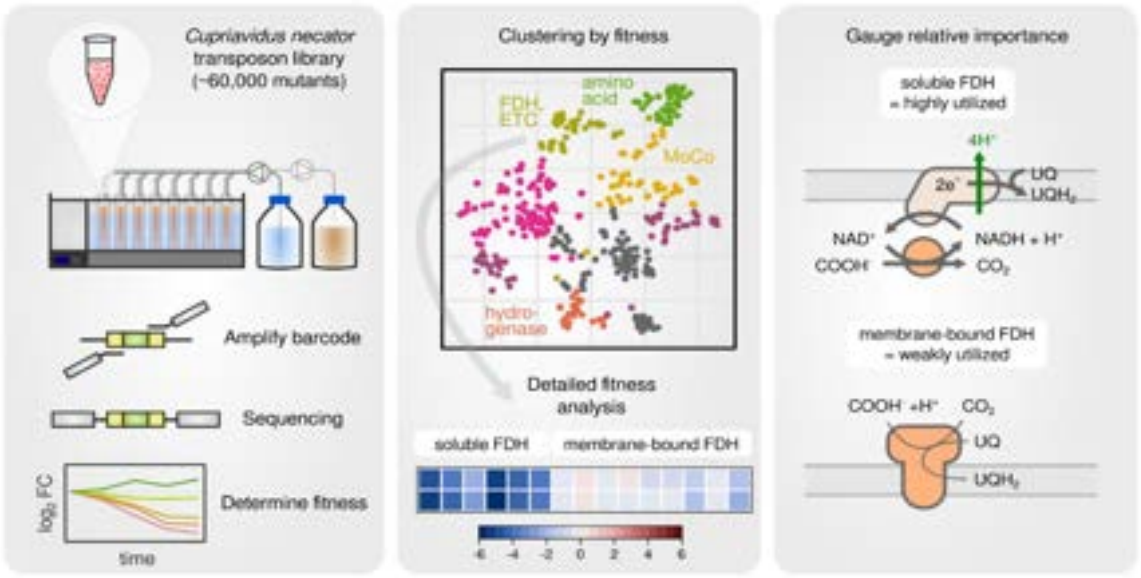


Figure 1: A barcoded transposon knockout library was constructed and screened in bioreactors (left). Gene fitness was analyzed by clustering and revealed strong differences in energy-related pathways (middle). Fitness scores of formate dehydrogenases (FDH) revealed importance of the soluble FDH (right).

Abstract ID: 1163 / Poster Number: 085.17P

Flavonoids Baicalein and Scutellarein as Potential Therapeutics Against MRSA

Presenting author: Ms. I-Fang Wang, Soochow University, Taipei, Taiwan

Co-authors: *Shin-Chun Yang*

The discovery of antibiotics has significantly helped inhibit pathogenic bacteria; however, their misuse has led to widespread drug resistance. Although this issue has persisted for years, finding effective methods to combat drug-resistant pathogens remains urgent. Flavonoids are well-known for their antibacterial, anti-inflammatory, and antioxidant properties. This study investigated their potential to inhibit MRSA biofilm formation, modulate bacterial populations, and regulate extracellular vesicles (EVs) within a localized microenvironment. Among the tested flavonoids, baicalein and scutellarein demonstrated significant antibacterial activity. They effectively suppressed MRSA growth and biofilm formation while influencing EVs secretion and bacterial community composition. Bacterial population analysis showed that these compounds significantly reduced MRSA levels while increasing the proportion of S. epidermidis, indicating their ability to modulate the skin microbiota. Further analysis revealed that baicalein and scutellarein altered the size, surface potential, and quantity of MRSA-derived EVs. These changes reduced the inflammatory response triggered by MRSA EVs, potentially weakening MRSA's pathogenic effects. In our investigation of the antibacterial mechanism, baicalein and scutellarein induced minimal reactive oxygen species (ROS) production in MRSA. Results from fluorescence microscopy analysis suggested that the antibacterial mechanism of baicalein and scutellarein might involve the inhibition of DNA synthesis. Based on our findings, baicalein and scutellarein show potential as candidate drugs for treating MRSA by regulating bacterial EVs secretion and interbacterial communication.

Abstract ID: 1531 / Poster Number: 339.15S

Flipping the switch: how a master regulator of central carbon metabolism activates pathogenesis

Presenting author: Prof. Andrew Roe, University of Glasgow, Glasgow, United Kingdom

Co-authors: *Kabo R. Wale, Nicky O'Boyle, Rebecca E. McHugh, Ester Serrano, David R Mark, Gillian R. Douce, James P.R. Connolly*

Introduction: In the attaching and effacing pathogens enterohaemorrhagic *Escherichia coli* (EHEC) and *Citrobacter rodentium*, virulence factors crucial for bacterial attachment to the colonic epithelium are encoded upon the Locus of Enterocyte Effacement (LEE). The LEE is regulated by a number of core-genome encoded transcription factors, one of which, PdhR, we here reveal is a key activator of the LEE which enhances host cell adhesion. We show that deletion of *pdhR* in EHEC affected the transcription of hundreds of genes, particularly those associated with pathogenesis and protein secretion. Further, we demonstrate that PdhR is essential for host colonization by *C. rodentium* in murine models. Our research highlights how core transcriptional regulators can be co-opted to control virulence factor expression in tandem with the cell's essential metabolic circuitry.

Objectives: In this study, we aimed to examine the intersection of host-specific metabolism and virulence regulation. Our research sought to identify potential regulators of virulence in EHEC and then characterise how these regulators impact bacterial virulence and colonisation within host cells.

Method: We utilised a number of molecular, cellular, and *in vivo* techniques in our research. In-frame deletions were made with Lambda red recombineering. GFP-promoter fusion reporter assays were performed, as was SDS-PAGE profiling of secreted proteins. HeLa cell adhesion assays and fluorescence microscopy was carried out on knockout and wildtype strains, as were animal colonisation experiments. RNA extraction was performed on both bacterial culture and murine tissue for RNA-sequencing and transcriptome analysis. Protein overexpression, immunoblot analysis, site-directed mutagenesis, and EMSAs were utilised to confirm the conserved LEE1 master regulatory region.

Findings: We demonstrate that the transcriptional factor, PdhR, contributes to LEE regulation in EHEC and that it activates EHEC T3SS expression to promote host-cell adhesion. Further we have identified that PhdR is a global regulator of transcription in EHEC which affects several infection-relevant loci, significantly, PdhR interacts directly

with the LEE master regulatory region to activate T3SS expression. This holds true in other attaching and effacing *E. coli*, as we show that the PdhR binding site within the LEE regulatory region is widely conserved in A/E pathogens.

Conclusion: Our research has discovered a new direct link between central metabolism and pathogenesis in A/E pathogens, demonstrating a likely strategy for optimising fitness within the intestinal pathogenic niche.

Abstract ID: 876 / Poster Number: 257.16P

Flotillin deficiency impairs P22 phage infection in *Salmonella enterica*

Presenting author: Tamara Alonso-Blanco, PhD, National Centre for Biotechnology, Spanish National, Madrid, Spain

Co-authors: *Elena Pajares-Martínez, María López-Bravo, Mar Cordero, Daniel Lopez*

Functional membrane microdomains (FMMs), analogous to eukaryotic lipid rafts, are specialized bacterial membrane regions enriched with specific lipids and proteins, playing a vital role in numerous cellular processes. A key component of FMMs, the scaffold protein flotillin, is essential for maintaining cellular homeostasis by stabilizing unfolded proteins under stress. This function becomes particularly critical during energy-depleted states, such as bacterial infections. In this study, we investigated the role of flotillin on stressed cells undergoing phage infection, using the pathogen *Salmonella enterica* as a model organism.

We assessed the infection capacity of three bacteriophages (P22, Det7, 9NA) in wild-type and flotillin-deficient *S. enterica* strains. While Det7 and 9NA showed no differences, P22 exhibited a significant reduction in plaque-forming units (PFUs) in the flotillin mutant. Further molecular analyses, including sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and gradient blue native polyacrylamide gel electrophoresis (BN-PAGE), showed no changes in protein quantity or oligomerization state in defective P22 phages. Surprisingly, DNA quantification showed a marked reduction in phages, suggesting attenuated phage proliferation in flotillin-defective strains.

Our findings reveal that flotillin is critical for efficient P22 phage proliferation during bacterial infection. These results highlight the importance of FMMs in all kind of bacterial stress responses including bacteriophage infection. This underscores a broader role of FMMs in modulating interactions with mobile genetic elements, expanding our understanding of bacterial adaptability and host-phage interactions.

Abstract ID: 1957 / Poster Number: 306.17P

Fluorescence-labelled Saccharomyces cerevisiae to study cross-species interactions in wine fermentation

Presenting author: Dr. Ernesto Petruzziello, University of Naples "Federico II", Departament of Agriculture Science, Portici (NA), Italy

Co-authors: *Chiara Vischioni, Giuseppe Blaiotta, Gianni Liti*

Wine fermentation is driven by complex microbial dynamics, with *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts shaping fermentation behavior and final product quality. While much is known about *S. cerevisiae*, the dynamics of multi-species fermentations remain a key area of research. This study introduces a new molecular approach to dissect the interactions between non-*Saccharomyces* species and *S. cerevisiae* in a synthetic fermentation system. We first genetically modified a natural strain of *S. cerevisiae* (F14-72) by integrating a green fluorescent protein (GFP) at the HO locus, which regulates mating-type switching, using CRISPR-Cas9 technology. Vectors pL12, p426, and yGAM were employed for precise gene editing. To analyze microbial interactions, the GFP-labeled strain was tested alone and in combination with five non-*Saccharomyces* species (*Metschnikowia pulcherrima, Lachancea thermotolerans, Torulaspora delbrueckii, Starmerella bacillaris and Hanseniaspora guilliermondii*) in synthetic must (200 g/L of initial sugar). Flow cytometry allowed real-time monitoring of population changes, with GFP fluorescence tracking viable *S. cerevisiae* cells and propidium iodide (PI) staining identifying dead cells. Fermentation kinetics were also monitored by both high-performance liquid chromatography (HPLC), to measure sugar reduction and ethanol formation, and by CO_2 loss to assess fermentative activity.In monoculture, *S. cerevisiae* completely metabolized sugars, while co-cultures demonstrated interactive effects that influenced its performance. Results revealed distinct competitive dynamics, with *S. bacillaris* consistently exhibiting higher viability and a negative impact on *S. cerevisiae* growth over a 10-day period. This research demonstrates the utility of CRISPR-Cas9-based GFP tagging for studying yeast interactions, providing a model to investigate competitive and cooperative behaviors in fermentation.

Abstract ID: 1519 / Poster Number: 425.15P

Fluorescent labeling of bacterial cell envelope and its application in membrane protein enrichment

Presenting author: Dr. Athanasios Saragliadis, University of Oslo, Oslo, Norway

Co-authors: *Anja Ruud Winther, Jack Leo, Dirk Linke*

Gram-negative bacteria are decorated on their surface with a double membrane: the outer and the inner membrane, which in turn encompasses the in-between periplasmic space. Each of these distinct organismal features contain various macromolecules such as lipids, proteins and carbohydrates which as a whole serve purposes such as solute transport, energy production, support protein secretion and lipid biosynthesis. These very important and impactful to the cell proceses, need to be investigated on many occassions for which one needs to isolate the bacterial membrane-envelope for multitude of scientific purposes.

We developed a biotechnological tool that allows for easy, fast and simple detection of membrane fractions and eventually monitor and evaluate their further enrichment.

Our tool relies on the use of genetically engineered strains to produce, on genomic level, fluorescently labeled inner and outer membranes for enrichment-monitoring purposes.

We started by generating the necessary constructs in plasmid form and eventually incorporated these constructs in the bacterial chromosome to remove the cellular burden of plasmid maintenance and facilitate simultaneous use of different plasmids. Thusly, we were able to observe and troubleshoot membrane enrichment using different established protocols such as sucrose gradient ultracentrifugation or preferential detergent solubilization.

Abstract ID: 1167 / Poster Number: 195.15P

FocA works as a bidirectional formic acid channel in Escherichia coli

Presenting author: Prof. Karen Trchounian, Yerevan State University, Yerevan, Armenia

Co-authors: *Liana Vanyan, Anait Vassilian, Anna Poladyan, Gary Sawers,*

FocA is a member of the formate-nitrite transporter family, widely present in prokaryotes, and plays a crucial role in the translocation of formic acid across the cytoplasmic membrane of fermentatively grown *Escherichia coli*. To elucidate its physiological role, total and DCCD-sensitive proton and potassium fluxes were measured in whole cells using ion-selective electrodes, and ATPase activity by measuring the amount of inorganic phosphate (Pi) produced during the ATP hydrolysis reaction by bacterial membrane vesicles in wild-type and *focA* mutant strains.

Our findings reveal that cells expressing the native FocA channel exhibit efficient proton efflux (~2.5 mmol min^{-1}), with 51% being DCCD-sensitive. In contrast, mutants lacking FocA showed reduced proton flux and a compensatory increase in ATPase-mediated activity. The $FocA_{H209N}$ variant, which translocates formic acid only outward, exhibited a 20% increase in proton efflux, while the $FocA_{T91A}$ variant, unable to export formic acid, showed no significant DCCD-independent flux. Potassium flux assays indicated a strong coupling between FocA and F_OF_1-ATPase activity, particularly during formic acid translocation. In wild-type cells, ATPase activity accounted for ~84% of total proton flux, supporting both potassium uptake and pH balance.

These findings demonstrate that FocA's bidirectional functionality is integral to balancing ion gradients and energy metabolism. The channel's ability to coordinate formate, proton, and potassium fluxes highlights its importance in maintaining cellular homeostasis and reveals potential targets for controlling microbial fermentation processes.

Abstract ID: 2692 / Poster Number: 069.16P

Food & Microbes, a food-taxa interaction database for the promotion of health-associated gut microbiota

Presenting author: Gabriel Garcia-Gamero, PhD, FISABIO, Valencia, Spain

Co-authors: *E. Daniel Leon Lopez, Giuseppe D'Auria, Vicente Perez Brocal, Mª Pilar Francino Puget*

Introduction: The gut microbiota is implicated in a multitude of high-impact biological processes. The ability to modulate gut microbiome composition has far-reaching implications in human health as dysbiosis is related to major diseases. Although the gut microbiome dependence on dietary factors is now well established, systematizing gut microbiome knowledge to effect health-promoting change is necessary and we lack systems that enable us to do so.

Objective: As a response to fill this gap, we are developing the Food & Microbes (F&M) database, a food-taxa interactions database. The final goal is the integration of this database with information on metabolites related to the different taxa and a microbiome-clustering database where disease-taxa relations and baseline data for a healthy reference population are available. This integration will provide the basis to develop algorithms that allow us to generate personalized food recommendations.

Method: F&M is an SQL relational database gathering information obtained from a wide range of different studies where the gut microbiomes of healthy individuals and patients (diabetes, Chron's disease, etc.) were characterized in response to different foods or diets. Information related to type of study and omic technique is used to establish confidence in each food-taxon relation.

Findings: Hundreds of food-taxon interactions have been included in the F&M database. F&M has been hand-curated but we are exploring the use of text-mining techniques to expand the amount of information available.

Conclusion: Further development of F&M and associated algorithms will facilitate the leveraging of food-microbiome interaction knowledge to enable personalized health-promoting dietary advice based on sound evidence.

Abstract ID: 1014 / Poster Number: 209.17P

Food Industry-Related Microbial Control Agents Can be Undermined by Antimicrobial Resistance Genes in Salmonella Typhimurium

Presenting author: Mr. Raúl Campillo, Instituto Agroalimentario de Aragón-IA2, Universidad de Zaragoza-CITA, Zaragoza, Spain

Co-authors: *Ivo García-Penas, Alberto Fau, Diego Gómez, Daniel Berdejo, Diego García-Gonzalo, Rafael Pagán*

Introduction: The high prevalence of antimicrobial-resistant (AMR) *Salmonella* spp. in food and human isolates manifests that the prevention and control agents applied in the agri-food industry might not be sufficiently impeding its dissemination. Interestingly, recent results obtained by our research group have suggested a potential link between antimicrobial resistance genes (ARGs) and acquisition of cross-protection to food industry-related microbial control agents, such as organic acids, natural antimicrobial compounds, or heat treatments. Assuming this hypothesis, *Salmonella* spp. acquiring ARGs may turn more tolerant to microbial control agents, facilitating their dissemination and access to the end consumer.

Objective: This study aimed to assess the impact of the mutated ARGs (*rpoD*, *cyaA* and *ramR*) from a CIP-resistant variant of *Salmonella* Typhimurium LT2 (SeT_{RV1}), obtained by cyclically exposing the parental strain (SeT) to CIP doses, in cross-protection to lethal treatments by several microbial control agents: lactic acid, carvacrol, and heat.

Methods: Based on SeT_{RV1}, single and double mutants of SeT (SeT_{rpoD}, SeT_{cyaA}, SeT_{ramR}, $SeT_{rpoD/cyaA}$; $SeT_{rpoD/ramR}$; $SeT_{ramR/cyaA}$) were obtained via transferring the ARGs into SeT's genome. The resulting constructions were submitted to lethal doses of lactic acid (1% v/v for 30 min) or carvacrol (200 µg/mL for 60 min) in flasks containing 10 mL of TSB, and to a heat treatment (54 ºC for 20 min) in tubes with 1 mL of McIlvaine buffer pH 7.0.

Findings: All mutated ARGs in SeT_{RV1} contributed to enhanced CIP resistance (from 2- to 7.5-fold), and to cross-tolerance to lactic acid (**Figure 1A**), carvacrol (**Figure 1B**), and heat (**Figure 1C**), confirming our hypothesis. The *rpoD* mutation contributed most to cross-protection to the three microbial control methods. For instance, while 3.50 $\log_{10}$ cycles of SeT population were inactivated upon the lactic acid treatment, only 0.71 of SeT_{rpoD} , 1.25 of SeT_{cyaA} and 2.66 of SeT_{ramR} were reduced. Only the simultaneous presence of all three mutations explained the phenotype of SeT_{RV1} against lactic acid and carvacrol.

Conclusion: Mutations occurred in SeT_{RV1} turned SeT into a CIP-resistant strain with acquired cross-protection to lactic acid, carvacrol, and heat treatments. These phenomena was due to ARGs frequently reported in AMR bacteria, coding for RNA polymerase subunit σ^D (*rpoD*), enzyme adenylate cyclase (*cyaA*), and regulatory protein RamR (*ramR*). Thus, genetic alleles of these ARGs might be increasing survival risk of *Salmonella* spp. along the agri-food chain, thereby facilitating them to reach the end consumer – thus also limiting antibiotic action during infection.

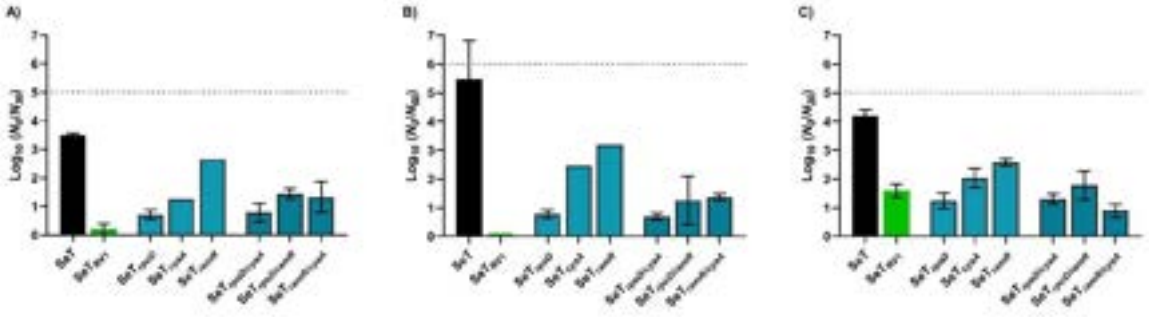


Figure 1: Log_{10} cycles of inactivation (log_{10} [N_0/N_t]) of *Salmonella* Typhimurium LT2 parental strain (■SeT), the CIP-resistant variant (■SeT_{RV1}), and its complemented strains (■SeT_{rpoD}, SeT_{cyaA}, SeT_{ramR}; ■$SeT_{rpoD/cyaA}$, $SeT_{rpoD/ramR}$, $SeT_{ramR/cyaA}$) after a lethal treatment with **A)** lactic acid; **B)** lethal carvacrol; **C)** heat. $N_0 \approx 3 \times 10^7$ CFU/mL. Horizontal dotted line represents the detection limit.

Abstract ID: 2644 / Poster Number: 250.15P

Forming mechanism of microbial complex systems: how to maintain functional stability

Presenting author: Hiroyuki FUTAMATA, PhD, Shizuoka University, Hamamatsu, Japan

Co-authors: *Takeshi Okada, Yasuhisa SAITO, Kenshi Suzuki, Masahiro Honjo*

Introduction and Objective: Microbial complex systems play important roles in wastewater treatment, agriculture, human health, and the like. We need to manage the system that change dynamically. A more detailed understanding of the mechanisms underlying the formation of microbial complex system exhibiting the functional stability is essential for the efficient management of microbial complex systems. To date, amplicon sequence analysis targeting 16S rRNA genes, statistical analysis such as a principal coordinate analysis (PCoA), and mathematical models allow us understood microbial community dynamics, however, which are insufficient for understanding of those mechanisms. Therefore, we need novel index.

Methods and findings: Soil microbial community were enriched under sequential batch cultures supplied with phenol as sole carbon and energy source. Phenol was not accumulated in any transferred cultures. PCoA showed succession states until the 3rd transferred cultures and stable states from the 5th to 10th transferred cultures. Novel quantitative parameters based on microbial population growth activity were developed, which indicated that the bacterial community was dynamic irrespective of the succession and stable states, in which vulnerable and resilience forces were detected. Mathematical models indicated the construction of metabolic networks, suggesting the stabilizing mechanism of the community structure. Core OTUs consisted of majorities, middle-class, and minorities. The abundance of the middle-class changed, whereas that of the others did not, which indicated that core OTUs maintained functional stability by changing metabolic networks. Some extremely low abundance OTUs were consistently exchanged, suggesting a role for scavengers.

Conclusion: These results indicate that stable states were formed by dynamic metabolic networks with member functioning to achieve robustness and plasticity.

Abstract ID: 258 / Poster Number: 386.16P

Fortification of vitamin B12 in various fruit pomace by in situ fermentation with the participation of propionic and lactic acid bacteria

Presenting author: Kamil Piwowarek, PhD, Warsaw University of Life Sciences, Warsaw, Poland

Co-authors: *Zuzanna Makowska, Krystian Rząd*

Introduction: People worldwide suffer from B12 deficiency and, thus, unpleasant health consequences. Therefore, new solutions to enrich the human diet with cobalamin (e.g., fermentation) seem necessary. The food industry generates millions of tons of by-products per year, a large part of which is fruit pomace. Microbiological activity (PAB and LAB) could increase pomace's nutritional value (e.g., B12), affect taste properties and increase its safety.

Objective: The study aimed to evaluate the possibility of fortifying vitamin B12 in various fruit pomace using PAB and LAB.

Methods: Monoculture (Propionibacterium freudenreichii DSM20271) and co-culuture (P. freudenreichii and Lactiplantibacillus plantarum ATCC10241) were carried out. Strawberry, grape, and blue honeysuckle pomace were used for fermentation, separately (120h, 30°C, pH≈6.5). The matrices were sterilized. Vitamin B12 production and bacterial growth were determined by microbiological and culture methods, respectively. Fruit side-streams were characterized in terms of nutrients for bacteria.

Results: The initial cobalamin content in the inoculated matrices (0h) was approximately 6-12 ng/g fresh matrix. The most efficient B12 production was observed in strawberry pomace (120h) in both monoculture (32.22±0.46 ng/g fresh matrix) and co-culture (46.98±2.62). In other variants, cobalamin production reached approx. 14.82-30.71 ng/g. Both PAB and LAB showed growth in the tested matrices – increase in the number of LAB by approx. 2 log units, whereas PAB maximum 0.33.

Conclusions: The tested pomace can be enriched with cobalamin by PAB monoculture and PAB/LAB co-culture. Managing pomace using fermentation can contribute to its use as valuable substrates in food production following sustainable development and zero-waste policy.

Abstract ID: 3587 / Poster Number: 229.16P

Fourier-Transform Infrared Spectroscopy (FTIR) as a tool for predicting Group A Streptococcus (GAS) emm-type using a Machine Learning model

Presenting author: Valeria Fox, PhD, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy

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Introduction: Since 2022, invasive Group A Streptococcus (GAS) infections have increased, caused by certain *emm*-types (e.g., *emm*1). Therapies may depend on *emm*-type, identified by sequencing, a costly and time-requiring technique. Recently, Fourier Transform Infrared Spectroscopy (FTIR) has emerged as a fast and cost-effective alternative for typing.

Objective: By Machine Learning (ML) algorithms, we aimed to create a model for *emm*-type prediction, to rapidly identify GAS strains more associated to invasive infections and timely guide the therapeutic approach.

Methods: Twenty-five GAS strains were analyzed by WGS and FTIR (IR Biotyper,Bruker Daltonics GmbH). Predictive model was built with IR Biotyper Software (1300–800cm-1 range), on a training dataset of 15 strains, belonging to 4 *emm*-types (1,3,4,6). The model was validated with 5 strains of the same *emm*-types and tested with 5 new strains, whose *emm*-type was determied by WGS.

Findings: An artificial neural network (ANN) model, trained for 200 cycles, was the most accurate (accuracy=87%). In particular, misclassification involved only 27% of spectra from *emm*1 strains, which were incorrectly identified as *emm*4. Nonetheless, when the model was validated, an accuracy of 100% was reached. Finally, when 5 new strains were tested, 2 were correctly identified as *emm*-type 1 and 4, while 3, belonging to *emm*12, 89 and 75 were misclassified, as they belonged to *emm*-types not included in the model.

Conclusions: We developed a model capable of accurately identifying the 4 most prevalent *emm*-types in our setting, confirming the potentiality of FTIR combined with ML in reducing diagnostic time and guiding therapies.

Abstract ID: 4147

From Bat to Worse: The Pivotal Role of Bats for Viral Zoonosis

Presenting author: H. Brüssow, KU Leuven, Leuven, Belgium

Zoonotic infections are increasingly observed and bats (Chiroptera) are playing a pivotal role here. The causal chain of events has been elucidated for Henipavirus (family: paramyxoviruses) infections. Deforestation combined with climate change has reduced the food sources of Pteropus fruit bats and attracted them to fruit trees planted around piggeries in Malaysia, transmitting Nipah virus to pigs as amplifying hosts and then to pig farmers and abattoir workers. Similar scenarios were seen in Australia where Pteropus bats transmitted Hendra virus to horses as intermediate hosts for human infections. Pteropus bats contaminated palm sap collected in Bangladesh with Nipah virus where fatal human-to-human transmissions occurred annually. Less direct evidence links coronaviruses carried by Rhinolophus bats with SARS and COVID-19 pandemics and a piglet epidemic in China. Rousettus bats living in caves transmitted the Marburg virus (family: filovirus) to miners in Africa. Most cases of human rabies in North America were caused by bat lyssaviruses (family: Rhabdoviruses). Bats are viral reservoir species for various virus families (reovirus, Hepacivirus of Flavivirus family, influenza A viruses). Bats are the only flying mammals which opened enormous evolutionary possibilities resulting in a worldwide radiation with 1400 species. Some bat species are represented by huge populations that come together in extremely crowded resting places that are conducive to viral transmission. Bats have evolved mechanisms that tolerate virus replication but suppress the associated pathology, making them healthy carriers for many viruses. It is speculated that with that strategy bats avoid an arms race with viruses for resistance and anti-resistance mechanisms. The excretion of viruses that are highly pathogenic for other mammalian orders could be used as biological weapons to defend their habitat against intrusion by mammalian competitors, including humans. This hypothesis might explain the increasing involvement of bat viruses in emerging infectious diseases observed in recent decades and expected in the future.

Abstract ID: 1827 / Poster Number: 310.15P

From Farm Waste to Food Security: Unveiling the Microbial Impact of Antibiotics in Agriculture

Presenting author: Dr. Oluwaseyi Samuel Olanrewaju, North-West University, Potchefstroom, South Africa

Co-authors: *Cornelius Carlos Bezuidenhout*

Introduction: Most animal-administered antibiotics are passed out directly by the animals through their feces. This forms a route of entry of antibiotics into agricultural farms when these feces are used as manure. Soil microbiomes are essential in helping plants combat various biotic and abiotic stresses they are exposed to, but understanding the impact of these antibiotics on the soil microbiome is crucial in using beneficial microbes for bioremediation.

Objective: Hence, this study focuses on the impact of sulfamethoxazole (SMX) and trimethoprim (TMP) on the microbiome of lettuce plants in the greenhouse. SMX and TMP were mixed with freshly collected cow manure. Pots were filled with soil mixed with the antibiotics-manure mixture, and the pots (each treatment in triplicates) were arranged using an RCBD design.

Method: After sample collection, DNA extraction, and analysis, the result showed that the antibiotics treatment significantly impacted the rhizosphere and endosphere bacteria community compared to the control.

Result: Diversity indices, alpha and beta diversity, showed that bulk soil has the highest bacteria diversity, followed by the rhizosphere, compared to the control soil. Likewise, the SMX-treated plants have the highest diversity compared to the TMP-treated plants and the control. The diversity between the bulk soil and the rhizosphere, the rhizosphere and the endosphere, SMX-treated plant, and TMP-treated plants are statistically significant at $p=0.5$.

Conclusion: This study identifies the key impacts of SMX and TMP on the lettuce bacteria community. This will enable and improve sustainable agriculture practices to enhance food security and human health.

Abstract ID: 417 / Poster Number: 415.15P

From Fungus to Factory: Recombinant Production of Soluble Lignin Peroxidase in E. coli

Presenting author: Mr. Abdul Basith Akbar Aly, Bharathiar University, Coimbatore, India

Co-authors: *Abdul Basith Akbar Aly, Parthiban Subramanian*

Background: Lignin peroxidase (LiP) is an enzyme mainly produced by the white-rot fungus Phanerochaete chrysosporium (Pc). It is well known for its robust ability to break down the complex polymer lignin found in plant cell walls. This unique capability makes LiP a valuable tool for bioremediation, biofuel production, and paper processing applications. However, challenges in the high-yield production of soluble and active enzymes constrain industrial utilization. Notably, this LiP hasn't been commercialized yet. This study successfully addressed these challenges by optimizing conditions for recombinant production in E. coli and its functional validation.

Objectives: To address the recombinant production challenges by optimizing the conditions for the expression of lignin peroxidase in E. coli.

Methods and results: Total of 10 LiPs are found in P. chrysosporium; among these, PcLiP4 has the highest catalytic activity. The PcLiP4 gene was de novo synthesized with a 6x his-tag, codon optimized and cloned in a bacterial expression vector, pDEST527, via GATEWAY cloning technology. Further, it was transformed to E. coli BL21 DE3 pLysS expression system. Important parameters optimized included, temperature at 30°C, IPTG concentration at 0.1 mM, and a post-induction time at 2 hours have significantly improved the solubility of rPcLiP4.

The rPcLiP4 was successfully produced with a yield 7 mg/ml in an soluble form, which was confirmed by SDS-PAGE and Western blotting. Veratryl alcohol oxidation assay demonstrated that the rPcLiP4 retained its catalytic activity. This approach provides a scalable and cost-effective strategy for LiP production to overcome the existing solubility barrier issues.

Conclusions: This study establishes a foundation for the industrial scale production of recombinant LiP facilitating efficient lignin valorization leading to sustainable biotechnological applications.

Abstract ID: 3552

From the pan into the fire: How seasonal drought reduces microbial diversity and functional potential in the Amazon rainforest

Presenting author: Ms. Jessica Finck, Max-Planck-Institute for Biogeochemistry, Jena, Germany

Co-authors: *Dan Frederik Lange, Beto Quesada, Bruno Takeshi, Fernando Dini Andreote, Gerd Gleixner*

Tropical rainforests such as the Amazon are of high importance as a global carbon sink. Due to its well-known nutrient limitation, the Amazon rainforest relies heavily on rapid microbial decomposition of biomass to release freshly available nutrients for plant growth. Despite the fundamental importance of decomposers for this ecosystem, little is known about the biodiversity of tropical soil microbiomes, their functional activity, and spatial and seasonal variability. We used amplicon rRNA sequencing and shotgun metagenomic sequencing to investigate differences between microbial communities of the Amazon's terra firme forests and much drier white-sand ecosystems during the dry and wet seasons of 2022 and the 2023 El Nino year. Using both sequencing datasets, we used Picrust2 and Maaslin3 to study season and site-specific shifts in the functional potential of microbiota, with focus on organic matter cycling. Bacterial communities differed significantly between seasons, displaying lower diversity in response to seasonal drought. In contrast, fungal diversity measures differed strongly between sites but were less affected by seasonal variation in the first year, indicating that drought was buffered more efficiently (Fig. 1A). However, this was not the case in the subsequent El Nino year, in which microbial diversity did not recover during the 2023 wet season and dropped below 2022 wet season levels during El Nino (Fig 1B). Fungal and bacterial communities alike also featured lower abundance of taxa involved in organic matter decomposition following seasonal drought. These changes were also reflected at the functional level, with samples collected during the dry season and at drier white-sand sites featuring lower abundances of organic matter decomposition-related pathways. Soil hydro-chemical data further emphasizes how prolonged drought may limit soil nutrient supply through local microbiomes. Our results suggest that the reduced nutrient availability and soil connectivity during drought and within the white-sand ecosystem lower microbial activity and functional redundancy, henceforth demonstrating the strong impact of ecosystem type and drought on tropical microbiomes and their functional capacities. Thus, our results highlight how the observed increase in droughts in the Amazon

rainforest may limit nutrient supply through the microbial community, limiting carbon sequestration in the ecosystem and resulting in negative consequences for the global climate system, and ultimately, rendering this vital system vulnerable to climate change induced disturbances.

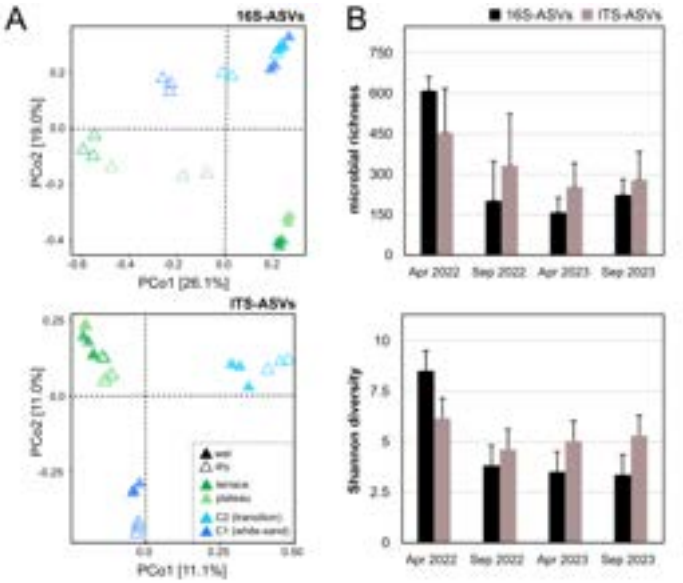


Figure 1: (A) Principal Coordinate Analysis of the bacterial (16S-ASVs) and fungal (ITS-ASVs) communities in the year 2022, based on Bray-Curtis dissimilarity. Green and blue triangles indicate Terra firme and white-sand ecosystem sites, respectively. (B) Alpha diversity measures of the bacterial (16S-ASVs) and fungal (ITS-ASVs) communities during different seasons over time.

Abstract ID: 3045 / Poster Number: 213.16P

Fully Immunocompetent Porcine Model of Complicated Skin and Soft Tissue Infection Caused by Carbapenem-Resistant Pseudomonas aeruginosa

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Introduction: Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) poses a significant threat to patients, often resulting in life-threatening infections. With increasing antimicrobial resistance, novel therapeutic strategies are urgently needed. Although animal models are crucial for preclinical studies, limited data are available for porcine models, more specifically for CRPA complicated skin and soft tissue infections (cSSTI).

Objective: The study presents a novel porcine model inducing and sustaining cSSTI caused by CRPA.

Methods: Six fully immunocompetent pigs (120 wounds) were used to develop wound infection and evaluate the progression of cSSTI in the model. Full-thickness skin defects (5 × 5 cm) with fascial incisions were surgically performed to introduce cSSTI. CRPA clinical strain FF2 (producing VIM carbapenemase) was used for wound infection development. Microbiological and histopathological assessment was performed by processing the tissue biopsy samples.

Results: The model demonstrated bacterial loads of 10^7 CFU/gram of tissue and higher with extensive areas of bacterial cells within the wound tissue and on its surface. The cSSTI fully developed within three days and remained as such for 14 days post-infection. The histopathological evaluation confirmed the full development of cSSTI. The samples were characterized by significant tissue infiltration by inflammatory cells, with a predominance of polymorphonuclear cells and macrophages.

Conclusion: This fully immunocompetent model of cSSTI caused by CRPA will serve in the development of novel therapeutical strategies (including antimicrobial peptides and enzymes, phage therapy, etc.) and corresponding preclinical studies. All immunological processes associated with the wound healing process (especially the inflammatory and proliferative phases) are preserved.

Abstract ID: 1498 / Poster Number: 205.15P

Functional and structural study of three sRNAs controlled by NikR and Fur in Helicobacter pylori

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Co-authors: *Andrea Vannini, Davide Roncarati, Vincenzo Scarlato*

Helicobacter pylori is a bacterium that colonizes the gastric tract, causing serious pathological conditions. Its ability to survive in the harsh gastric environment is facilitated by a regulatory program that integrates both transcriptional and post-transcriptional control mechanisms. The transcriptional regulators NikR and Fur are crucial for maintaining intracellular homeostasis of nickel and iron, which are essential co-factors for enzymes for acid acclimation, energy metabolism, and oxidative stress resistance. NikR and Fur also regulate other genes involved in different cellular responses and the expression of small non-coding RNAs (sRNAs) Nrr1, Nrr2, and Nc2090.

This project aims to functionally and structurally characterize these sRNAs to understand their role in post-transcriptional regulation of targeted transcripts and determine if they contribute to the indirect regulons of Fur and NikR.

These sRNAs were initially characterized determining their length, predicted secondary structures, transcript stability at different growth stages, and expression levels during bacterial growth. To investigate their roles in cell regulation, the single-sRNA knockout strains were assessed for their growth rate, whole-transcriptome profile by RNA-sequencing, and proteome changes, in comparison to the WT strain. The interactome of each sRNA was initially predicted through bioinformatic analysis informed by transcriptome and proteome data. Furthermore, the in vivo interactome is currently being determined adapting the MS2 affinity purification approach coupled with RNA-sequencing (MAPS) protocol to our system.

Therefore, this multi-faceted approach will integrate, validate, and strengthen the results, characterizing the sRNA regulatory role and establishing a robust pipeline to study these factors in *H. pylori* and related bacteria.

Abstract ID: 1350 / Poster Number: 351.16P

Functional characterization of Limosilactobacillus vaginalis BC17 and its derivatives as food supplements

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Introduction: Early gut colonization by bifidobacteria, typically occurring in vaginally born infants but not in those delivered via C-section, is crucial for maintaining a general state of health. Supplementation with lactobacilli of vaginal origin, which naturally come into contact with vaginally born infants, or their derivatives (postbiotics), represents an intriguing strategy to promote proper gut microbiota development.

Objective: To investigate the health-promoting properties of the vaginal strain Limosilactobacillus vaginalis BC17 and its postbiotics (cell-free supernatant, CFS, or heat-killed cells, HK), with the ultimate aim of developing postbiotic-based oral formulations. The project was funded by the European Union, NextGenerationEU (AlmaValue project LATONA, CUP ID C38H23000770002).

Methods: The safety of L. vaginalis BC17 and postbiotics was evaluated through genome analysis, compatibility with intestinal cell lines (Caco-2 and HT-29), and genotoxicity assessments. The effects on the adhesion of beneficial bifidobacteria and enteropathogens to Caco-2 cells and anti-inflammatory properties were indagated. Oral tablets were formulated by combining CFS with HK or live probiotic alongside maltodextrin.

Findings: BC17 and postbiotics proved to be safe for human use. They enhanced bifidobacteria adhesion by up to 250% while reducing enteropathogens adhesion by 40-70%. They exhibited significant anti-inflammatory effects, decreasing nitric-oxide production in macrophages by 40-50% and protecting intestinal cells from SDS-induced stress. The beneficial properties were preserved even after incorporation into fast-dissolving tablets.

Conclusion: BC17 and derivatives showed promising properties, particularly prebiotic potential, and can be easily incorporated in suitable oral formulation, paving the way for their application as food supplements.

Abstract ID: 2512 / Poster Number: 243.15P

Functional diversity in deep ocean basins linked to the absence of phototrophs

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Co-authors: *Francesco Ricci, Chris Greening*

Pelagic dark waters (depths > 200 m) harbor ~70% of the microbial cells in the ocean. However, there is still a lack of global understanding of the biogeochemical cycles and functional roles of dark ocean microbiome. In this study, we tested the hypothesis that the absence of light in deeper water reduces the abundance of photosynthetic microbes, thereby freeing resources for other microbial guilds, which in turn fosters higher functional diversity. Using 20 global ocean profiles ranging from surface waters to the bathypelagic zone (~4,000 m), we observed an increase in the abundance of key nutrients with depth. Genes associated with phototrophy, the Calvin-Benson-Bassham cycle and nitrogen fixation (*rho*, *psaA*, *psbA, rbcL* and *nifH,* respectively) declined rapidly with depth. Conversely, the abundance of genes related to alternative carbon fixation pathways such as the rTCA and 4-HB cycles (*aclB* and *hbsT*), nitrite reduction (*nirK*), and the oxidation of carbon monoxide (*coxL*), hydrogen (NiFe.G1 and G2), sulfur (*sqr*) and ammonia (*amoA*) increased with depth. More broadly, functional alpha diversity rose with depth, peaking in the bathypelagic zone. These results were consistent with over 4,000 non-redundant metagenome-assembled genomes (MAGs) reconstructed in this study. Altogether, our findings suggest that the absence of photosynthetic organisms in the dark ocean enhances nutrient availability for other functional guilds, leading to remarkable metabolic diversity. This underscores the critical role of deep-sea microbial communities in sustaining global biogeochemical cycles.

Abstract ID: 3326

Functional gene redundancy as a response strategy to variable substrate concentrations in marine PAH-degrading Cycloclasticus spp

Presenting author: Dr. Esteban Gongora, ISWA, University of Stuttgart, Stuttgart, Germany

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Polycyclic aromatic hydrocarbons (PAH) are common pollutants in marine environments, and PAH concentrations varies over several orders of magnitude, with microbial degradation playing a crucial role in attenuation [1, 2]. Notably, large-scale oil spills account for only 9% of annual petroleum hydrocarbon emissions, while 40% arise from chronic, often diffusive small-scale pollution events like shipping discharges, rivers and runoff, and atmospheric deposition [4, 5]. Despite the significance of these low-concentration inputs, the processes governing the biodegradation of low-dosage PAHs remain poorly understood. To address this knowledge gap, our work combined numerical modeling and laboratory experimentation, investigating both biochemical PAH degradation pathway dynamics and environmental system responses.

Process-based numerical modeling with parameter values derived based on literature data [5] was used to simulate biomass growth and naphthalene degradation by *Cycloclasticus spp.* under repeated low-dosage naphthalene pulses compared to single high-dosage inputs [6]. Results indicated that pulsing frequency and dosage concentration significantly influence naphthalene degradation efficiency, with dissolution kinetics limiting biodegradation and bacterial growth. Under the assumption of first-order degradation kinetics, high-dosage inputs resulted in more rapid degradation, suggesting that repeated low-dosage inputs could potentially lead to PAH accumulation in pristine environments depending on the relation of degradation half-life and input frequency.

To assess low-dosage PAH impacts, functional gene expression levels in *Cycloclasticus pugetii* strain PS1 were quantified under varying PAH loads in laboratory experiments [7, 8]. High concentrations (200 mg L^{-1}) revealed substrate-independent expression of three key functional genes (i.e., *rhd2a*, *rhd3a*, and *pahE*) during degradation of naphthalene and phenanthrene and in no-PAH control incubations [*7]*. RNA sequencing experiments with high and low naphthalene doses (100 versus around maximal solubility at 30 mg L^{-1}) revealed the transcription of 15 genes involved in the initial degradation step,

demonstrating genetic redundancy. Some genes were transcribed during active degradation, while others were expressed under naphthalene-starvation conditions [8]. These findings suggest two distinct enzymatic systems for naphthalene degradation: one for "background" degradation through substrate-independent gene transcription, and another for a "rapid response" to naphthalene exposure. This flexibility may help specialized PAH degraders like *Cycloclasticus spp.* to adapt to varying pollutant concentrations.

Our work highlights that PAH-biomarker gene transcription does not always correlate with degradation activity, necessitating careful interpretation of (meta-)transcriptomic data. The results also emphasize the significant impact of PAH dosage and frequency of exposure on biodegradation, and improves our understanding of PAH fate in marine ecosystems. By combining numerical modeling with lab studies, this research offers key insights for environmental risk assessment and bioremediation strategies for diffuse oil contamination.

Abstract ID: 3027 / Poster Number: 163.17P

Functional insights into Rv0783c of Mycobacterium tuberculosis: a multidrug efflux pump with a prominent role in biofilm formation

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Introduction: A critical factor for the emergence of multidrug-resistant tuberculosis (MDR-TB) is the activity of efflux pumps that reduces drug efficacy by extruding antibiotics from the cells. Therefore, it is crucial to understand the underlying mechanisms of drug efflux in mycobacteria.

Objective: This study investigates the contribution of Rv0783c of *Mycobacterium tuberculosis* that is apparently upregulated in several MDR-MTB strains.

Methods: The MTB gene, *rv0783c*, was cloned in pBAD18-Cm and pMIND vectors for *in-vivo* expression. The effects of rv*0783c* expression in *Escherichia coli* & *Mycobacterium smegmatis* on antibiotic resistance were elucidated by determining minimum inhibitory concentration (MIC) using micro-broth dilution. Intracellular antibiotic accumulation and energy-dependent real-time efflux were assayed *in vivo*. Additionally, biofilm formation with expressed *rv0783c* was evaluated using crystal-violet staining and microscopy.

Results: Ectopic expression of *rv0783c* in *E. coli* & *M. smegmatis* have increased the resistance to various antibiotic classes, namely, fluoroquinolones, aminoglycosides, beta-lactams, tetracyclines, anti-TB drugs and dyes. The addition of carbonyl-cyanide-m-chlorophenylhydrazone (CCCP), proton-motive force (pmf) inhibitor, eventually reversed the resistance gained, demonstrating pmf dependence of Rv0783c. *E. coli* & *M. smegmatis* cells expressing *rv0783c* show lower intracellular accumulation of dye (EtBr), fluoroquinolones (sparfloxacin, norfloxacin, lomefloxacin), Bocillin FL, which suggests the involvement of Rv0783c in drug efflux. Real time assays with EtBr & Nile-Red efflux further demonstrate the energy-dependence of Rv0783c. Interestingly, upon *in trans* expression of *rv0783c* the host *E. coli* & *M. smegmatis* cells form enhanced biofilms (48% & 59%, respectively) than that of WT.

Conclusion: Rv0783c might be a pmf-dependent multidrug-transporter, capable of extruding structurally unrelated antibiotics and can facilitate biofilm formation.

Abstract ID: 4145

Functional microbiomics of the gut-brain axis: industrial perspective about future interventions and analytics

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Brain health solutions for mental performance, metal well-being, sleep neuro-protection and healthy ageing consist nowadays mainly of vitamins, omega-3 and carotenoids. New avenues for interventions come to life around the microbial metabolites produced by the gut microbiome. Modulation of the microbiome contribute to differential metabolic output. Examples of the gut metabolites of interest are primary metabolites such as short chain fatty acids, but also secondary metabolism compounds such as amino acids-based metabolites or polyphenols for example. Foundational work about the identification of gut microbes biosynthetic pathways producing these metabolites of interest was published by Vales-Colomer (2019).

Industrial application/interventions on the gut-brain axis can rely on microbiome modulations using probiotics, prebiotics, synbiotics and postbiotics. However more physiologically relevant technologies combined with augmented metabolic-driven translation are needed to accelerate and de-risk the innovation discovery pipeline.

New tools about metabolic network mapping, based on broad metabolomics facilitate physiologically-relevant translation, and allow global dynamic or differential metabolic footprinting, that ultimately supports elucidation of mode of action and identifies basis for novel intellectual property claims.

Abstract ID: 2696 / Poster Number: 321.17P

Functional soil microbial profile in lettuce (Lactuca sativa L.) grown with a combination of compost, biochar and PGPR

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Co-authors: *Luciano Orden, José Antonio Saez Tovar, Lucía Valverde Vozmediano, Maria Angeles Bustamante Muñoz, Encarnacion Martinez Sabater, Diego Cunha Zied, Raul Moral Herrero*

Background: Compost, biochar and biological inputs are tools that, if properly managed, can be effective in making agricultural systems more sustainable and resilient.

Objective: We investigated the combinations effect of compost, biochar and plant growth-promoting rhizobacteria (PGPR) on the functional soil microbiota profile in a lettuce trial.

Materials and Methods: The experiment was conducted in a controlled growth chamber, where lettuce seedlings were transplanted into pots. Each pot was filled with 1,3 kg of OECD standard soil and fertilized with the proposed treatments. These treatments included: the application or absence of 200 kg ha^{-1} of nitrogen fertilizer in the form of synthetic NPK (15-15-15), compost, or organo-mineral pellets (7-7-7); the addition or absence of 10 tha^{-1} of biochar derived from olive mill waste; and the inoculation or non-inoculation of the soil with *Azospirillum brasilense* + *Pseudomonas putida* (Az+Pp) or *Bacillus megatherium* (Bm). The test was carried out in triplicate and a homogeneous soil sample was taken from each pot at the end of the harvest. Serial dilutions were prepared in saline solution and plated on different media: PDA and NA (general microbial community), YMA (for rhizobia), Pikovskaya agar (for phosphate solubilizers), Aleksandrow agar (for potassium solubilizers). Analyses were performed in duplicate, using dilutions between 10^{-4} and 10^{-6}.

Results and Conclusion: The results showed that applying microorganisms (Az+Pp or Bm), compost, and organomineral pellets enhanced the microbial community, while pellet and biochar improved rhizobia, phosphorus, and potassium solubilizers ($p<0.05$). These tools have been show a feasible strategy to improve soil biological health and fertility.

Abstract ID: 2649 / Poster Number: 389.15P

Functional study of the Planctomycetes division by budding and developed endomembrane systems

Presenting author: Luis Felipe Muriel, PhD, Institut Pasteur de Lille, Lille, France

Co-authors: *Angeline Reboul, Damien Devos*

The *Plantomycetota*, *Verrucomicrobiota*, and *Chlamydiota* (PVC) superphylum is a supergroup encompassing highly diverse bacteria with different cellular biology and relevance for medicine, basic research, and biotechnology. *Planctomycetota* members share several features that are rare in bacteria but common in eukaryotes and archaea. Some of these traits include budding division and the presence of developed endomembrane systems, linked to endo- and phago-cytosis-like phenomena. Additionally, some membrane-coat-like (MC) proteins, which are structurally similar to the eukaryotic clathrin protein, have been identified to be in close interaction with the PVC endomembrane system. Thus, the study of PVC bacteria is of great interest for understanding the evolutionary aspects of eukaryotic compartmentalization.

In this study, we aim to characterize the growth of these bacteria and the function of the complex endomembrane systems and MC proteins in *Gemmata obscuriglobus* and *Planctopirus limnophila*, two representative species of the *Planctomycetota* phylum.

We show that the bud of *P. limnophila* seems to always occur at the same pole, and the daughter cell is entirely composed of newly synthesized material. To understand the role of MC proteins, we constructed strains carrying a fusion between an MC protein and the msfGFP fluorescent reporter. *In vivo* expression in both *G. obscuriglobus* and *P. limnophila* showed periplasmic localization and/or contact with membranes, distinct from the cytoplasmic expression of the non-fused msfGFP control. These results open great perspectives in PVC bacteria, from understanding the growth of the bud to the interaction of MC proteins with the membranes or membrane-associated proteins, and associated phenomena.

Abstract ID: 2801 / Poster Number: 323.17P

Functional traits of consortia of Propionic Acid Bacteria and Lactic Acid Bacteria as starters for faba bean (Vicia faba L) fermentation

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Co-authors: *Shahana Akhter, Maria Deng, Prabin Koirala, Minnamari Edelmann, Pekka Varmanen, Rossana Coda*

Background: There is a growing need for food from sustainable sources, and fermented plant-based options like faba beans provide a promising solution. Faba beans are high in protein and fibre and could be used more in food products, however, some negative attributes remain, such as antinutritional factors and flavour. One potential solution is to use starter cultures able to hydrolyse raffinose oligosaccharides (RFOs), enhance protein solubility, and potentially synthesize bioactive compounds such as vitamin B12, which can improve nutritional properties. To achieve this, it is essential to specifically design the fermentation process focusing on the interaction between starter microbes, raw materials, and processing conditions.

This study aims to design microbial consortia of lactic acid bacteria (LAB) and propionic acid bacteria (PAB) to ferment faba bean to improve its potential nutritional benefits.

Method: Strains of LAB and PAB selected based on their phytase, peptidase I, aminopeptidase N and beta-glucosidase enzyme activity, growth rate, reduction of pH and production of acids and vitamin B12 were used in consortia to ferment faba bean model media. Strain performance was assessed using plating methods and HPLC analysis (sugars, acids and vitB12).

Results: Preliminary results show that in 4 consortia of PAB and LAB, both the starters were able to grow in the medium. The consortia were able to ferment the medium, synthesize organic acids and vitB12 and decrease RFOs to different extents.

Conclusion: PAB and LAB consortia successfully fermented faba bean model media, confirming their feasibility as starters for faba bean based fermented food.

Abstract ID: 3728

Fungi and plastics can co-exist? Stories of an uncommon duo

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Despite the current efforts to limit the impact of plastic on the environment, we cannot oversee the actual risk the entire ecosystem is exposed to. Bioplastic has surely mitigated the problem but their fate in the environment cannot be underestimated. Indeed, without a proper disposal, they may trigger undesirable effects on terrestrial and aquatic organisms. Nature is not stand unarmed but actually react to it. Knowing these responses may help us to develop new solution and optimize the management process.

The mycobiota associated to the plastisphere is indeed poorly-studied, with very few information available about the degrading skills of these organisms. The goal of the present research was to investigate extreme polluted environments, including plastic-polluted landfill soil and organic waste treatment plants, as well as marine water and sediments. The microbial community was studied through culturomic and metagenomic approaches, focusing the attention of both traditional polymers and bioplastic.

The isolated fungal community differed a lot, with a strong role of the site, the plastic material and environmental parameters as the salinity. Hundred strains were isolated, mostly associated with the Ascomycota phylum. Despite the filamentous fungi were very abundant, yeasts were not neglectable (approx. 10%). Studies confirmed that environmental parameters differently affect the microbial community (Figure 1).

Being capable of living in the plastisphere cannot be considered as an axiom of being capable of transforming plastic. Studies were focused on the evaluation of this phenomenon: growth screenings were followed by liquid culture to quantitatively measure the polymer transformation yields. Many fungal strains (i.e. *Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, Purpureocillium*) showed the capability to degrade plastic polymers with the consequent production of monomers.

Fungi were also tested against different polymers and in peculiar environments, including sea water, agricultural soils and organic treatment plants. In a model marine environment, selected fungi did transform bioplastics, even though halving the yields

obtained in fresh water. Moreover. when used as pre-treatment of plastic for further anaerobic digestion, they helped to strengthen and fasten bioplastics degradation.

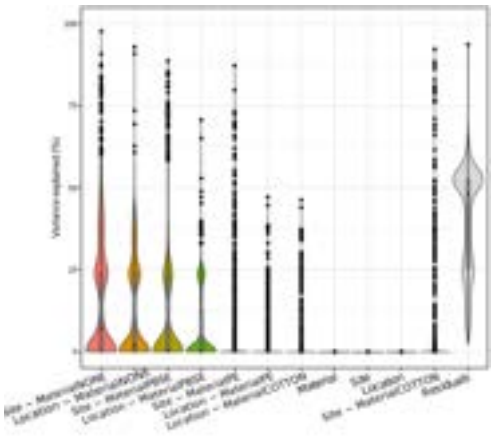


Figure 1: Variance partitioning analysis on the fungal community and associated metadata.

Abstract ID: 2117 / Poster Number: 016.15P

Fungi inhabiting the microbialites of the alkaline lake in Pantelleria island (Italy): biodiversity across a submerged-emerged transect

Presenting author: Dr. Flavia Pinzari, Consiglio Nazionale delle Ricerche, Montelibretti (Rome), Italy

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The Bagno dell'Acqua alkaline lake in Pantelleria island, Italy, is characterized by CO_2 emissions, alkaline waters with a pH of around 9, and strongly oxidizing conditions. The lake is home to actively growing microbialites rich in calcium carbonates and silica precipitates. The lake's sediments are dominated by Mg-smectite clays associated with K-feldspars and Ca-carbonates, and the presence of volcanic glass and minor phases like pyroxene and hematite has been observed. These extreme conditions impact the fungal diversity in the sediments and microbialites, offering insights into the adaptation of fungi to high pH values. A transect from the lake's margins to the line of vegetation was used as a sampling scheme to evaluate the impact of alkaline water on the fungal communities in the first 5 cm of sediment depth. High-throughput sequencing of the fungal internal transcribed spacer 2 (ITS2) molecular marker region, performed with the Illumina NovaSeq PE250, was applied to compare the alpha and beta biodiversity of the microbialite samples. Fungi were also isolated using classic culturing and the lake sediment as the culture medium. The strains isolated from the submerged sediments are entirely different from those obtained from the microbialites that were exposed to the air at the time of sampling, suggesting the overlap of two distinct fungal communities, those that colonize the surfaced material (airborne species) and instead the species that seem to inhabit the submerged plant material. The latter appeared poorly pigmented compared to the species isolated from the vegetation line.

Abstract ID: 2666 / Poster Number: 357.16P

Fusobacterium nucleatum promote subspecies- and strain-specific cancer cell proliferation

Presenting author: Chiara Nicoletti, PhD, Università cattolica del Sacro Cuore, Roma, Italy

Background: Accumulating evidence shows that the oral pathogen *Fusobacterium nucleatum* is involved in the progression of an increasing number of tumor types. The mechanisms underlying tumor exacerbation by *F. nucleatum* include enhancement of cell proliferation that promotes metastasis.

Objective: To characterize different subspecies and strains of *F. nucleatum* for their ability to promote proliferation of cancer cells.

Methods: Isolation in culture, identification by MALDI-TOF and by genetic analysis of Fusobacteria from clinical specimens. Infection of Caco-2, HN-13 and MCF-7 cancer cells with *F.nucleatum* ATCC 25586 and a set of strains of *F.nucleatum* ssp: *nucleatum*, *animalis*, *polimorphum* e *vincentii* isolated from OSCC patients. Cloning of the pVopO-GFP plasmid whit the *F. nucleatum* ATCC strain. Evaluation of cell proliferation by staining nuclei and cell count using the Cytation instrument. Assessment of intracellular persistence by CFU count. Cell migration and proliferation also assessed by wound healing tests.

Result: We generated a fluorescent GFP-expressing *F. nucleatum* ssp *nucleatum* ATCC strain and defined the experimental conditions to assess the biological effects using Caco-2 cells: we used different MOI, with the presence or not of antibiotics during the incubation period and measured intracellular and extracellular CFUs. A significant cell proliferation was measured 24h post-infection. We then infected the Caco2, HN-13 and MCF-7 cell lines with 37 Fusobacterium strains of different subspecies, isolated from OSCC patients. The results obtained indicate that *F. nucleatum* subsp. *animalis* and *F. nucleatum* subsp. *nucleatum* can promote the highest proliferation, as measured as increase in the total number of cells, the scratch assay and the invasion assay.

Conclusion: The genetic characterization of these strains coupled with the molecular characterization of the cellular pathways on tumor cells involved in these processes will help us unravel the impact of bacterial variability in the tumorigenic properties of this emerging bacterial species.

Abstract ID: 3366 / Poster Number: 334.17P

Gene essentiality in the solventogenic Clostridium acetobutylicum DSM 792

Presenting author: Dr. François Wasels, IFP Energies nouvelles, Rueil-Malmaison, France

Co-authors: *Alexandre Delarouzée, Nicolas Lopes Ferreira, Chloé Baum*

Introduction: *Clostridium acetobutylicum* is a solventogenic, anaerobic, gram-positive bacterium that is commonly considered the model organism for studying Acetone-Butanol-Ethanol (ABE) fermentation. The need to produce these chemicals sustainably and with a minimal impact on the environment has revived interest in research on this bacterium. The recent development of efficient genetic tools allows us to better understand the physiology of this microorganism, aiming to improve its fermentation capacities.

Objective: Knowledge about gene essentiality would guide future genetic editing strategies and support the understanding of crucial cellular functions in this bacterium.

Method: In this work, we applied a transposon insertion site sequencing (TIS) method to generate large mutant libraries containing millions of independent mutants with unique insertion sites, allowing us to identify essential genes.

Findings: In total, we identified a core group of 418 critical genes needed for *in vitro* development on rich media containing glucose as a carbon source.

Conclusion: Future research on this model organism will be guided by the data provided in this work, which will serve as a valuable resource for the community.

Abstract ID: 2592 / Poster Number: 313.16F

Genetic and phenotypic diversity of Streptococcus spp. strains isolated from a low-moisture Mozzarella production site

Presenting author: Ms. Vittoria Piccini, Technical University of Denmark, Copenaghen, Denmark

Co-authors: *Martin Laage Kragh, Yinghua Xiao, Søren Dollerup Nielsen, Ellen Wemmenhove, Lisbeth Truelstrup Hansen*

Streptococcus thermophilus is a lactic acid bacterium (LAB) used as starter cultures (starters) in fermentations in the dairy industry. However, non-starter wild type (WT) *S. thermophilus* can survive thermal treatments, produce biofilm and hence colonize dairies to contaminate processing lines and final products, including cheese whey which is later used for various high-value ingredients.

This study aims to explore key genomic differences between *S. thermophilus* starters and WTs and map the diversity of WT strains in a cheese plant. Furthermore, phenotypical characteristics of dairy isolates, such as heat resistance and biofilm formation were investigated.

In this work, we analysed organic and conventional milk samples (total sampling rounds n=6) after heat treatment from different locations (n=7) in a cheese plant. *Streptococcus* spp. strains were identified by MALDI-TOF MS and the genomes of 136 representative isolates sequenced. Biofilm formation capacity of relevant strains was evaluated through safranin staining and heat resistance experiments were performed in skim milk.

Results show that *Streptococcus* spp. were present in 57% of the samples. According to whole genome phylogeny and average nucleotide identity, most *Streptococcus* spp. strains could be grouped into four major clusters. Twelve presumed WT strains had between 150-200 single nucleotide polymorphism differences compared to known starters, which may suggest starters at some point have adapted to the dairy environment. Knowledge of genotypes, and phenotypic diversity will be used to design of a detection method and mitigative strategies for control of WT *Streptococcus* spp. to ensure product quality.

Abstract ID: 2215 / Poster Number: 161.15P

Genetic determinants of rhodibalamin resistance in Cutibacterium acnes and Pseudomonas aeruginosa

Presenting author: Dr. Michael Paxhia, Quadram Institute Bioscience, Norwich, United Kingdom

Co-authors: *Keith Turner, Shikha Saha, Sree Javvadi, Mark Webber, Martin Warren*

Cobalamin (Vitamin B_{12}) requires cobalt for its activation and use as a coenzyme, however inactive chemical analogues can by synthesized with other metals such as rhodium in the corrin ring. Collectively these are classified as B_{12} antivitamins. Previous work has shown that several bacterial species are sensitive to these antivitamins, however the molecular mechanisms underlying the sensitivity and resistance have so far not been characterized. Two opportunistic pathogens, *Cutibacterium acnes* and *Pseudomonas aeruginosa*, were found to be sensitive to rhodibalamin. Monitoring the effect of antivitamin exposure on cobalamin pools in the cell shows that *C. acnes* converts several forms of rhodibalamin into adenosylrhodibalamin (AdoRhbl), which corresponds with differences in the inhibition of growth of this organism. Isolation of spontaneous suppressors of *C. acnes* and transposon-directed insertion site sequencing (TraDIS) in *P. aeruginosa* were performed to better understand the downstream effects of rhodibalamin on either organism. Mutants in transporters or cobalamin-dependent transcriptional regulation suggest that the effect of rhodibalamins on these organisms is coordinated by accumulation of AdoRhbl and its regulatory effect on cobalamin riboswitches, predicting physiological changes that lead to a lack of growth. This work expands our understanding the mechanism of inhibition of rhodibalamins across two opportunistic pathogens and provides insights into their underlying cobalamin regulatory networks.

Abstract ID: 2979 / Poster Number: 063.15P

Genetic diversity and population structure of italian Candida albicans isolates of animal origin

Presenting author: Letterio Giuffrè, PhD, University of Messina, Messina, Italy

Co-authors: *Silvia Crotti, Lucilla Lo Re, Deborah Cruciani, Alice Ranucci, Ylenia Lascala, Orazio Romeo*

Introduction and Objective: *Candida albicans* is recognized as a human and animal pathogen of critical priority worldwide and despite the “One-Health” rhetoric integrating human, animal, and environmental health, to date, there is an evident paucity of studies exploring the genetic diversity of this important pathogen in veterinary and environmental settings.

Method: In this study, 41 *C. albicans* isolates were collected from several animals (hedgehog, porcupine, badger, wolf, roe deer, and fox). After conventional phenotypic identification and further characterization via MALDI-ToF and/or molecular techniques (*hwp1* gene-amplification), the isolates were genotyped using the "ABC typing" method and the official Multi-Locus Sequence Typing (MLST) scheme. Moreover, the configuration of the mating-type locus (MTL) was also analyzed.

Findings: All isolates were heterozygous at the MTL locus (MTL-a/α). MLST analysis revealed significant genetic diversity, identifying 116 alleles (20 novel) and 36 Diploid Sequence Types (DSTs), including 28 previously undescribed genotypes. Phylogenetic and clonal-complex analyses did not identify any animal-specific genetic clusters as some DSTs were also found in human isolates, suggesting a wider-host flexibility for these genotypes. Most isolates (27) were classified as genotype B, 10 were genotype A, and the remaining 4 showed an atypical genetic pattern not reported before. One of these atypical *C. albicans* isolates was selected for whole-genome sequencing.

Conclusion: The findings of this study highlight wildlife animals as a potential reservoir of novel *C. albicans* genotypes, some of which may have significant implications for public and veterinary health.

Abstract ID: 2734 / Poster Number: 322.17P

Genetic improvement of Lachancea thermotolerans for table olive fermentation by mixing for hybridization with Wickerhamomyces anomalus

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Co-authors: *Benjamin Vázquez-Conejo, Joaquin Bautista Gallego*

Yeasts play an important role during the whole production process of table olives, helping lactic acid bacteria (LAB), improving the organoleptic profile, etc. Unfortunately, yeasts have poor efficiency in completing table olive fermentation without LAB. However, Lachancea thermotolerans (Lt) have been proposed as a new tool to improve the table olive performance due to, among others, its high capacity to acidification. This fact can improve deeply the initial stages of the fermentation process, considerably reducing the time required to achieve the final conditions of microbiological safety.

Due to this goal, strains with high resistance to sodium chloride and pH were selected and their genetic improvement by hybridization of Lt with Wickerhamomyces anomalus (Wa) was assayed. The new yeast mixture clones (Wa-mixed Lt) showed an intermediate phenotype between both parent yeasts for some relevant biotechnological properties, such as resistance to sodium chloride, pH, copper, SH_2 production, as well as flocculation potential. These properties varied depending on the specific Wa-mixed Lt clone. Several mixture clones showed improved capability for table olive fermentation as compared to the Lt parent strain, approaching the fermentation capability of the Wa parent strain but with a higher production of acids. Finally, those table olives fermented by the hybridized yeasts showed the best organoleptic quality score.

The new selected Wa-mixed Lt yeasts showed improved and a better performance than their parent yeasts. Additionally, they are stable enough to be marketed for industrial table olive and other vegetable production.

Abstract ID: 2041 / Poster Number: 109.17P

Genetic insight of fluoroquinolone-resistant group B Streptococci isolates in Serbia

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Introduction: Group B streptococci (GBS) can asymptomatically colonise the intestinal and genitourinary tracts of humans, or cause neonatal infections and emerging infections in the elderly. Although penicillin is the first therapeutic choice, multidrug-resistant (MDR) strains resistant to quinolones are also reported.

Objective: This study aimed to detect the molecular characteristics of antimicrobial resistance and genotypes of fluoroquinolone-resistant GBS strains in Serbia.

Method: The study included 23 confirmed quinolone-resistant GBS isolates from the collection of the National Reference Laboratory for Streptococci Serbia in the period 2015. to 2023. The resistance mechanisms to macrolides, tetracyclines, and capsular types (CPS) were determined by multiplex PCR, while the quinolone resistance was detected by sequencing.

Findings: The main fluoroquinolone resistance determinant was substitutions on the *parC* gene (95.6%), primarily S79F, while substitutions on the *gyrA* gene were detected in 52.2% (primarily S81L). Macrolide resistance genes (*ermA, ermB, and mefA*) were evenly detected in 34.8%, while the *tetM* gene was primarily responsible for tetracycline resistance (78.3%). The dominant CPS type was type V – 52.2%. The majority of strains were MDR (60.9%), simultaneously resistant to fluoroquinolones, macrolides, and tetracyclines. Among them, CPS type V was also the most frequent (71.4%), while CPS types III, II, and IV accounted for 28.6%.

Conclusion: The present study indicates that GBS CPS type V is the main lineage responsible for the spread of fluoroquinolone-resistant strains in Serbia, with the *parC* gene (S79F) primarily affected. The same lineage is responsible for the spread of MDR strains, carrying both macrolide and tetracycline resistance.

Abstract ID: 2028 / Poster Number: 108.17P

Genetic landscape of Streptococcus uberis isolated from bovine mastitis; typing and antibiotic resistance in-depth analysis

Presenting author: Dr. Alicia Romano', Agroscope, Bern, Switzerland

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Bovine mastitis remains the leading cause of economic loss at the dairy herd level. *Streptococcus uberis* is one of the most important mastitis pathogens and has shown increased resistance to antibiotics. Though clinically relevant, *Strep. uberis* is still poorly characterized in terms of subtypes and their genotypic and phenotypic antimicrobial resistance (AMR) profiles.

The aim of our study was to characterized genotypically and phenotypically 109 *Strep. uberis* mastitis strains.

All strains were subjected to whole genome sequencing and tested for phenotypic antimicrobial resistance using the minimum inhibitory concentration (MIC) assay. The WGS data were used for subtyping the strains by MLST, evaluating their genotypic AMR profile and presence of mobile genetic elements using bioinformatics tools. RAPD-PCR (Random Amplified Polymorphic DNA-PCR) was used as an additional method for genotyping 43 strains for which supplementary clinical data had been collected.

Our results showed high level of variability in the genotypes identified by both MLST and RAPD-PCR methods. Regarding antibiotic resistance, seventy strains (64%) were susceptible to all the 26 antibiotics tested; thirty-nine strains were resistant to cephalosporins (2%), lincosamides (12%), macrolides (12%), and tetracycline (33%). Eleven strains (10%) were resistant to three different classes of antibiotics (multi-drug resistant strains). Fifteen ARGs were detected with a majority related to tetracycline resistance (*tetL, tetM*, and *tetO*) carried by mobile genetic elements integrated in the genome.

The present study explains the observed phenotypic tetracycline resistance of *Strep. uberis* at the genetic and genomic level and demonstrates a non-understood phenomenon of a high Sequence Type diversity.

Abstract ID: 3626 / Poster Number: 149.15P

Genetic variability of Bacillus spp. using REP-PCR marker

Presenting author: Adriane Wendland, PhD, Brazilian Agricultural Research Corporation – Embrapa, Goiania, Brazil

Co-authors: *Enderson Ferreira, Kassia Lorrany M. De Paula*

The study analyzed the genetic variability of Bacillus spp. using the REP-PCR technique. Twenty-seven isolates from the Embrapa Arroz e Feijão microbial collection were examined. DNA was extracted and amplified using the REP (Repetitive Extragenic Palindromic) primers under specific thermocycler conditions. The resulting PCR products were separated via electrophoresis and visualized under UV light. A binary matrix (presence/absence of bands) was used to calculate genetic distances based on Jaccard's similarity index with R software. A dendrogram was constructed using the UPGMA algorithm, with node consistency evaluated through 10,000 bootstraps. The analysis revealed 23 unique fingerprint profiles among the isolates, with bands ranging from 300 to 2100 bp. The number of bands per isolate varied from 0 to 7, and the isolates were grouped into six main clusters. The results demonstrated significant intra- and interspecific genetic diversity within the genus. This highlights the effectiveness of REP-PCR as a molecular tool for characterizing Bacillus spp. and identifying genetic variability. The study confirms the potential of REP-PCR not only for bacterial genotyping but also for broader applications, emphasizing its role in studying beneficial bacteria used in agriculture. The findings provide a basis for further exploration of Bacillus spp., aiding strain selection for agricultural purposes.

Abstract ID: 2480 / Poster Number: 255.17P

Genome Analysis and Functional Insights into Methylomonas sp. QW1: A Rapidly Growing Methanotroph with Secondary Metabolite Biosynthesis Potential

Presenting author: Mr. Si Chul Kim, Kunsan National University, Gunsan, South Korea

Co-authors: *Ye Jin Gwak, Gi Wook Hong, Hyo Jung Lee*

Methylomonas sp. QW1 was isolated from a rice paddy in Taean-gun, South Korea (37°05'40.7"N, 129°01'24.6"E) and demonstrates the ability to rapidly utilize methane and methanol as carbon sources. Whole-genome sequencing using Illumina and PacBio revealed a genome size of 4,928,131bp with a G+C content of 51.7mol%. The strain harbors complete pathways for the Embden-Meyerhof-Parnas (EMP), pentose phosphate (PP), Entner-Doudoroff (ED), and tricarboxylic acid (TCA) cycles, highlighting its metabolic versatility. Functional genome annotation revealed the presence of both particulate methane monooxygenase (pMMO) and soluble methane monooxygenase (sMMO), suggesting enhanced efficiency and flexibility in methane oxidation under diverse environmental conditions. Genomic analysis using antiSMASH 7.0 revealed pathways for synthesizing value-added secondary metabolites, including pyrroloquinoline quinone (PQQ) and squalene, utilizing methane as a one-carbon source. Comparative genomic analysis of 11 Methylomonas strains identified 80 unique Clusters of Orthologous Groups (COGs) in QW1, with 37.5% categorized as “function unknown” and 23.75% related to replication, recombination, and repair. The high proportion of replication-related genes may correlate with the strain’s rapid growth capability. This study provides a comprehensive genomic perspective on *Methylomonas* sp. QW1, revealing its genetic traits, secondary metabolite biosynthetic pathways, and metabolic potential. These findings offer valuable insights into the biological capabilities of methanotrophs and their potential applications in biotechnology and sustainable methane utilization.

Abstract ID: 2473 / Poster Number: 139.15P

Genome analysis and Phylogenetic Characterization of Lacrimispora faecalis sp. nov., a Novel Acetogen isolated from the Cow Feces, South Korea

Presenting author: Ms. Ye Jin Gwak, Kunsan National University, Gunsan-si, South Korea

Co-authors: *Hyo Jung Lee, Si Chul Kim, Gi Wook Hong*

Strain KS-F26, a strictly anaerobic acetogen, demonstrates the ability to convert carbon dioxide (CO_2) into multi-carbon compounds via the Wood-Ljungdahl pathway. The strain was isolated from cow feces and enriched in AC-B1 medium at 37°C under H_2:CO_2:CO (65:15:20) as the energy and carbon source. Cells were motile, rod-shaped without oxidase and catalase activity. Strain KS-F26 growth was observed at 20-40 ℃ (optimum, 37 ℃), with 0-8 % (w/v) NaCl (optimum, 1%) and pH 5.0-10.0 (optimum, pH 10.0). Phylogenetic analysis of the 16S rRNA gene showed strain KS-F26 was located within the genus *Lacrimispora*. Whole-genome sequencing using Illumina and PacBio revealed a genome size of 4,928,819 bp with a G+C content of 43.4 mol%. Pan-genome analysis with genome of all strains belonging into *Lacrimispora* identified 4,282 genes, including 1,762 core genes and 327 unique genes of strain KS-F26. Functional annotation using COG classified 4,224 genes into 20 categories, with “Functional unknown (S)” as the most abundant. CAZyme analysis identified 94 carbohydrate-active enzymes (49 GHs, 37 GTs, 4 CBMs, 4 PLs), supporting the strain's metabolic versatility. Secondary metabolite biosynthesis and metabolic gene clusters predicted using anti-SMASH and gut-SMASH revealed 3 BGCs (ranthipeptide, cyclic-lactone-autoinducer) and 17 MGCs (e.g., xanthine dehydrogenase, Putrescine2spermidine). Genome analysis confirmed the presence of genes for the Wood-Ljungdahl pathway, underscoring strain KS-F26's CO_2 metabolism and biofuel production potential. Based on phenotypic, chemotaxonomic, and molecular analysis, strain KS-F26 is proposed as *Lacrimispora faecalis* sp. nov. These findings provide critical insights into acetogen genetics and their applications in sustainable energy.

Abstract ID: 144 / Poster Number: 054.17P

Genome mining of verotoxigenic Escherichia coli O157:H7 NWU_A_F_E. coli_201 from cattle faeces: elucidating the resistome, virulome, and non-LEE effector (nle) determinants

Presenting author: Prof. Collins Njie Ateba, University of Mpumalanga, Mbombela, South Africa

Co-authors: *Adia Kagandagaza Kabelinde, Daniel Jesuwenu Ajose,*

Verotoxigenic Escherichia coli O157:H7 (VTEC O157) are important foodborne pathogens that are of great concern to public health. The characterisation of virulence determinants in VTEC O157:H7 strains has predominantly focused on prominent genes, including those associated with Shiga toxin and intimin, which are located within the locus of enterocyte effacement (LEE). This study employed whole genome sequencing (WGS) as a means to identify and characterise non-LEE effector genes in a VTEC O157:H7 strain NWU_A_F_E. coli_201. The genome of NWU_A_F_E. coli_201 obtained from cattle faeces was subjected to WGS analysis. WGS analysis confirmed that the NWU_A_F_E. coli_201 isolate is an E. coli of the serogroup O157with a genome size of 5,463,336 bp, GC content of 50.42% and 400 contigs. The genome harboured some virulence genes including the haemorrhagic E. coli pilus (hcpA), and the type I fimbriae (fimA and fimI) Notably, a total of 12 non-LEE effector genes including extra spindle poles-like (espL1and espL2), epithelial splicing regulatory protein (espR1 and espR3), and effector protein (espY4 and espY5) were detected in the genome. Moreover, antibiotic resistance genes coding for different classes of antibiotics such as trimethoprim (folA, dfr), sulfonamide (folP), quinolone (gyrA, gyrB, and norA), and broad spectrum resistance to aminoglycoside, phenicol and tetracycline (ykkCD) were detected. The identification of these non-LEE virulence genes as well as determinants encoding several drug resistant traits in the genome of NWU_A_F_E. coli_201 highlights its public health significance.

Abstract ID: 627 / Poster Number: 387.16P

Genome-based classification of Streptomyces tatrensis sp. nov., actinomycetes isolated from moonmilk in Szczelina Chochołowska cave of Tatra Mountains in Poland

Presenting author: Prof. Patrycja Golińska, Nicolaus Copernicus University in Toruń, Toruń, Poland

Co-authors: *Patrycja Bielańska, Elaine Romines, Łukasz Rąbalski, Grzegorz Węgrzyn*

Introduction: The genus Streptomyces is the largest taxon of the phylum *Actinomycetota* which members are widely distributed in the environment.

Objective: taxonomic position of two actinomycete strains isolated from moonmilk of cave in Poland and their genome-mining were subjected to study.

Method: The comparative studies based on 16s rRNA sequences and phylogenomic studies were carried out for isolates 2.9T and 4.24 to check their relationships with members of the genus Streptomyces. Their genome sequences were analysed for biosynthetic gene clusters (BGCs) and stress related genes. The isolates were analysed for morphological, cultural, chemotaxonomic and phenotypic properties.

Findings: The isolates exhibited morphological and chemotaxonomic properties consistent with their classification in the family Streptomycetaceae. Comparative 16S rRNA gene sequence studies showed that isolates were most closely related to the type strains of Streptomyces avidinii, S. cirratus, S. nojiriensis, S. spororaveus, S. subrutilus, S. vinaceus and S. xanthophaeus (99.72% similarity). The average nucleotide identity and genomic DNA–DNA hybridization values showed closest relationship of the isolates to the type strain of S. nojiriensis, but these values were well below the cut-off points recommended to assign bacterial strains to the same species. Genome sequences of the isolates were rich in BGCs predicted to produce novel specialized metabolites and stress-related genes which provided an insight into how they have adapted to the conditions that prevail in cave.

Conclusion: Consequently, it is proposed that the isolates be recognized as a new Streptomyces species, Streptomyces tatrensis sp. nov., with isolate 2.9T as the type strain.

Abstract ID: 1826 / Poster Number: 046.15P

Genome-resolved insights into the microbial diversity in the Alaskan tundra through single-cell genomics and long-read metagenomics

Presenting author: Nu Ri Myeong, PhD, Korea Polar Research Institute, Yeonsu-gu, South Korea

Co-authors: *Mincheol Kim*

Permafrost, a unique habitat for cold-adapted microbiomes, is a layer of soil that remains frozen for at least two consecutive years and is primarily found in high-latitude regions of the Northern Hemisphere. It harbors a highly diverse microbial community that plays crucial roles in carbon cycling, methane production, and adaptation to extreme environmental conditions. However, the distribution and functional potential of many permafrost microorganisms remains unexplored. In this study, we employed various genomic techniques, including both short-read and long-read metagenomics, as well as single-cell genomics, to uncover the unexplored taxonomic and functional diversity of the active layer and permafrost in the Alaskan moist tundra. This approach led to the reconstruction of 485 high-quality genomes, including numerous novel species with unique metabolic capabilities, which are essential for understanding carbon cycling in permafrost environments. Notably, methanogens, including a novel species of *Methanoflorens*, were abundant and displayed region-specific adaptations, suggesting ecological divergence among methanogens across different permafrost sites. Furthermore, a strong correlation was observed between syntrophic degradation activity by bacteria and permafrost methanogens, indicating the interconnected nature of these microbial communities in carbon cycling processes. These organisms harbor a diverse range of genes associated with carbon cycling, including pathways for decomposing complex organic compounds, which are essential for carbon and methane cycling in permafrost ecosystems under warming conditions. Our study enhances the understanding of permafrost microbial ecosystems, shedding light on their metabolic versatility and potential responses to ongoing environmental changes.

Abstract ID: 3683 / Poster Number: 219.17P

Genomic analyses reveal Staphylococcus aureus persists within the bladder of individuals with long-term urinary catheters despite antimicrobial treatment and catheter changes

Presenting author: Dr. Jennifer Walker, The University of Texas Health Science Center at Houston, Houston, United States

Co-authors: *Blake Hanson*

Introduction: Urinary catheters (UCs) are the most frequently placed medical devices, with million used across the world every year[1,2]. Their widespread use is due to their efficacy in the clinical management of hospitalized and critically ill patients and at improving the quality of life for millions of individuals, including those with neurogenic bladder dysfunction and the elderly. However, UCs increase the risk of developing symptomatic catheter-associated urinary tract infection (CAUTI) as well as asymptomatic bacteriuria (ASB) – the presence of bacteria in the urine – with virtually all long-term UCs (LTUCs; placed >30 days) colonized[2-5]. CAUTIs are common, costly, and associated with increased morbidity and mortality. In contrast to urinary tract infections (UTIs) in healthy individuals, which are primarily caused by *Escherichia coli* (>70%), both CAUTI and ASB are caused by a wide range of uropathogens, with most only making up between 2-10% of cases. Problematically, these uropathogens are often drug resistant, making empiric treatment difficult, and can serve as a reservoir for antimicrobial resistance (AMR) when they persist as ASB. As UC use is expected to increase due to the aging population, greater insights into how these other uropathogens cause CAUTI or persist during ASB is urgently needed, especially since most mechanistic studies still focus on UTIs in healthy urinary tracts with *E. coli*.

Objectives: Using clinically derived, longitudinally collected isolates from individuals with long-term urinary catheters, we explored how *Staphylococcus aureus*, the most frequent cause of ASB, persists within the catheterized bladder, despite exposure to anti-staphylococcal antibiotics or regular catheter changes.

Methods: Whole genome sequencing and bioinformatics analyses were performed to determine phylogeny, including sequence types (STs), AMR patterns, and virulence factor carriage, and identify genomic adaptions under selection in the catheterized bladder.

Results: A total of 142 *S. aureus* isolates were longitudinally collected from 20 participants, with an average of seven isolates per person. The majority of the isolates

were ST5 (**Fig. 1; inner most circle**) and were drug resistant, with an average of 12 antibiotic resistance genes, including 50% that carried *mecA* (**Fig. 1; outer most band**), making them methicillin resistant (MRSA). Phylogenetic analyses demonstrate that the isolates cluster by participant, with all but two participants maintaining the same ST throughout the study (**Fig. 1; inner band**). Further analyses revealed that isolates from between participants had tens of thousands of SNP differences. However, isolates from within participants had fewer than 30 single nucleotide polymorphisms (SNPs) despite being isolated weeks or months apart, suggesting these are the same strain persisting over time. Notably, half of the participants received antibiotic and all had at least one catheter change during the study.

Conclusions: This study indicates *i) S. aureus* persists within individuals with LTUCs, despite antimicrobial exposure and/or UC changes and *ii)* that most of these isolates are MRSA, which provides a reservoir of AMR within the urinary tract.

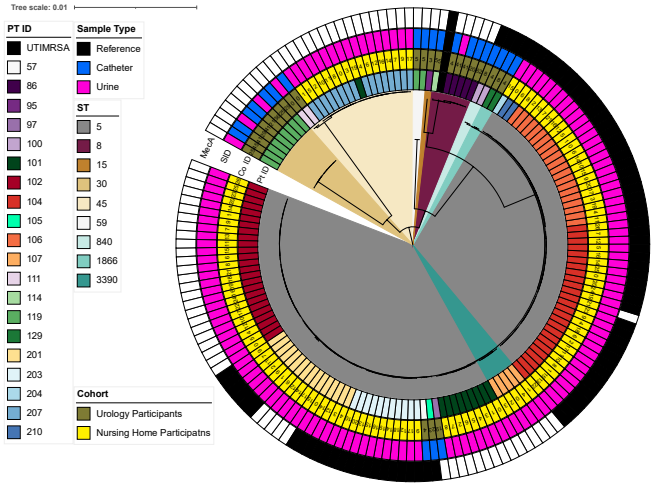


Figure 1: Phylogenetic analysis of S. aureus LTUC isolates. Phylogenetic analysis of all isolates displaying isolates clustering of isolates with the same ST within a participant.

Abstract ID: 577 / Poster Number: 284.16F

Genomic and phylogenetic characterization of lytic coliphages isolated from urban wastewater

Presenting author: Carlos Omar Lomeli Ortega, PhD, Catalan Institute for Water Research (ICRA), Spain, Girona, Spain

Co-authors: *José Luis Balcázar, Mario Muñoz Ramírez, Carles Borrego More*

Bacteriophages (phages) are the most abundant entities among Earth's biological forms, known for their ubiquity and role in environmental processes linked to their bacterial hosts. Their presence in wastewater and the human gut highlights their significance as ecosystem modulators and drivers of horizontal gene transfer. Phages have extensively been studied in biotechnology and medicine for their potential role as antibacterial agents, particularly against antibiotic-resistant bacterial pathogens. This study focuses on the genomic and phylogenetic characterization of three lytic phages (P1, P2, and P5) infecting Escherichia coli and isolated from urban wastewater.

Phylogenetic trees based on the amino acid sequences of the major capsid protein and terminase large subunit placed phages P1 and P2 within the genus Tequatrovirus, whereas phage P5 was classified within the genus Felixounavirus. Key findings provide insights into the replication cycle, genomic composition, and phylogenetic relationships of the phages. Comprehensive genome annotationrevealed genes associated with the lytic cycle, while genes encoding proteins related to lysogeny, virulence. or antibiotic resistance were absent. This observation confirms their biosafety for future biotechnological and clinical applications (e.g., phage therapy). Overall, the three isolated coliphages represent new members of their respective genera, providing valuable resources for advancing phage-based technologies.

Abstract ID: 3187 / Poster Number: 273.15P

Genomic and Safety Characteristics of Enterococcus lactis Strains from Korean Traditional Fermented Soybean Foods

Presenting author: Mr. Dae Gyu Choi, Chung-Ang University, Seoul, South Korea

Co-authors: *Dong Min Han, Che Ok Jeon*

Enterococcus is a genus that includes pathogenic microorganisms such as *Enterococcus faecium* and *Enterococcus faecalis*. However, certain species within this genus exhibit fewer pathogenic traits and are considered beneficial microorganisms. Notably, *Enterococcus lactis* has demonstrated a close phylogenetic relationship with *E. faecium* while possessing relatively fewer pathogenic genes. Previous studies have reported a high prevalence of *E. lactis* in traditional fermented foods in Korea. In this study, we isolated 25 *E. lactis* strains without redundancy from various fermented soybean blocks (Meju) obtained from traditional markets in Korea and conducted a comprehensive analysis of its genetic characteristics and strain safety.

Due to the high similarity of 16S rRNA sequences between *E. faecium* and *E. lactis*, distinguishing these two species poses a challenge. To address this, we employed species-specific primers as a novel classification method for microbial isolates. The genome of the isolated *E. lactis* strains had an average size of 2.81 Mb (± 0.08), containing 2,826 genes (± 170). Phylogenetic analysis confirmed that *E. lactis* strains from fermented soybeans belong to the same lineage as other *E. lactis* strains. Furthermore, the isolates exhibited resistance to gentamicin, kanamycin, and macrolide antibiotics while lacking pathogenic genes or enzymatic activities associated with virulence. This study demonstrates that strains isolated from traditional Korean fermented soybean blocks (Meju) are non-pathogenic, as evidenced by genetic and phenotypic characterization. Furthermore, the strains exhibit enzymatic activities potentially crucial to fermentation, offering insights into the role of *Enterococcus* in the fermentation process and the development of starter cultures

Abstract ID: 2783 / Poster Number: 249.16P

Genomic characterization and pathogenicity assessment of Escherichia coli isolates collected from artisanal foods of animal origin

Presenting author: Frédérique Pasquali, PhD, Alma Mater Studiorum- Università di Bologna, Bologna, Italy

Co-authors: *Cecilia Crippa, Gerardo Manfreda*

Escherichia coli is one of the most studied model microrganisms showing high genomic plasticity. Its great capacity of adaptation has led to the emergence of pathogenic variants. Although many virulence factors are well known, prediction of specific pathotypes based on presence/absence of specific virulence genes is challenging. Within the OnFoods project (www.onfoods.it), the aim pf the present study was to evaluate the virulome and pathogenicity of foodborne *E. coli* isolates in order to predict their potentialities as foodborne hazards. *E. coli* were collected from salami and soft cheese artisanal productions from semi-finished and finished food as well as the processing environment. A phylogenomic tree on E.coli newly sequenced genomes was built including publicly available genomes for comparison. Their virulome was assessed along with their pathogenicity by using genomics approaches coupled with a pathogenicity assessment using *Galleria melonella* as an *in vivo* infection model. Based on the presence of the locus of enterocyte effacement (LEE) and the absence of *stx1*, *stx2* and *bfp* genes, three genomes were identified as belonging to atypical enteropathogenic *E. coli* (aEPEC). Additionally, two genomes carrying the *iutA* gene and one operon coding for the P fimbriae, were predicted as potential ExPEC strains. Seventy-five and 20 % of *Galleria melonella* larvae survived after 5 days post infection when inoculated with the two ExPEC strains respectively, whereas the survival rate of larvae challenged with the three aEPEC strains was 50, 60 and 75% respectively. The diversity in mortality rates within aEPEC and mostly within ExPEC strains, reinforced the great complexity and challenge in idenfying minimum virulence gene pattern associated to a specific pathotype in *E. coli*. The ExPEC strain with the lowest survival rate (20%) carried the *pix* operon which has significant sequence homology to the most frequently identified *pap* operon coding for the P fimbriae. The *pix* operon was already identified in Urinary Tract Infections (UTI) related *E. coli* strain. Although more studies will be needed to assess the key virulence markers associated to the highest pathogenicity of *pix* positive and *iutA* positive ExPEC strain, this study reinforce the importance of validation of genomic-based

predictions with *in vivo* infection models for prioritization of foodborne *E.coli* potentially hazardous for humans.

Abstract ID: 1389 / Poster Number: 309.15P

Genomic diversity and concurrence of Acidithiobacillia class bacteria along a pH and electrochemical gradient

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Co-authors: *Camila Rojas-Villalobos, Dilanaz Arisan, Fernando Diaz, Juan Duarte, Sofia Reyes-Impellizzeri, Sebastian Pacheco, Celia Larrea, Simon Beard, Matias Castro, Gallardo Yasna, Hector Carrasco, Ricardo Ulloa, Barrie Johnson, Pedro Temporetti, Alejandra Giaveno, Pedro Sepulveda, Ana Moya-Beltrán, Raquel Quatrini*

Hydrogeothermal environments, including the Copahue-Caviahue volcanic complex, provide steep pH and electrochemical gradients that drive microbial diversification and adaptation. These ecosystems offer an ideal setting for investigating eco-evolutionary processes shaping acidophilic prokaryotic communities. Here, we applied a high-resolution metagenomic approach to elucidate the diversity, distribution, and ecological structuring of *Acidithiobacillia* populations, key drivers of biogeochemical cycling in acidic environments.

Using an *in-house* computational pipeline integrating different binning and refinement software, we reconstructed 1,081 unique metagenome-assembled genomes (MAGs) from over 50 metagenomes, 15% of which met medium-to-high quality standards (45% high quality, 54% medium quality).

Acidithiobacillia accounted for up to 60% of total microbial reads in the most acidic habitats, underscoring their ecological dominance. Taxonomic profiling revealed well-known species – including *Fervidacidithiobacillus caldus* and *Igneacidithiobacillus copahuensis* – coexisting with previously undescribed clades of iron oxidizing lineages. These clades formed distinct guilds in two out of six habitats profiled, suggesting localized ecological specialization. Comparative genomic analysis across the pH gradient revealed fine-scale genetic differentiation, indicating ongoing diversification driven by local stressors.

Community assembly analysis suggested the involvement of both stochastic and deterministic factors, with the prominence of species replacement. Variable intra-specific genomic diversity in *Acidithiobacillia* members between habitats further highlighted environmental filtering as a key driver, potentially reflecting locally adapted populations across the gradient.

By bridging ecological and genomic perspectives, this study provides a framework for understanding how microbial populations partition, assemble and evolve in extreme environments.

Abstract ID: 1793 / Poster Number: 219.15P

Genomic epidemiology of Helicobacter pylori in Colombian populations with different risk of gastric cancer

Presenting author: Prof. Doc. Alvaro J Pazos M, Universidad de Nariño, PASTO, Colombia

Co-authors: *Kevin Andres Guzmán Bastidas, Vanessa Pabon, Arsenio Hidalgo,*

Introduction

Helicobacter pylori is a gram-negative bacterium infecting 50% of the global population and associated with gastric diseases. However, only 1–3% of infected individuals develop gastric cancer, influenced by virulence genes, environmental factors, and host genetics. In Colombia, gastric cancer incidence is notably higher in the Andean region (150 cases per 100,000) compared to the Pacific coast (6 per 100,000).

Methodology

Data on mortality, population, and altitude in Nariño were collected from sources such as DANE and IDSN. Human ancestry profiles were analyzed for both regions. Mortality maps were created, and *Helicobacter pylori* genomes were phylogenomic analyzed using J99 as a reference strain. Phylogenetic trees were constructed in MEGA XI and visualized with FigTree. Spearman correlations between mortality and altitude were conducted in R 3.3.1.

Results

From 2011 to 2020, gastric cancer mortality rates in Nariño varied significantly. The Andean region had the highest rates (La Cruz: 44.8; Iles: 41.5; Belén: 40.8 per 100,000), while the Pacific coast had the lowest (Mosquera: 0.8; Magüí Payán: 0.4; La Tola: 1.3 per 100,000). Mortality was significantly correlated with altitude ($p<0.05$). High mortality in the Andean region was linked to Amerindian-European ancestry and *Helicobacter pylori* strains (hspColombia_Andes). Conversely, the Pacific coast, with African ancestry and hspColombia_PacificCoast strains, showed lower mortality.

Conclusion

Differences in gastric cancer mortality between these regions are tied to altitude, ancestry, and bacterial strains. High-altitude Andean municipalities face greater risk due to *Helicobacter pylori* (hspColombia_Andes), while the Pacific coast shows lower mortality linked to African ancestry and hspColombia_PacificCoast strains.

Abstract ID: 2804 / Poster Number: 391.15P

Genomic evolution and symbiotic adaptations in Tricholoma matsutake

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Co-authors: *Mi-Jeong Park, Eun-Kyung Bae, Min-Jeong Kang, Ki-Tae Kim*

Tricholoma matsutake, also known as pine mushroom, is an ectomycorrhizal fungus with significant market worth. It relies on a symbiotic relationship with pine trees for its growth, making its cultivation challenging. To get insights into the evolutionary and functional adaptation mechanisms of *T. matsutake*, we conducted a comprehensive comparative genomic analysis of *Tricholoma* species, focusing on *T. matsutake*. We analyzed genomic data from 19 assemblies across 13 species, including 8 *Tricholoma* species, to pinpoint genus-, species-, and strain-specific genes. Phylogenomic studies revealed that *T. matsutake* is part of a monophyletic group, appearing as a sister clade to *T. bakamatsutake*. Analyses of gene family expansions and contractions revealed unique evolutionary pressures on *T. matsutake*, especially the loss of genes associated with tryptophan metabolism and the acquisition of genes associated with iron ion homeostasis. These findings suggest that the fungus has developed adaptive strategies to survive in its specific ecological niche, such as a nutrient-poor environment. Furthermore, the decrease in secreted proteins and carbohydrate-active enzymes highlights the host-dependent nature of *T. matsutake* and related species. This study improves our understanding of evolutionary mechanisms that influence the genetic structure and functional adaptation of *T. matsutake*.

Abstract ID: 2466 / Poster Number: 236.15P

Genomic Insights into Methyloparacoccus murelli W16 Isolated from a wastewater treatment plant

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Co-authors: *Hyo Jung Lee*

Methanotrophic bacteria, known for their ability to oxidize methane play a crucial role in reducing methane emissions and offer significant potential for bioconversion applications. Understanding their functional capabilities necessitates the isolation and genome sequencing of methanotrophic bacteria. In this study, we report the isolation and complete genome sequencing of *Methyloparacoccus murelli* W16, a type I methanotroph enriched and isolated from a wastewater treatment plant. The complete genome of strain W16 comprises 3,375,919 bp with a G+C content of 65.0% and 3,127 coding sequences. Genome annotation revealed that strain W16 contains genes encoding particulate methane monooxygenase (pMMO), a key enzyme responsible for the oxidation of methane to methanol. In addition, strain W16 contained a complete set of genes encoding major pathways for formaldehyde assimilation into cell biomass, including ribulose monophosphate (RuMP), Embden-Meyerhof-Parnas (EMP), Enter-Doudoroff (EDD), and phosphoketolase (xfp)-based pathways. However, the strain lacked the gene encoding serine-glyoxylate aminotransferase (sga), resulting in an incomplete serine pathway. Strain W16 harbors genes responsible for the production of various organic acids, which are expected to stabilize cellular redox potential under hypoxic conditions (oxygen-limiting) by shifting its metabolism toward fermentation and producing diverse extracellular organic acids. These by-products are ecologically significant as they may serve as growth substrates for surrounding heterotrophic microorganisms. This study represents the first genomic report of *Methyloparacoccus*, providing valuable insights into its metabolic capabilities and potential applications in methane bioconversion and greenhouse gas mitigation.

Abstract ID: 2797 / Poster Number: 338.17P

Genomic insights into prokaryotic diversity in hypersaline and metal-contaminated soils

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Co-authors: *Cristina Galisteo, Fernando Puente-Sánchez, Stefan Bertilsson, Rafael R de la Haba, Antonio Ventosa*

The hypersaline soils of the Odiel Saltmarshes Natural Area in Southwest Spain harbor highly diverse microbial communities adapted to extreme conditions. However, their genomic diversity remains largely unexplored. In addition to high salinity, these soils are contaminated with heavy metals, making them a hostile environment of great interest for studying extremophilic microorganisms and their metabolic adaptations.

This study aims to characterize the prokaryotic populations of the Odiel Saltmarshes by reconstructing their genomic sequences from metagenomic datasets. We analyzed 18 metagenomes and reconstructed over 4,000 metagenome-assembled genomes (MAGs). They were evaluated for quality based on completeness and contamination thresholds, and taxonomically classified. Comparative genomic analysis was performed on a subset of high-quality MAGs to investigate their metabolic adaptations to hypersaline and heavy metal-rich conditions.

Of the reconstructed MAGs, 273 were classified as medium-quality (≥50% completeness; <10% contamination), while 11 met high-quality standards (>90% completeness; <5% contamination). Bacteria-assigned MAGs were generally more complete and less contaminated than those classified as Archaea. Among the high-quality MAGs, only one could be identified at the species level (*Pseudomonas taetrolens*), while the others were classified at the family level or higher. Comparative genomic analysis of six MAGs revealed key metabolic traits related to survival in extreme salinity and heavy metal conditions.

This study provides new insights into the microbial diversity of hypersaline environments and expands the catalog of known prokaryotic genomes. The detailed characterization of six novel Candidatus taxa highlights the unique adaptations of these microorganisms, contributing to our understanding of life in extreme habitats.

Abstract ID: 849 / Poster Number: 272.16F

Genomic investigation and engineering of microbial biosynthetic gene clusters with potential biotechnological interest

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Genome mining has revolutionized natural product discovery through identification of novel Biosynthetic Gene Clusters (BGC). Furthermore, advancements in Synthetic Biology facilitate BGC activation via direct cloning and heterologous expression. To investigate microbial biosynthetic capacity, this study employs a taxonomically diverse collection of 84 bacterial isolates, 52 of which belong to the phylum of *Actinomycetota*. The genomic sequences were determined by Nanopore technology, followed by 16s rRNA phylogenetic classification. The generated genome assemblies were computationally analyzed using antiSMASH and BiG-SCAPE to predict BGC distribution, confirming the biosynthetic robustness of streptomycetes, harboring 20-70 BGCs per genome. Continuous clusters of 20-90Kb, predicted to generate antibiotics, were prioritized for cloning through in-gel Cas9-assisted targeting of chromosome segments (CATCH) coupled with Gibson assembly ligation to a suitable BAC vector for subsequent transformation to *Escherichia coli*. The genomic sequences at the boundaries of each BGC directed the design of specific sgRNA oligos for intact BGC isolation, as well as the preparation of vectors with homologous overhangs for cloning. Currently, pilot cloning of 20Kb clusters, such as those of albaflavenone and 7-prenylisatin, is in progress, requiring optimization of procedures given the well-documented challenges associated with manipulation of *Streptomyces* cells and genomes. The long-term objective of this study is the heterologous expression of cloned BGCs to identify novel secondary metabolites of prospective therapeutic value, mainly as antibiotics. The research project was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the “2nd Call for HFRI Research Projects to support Faculty Members & Researchers” (Project Number: 3712).

Abstract ID: 3864 / Poster Number: 125.16P

Genomic Stability and Antimicrobial Resistance (AMR) of Enterococcus spp. from two Healthy European Infant Cohorts

Presenting author: Mr. Muhammad Hassan Saeed, Technical University of Munich, Freising, Germany

Introduction: *Enterococci* are common gut microbiota members. Several are often harmless commensals, but become opportunistic pathogens in perturbed gut microbiomes (e.g., antibiosis).

Objectives: Given the significant increase in antimicrobial resistance (AMR) in ailing and healthy individuals, predicted to cause 10M deaths every year by 2050, the resistome of healthy infants is of great interest. It is unclear whether certain genomic traits (e.g. AMR, phages, plasmids, virulence factors, etc.) are restricted to humans or more widespread and vary geographically.

Methods: To address this, we analyzed 100 *Enterococcus* strains, isolated from two healthy human infant cohorts from Spain or The Netherlands. We used phenotypic screenings (antibiotic susceptibility testing (AST) and survival assays) and bioinformatics approaches (genomics and pangenomics).

Results: We found a rich reservoir of genomic traits with minimal cohort-specific differences and similar metabolic profiles in case of *E. faecalis*. Epxs, pore-forming toxins associated with pathogenicity, were found in *E. hirae* strains. AST indicated 28% of Spanish strains were gentamicin resistant, compared to 5% of strains from The Netherlands. The latter isolates also had further resistances. *Caenorhabditis elegans,* when fed with different *E. faecalis* strains, displayed mortality at various rates; thus, some strains are likely potential pathogens. Public genomes of multi-drug resistant strains revealed no geographic or temporal pattern.

Conclusion: Owning to their adaptability, *Enterococcus* spp., including their ARGs, are highly mobile across Europe and beyond. To mitigate the growing threat, the probiotic potential of harmless *Enterococcus* ssp. should also be explored in order to supersede potentially pathogenic variants.

Abstract ID: 1690 / Poster Number: 137.16S

Genomically diverse Stenotrophomonas maltophilia isolates are lysed by specialist and generalist phages

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Co-authors: *Daria Van Tyne, Joanna Goldberg*

Introduction: *Stenotrophomonas maltophilia* complex (Smc) is an emerging Gram-negative bacterial pathogen broadly distributed in many natural environments (e.g. soils and lakes) and anthropogenic settings (e.g. hospitals)[1,2]. Blood infections caused by this pathogen can have mortality rates above 65%[3]. Smc primarily affects people that are immunocompromised or suffer from conditions like cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and cancer; however, immunocompetent people can also be affected[4–8]. Infections in people with CF or COPD can lead to increased mortality and worse symptoms[4,6]. Isolates are often multidrug-resistant, infections are difficult to treat, and alternative therapies are urgently needed[2]. Smc strains exhibit high genomic diversity and have been classified into 23 monophyletic lineages (also called genomospecies)[9]. The average nucleotide identity between strains from different genomospecies is <95%[9].

Bacteriophage (phage) therapy has been successfully used to treat multidrug-resistant infections with minimal side effects[10]. This treatment approach uses phages, which are viruses that infect and kill specific bacteria (generally from a single species) but do not affect human cells or the human microbiome[10]. Few phages that lyse Smc isolates have been described, and it is unknown how strain diversity affects phage infectivity.

Objective: We sought to characterize the infectious range and genomic structure of diverse Smc phages.

Methods: We isolated a lytic Smc phage from soil in Atlanta, Georgia (Lsmp1) and five lytic phages from wastewater in Pittsburgh, Pennsylvania (STM02, STM03, STM04, STM05, and STM06). We determined the host range of these phages on 25 isolates from diverse Smc genomospecies using infection assays. Finally, we sequenced and compared the phage genomes from this study.

Findings: We determined that phages STM02, STM03, STM04, and STM05 are generalists and can lyse multiple, but distinct Smc strains from distinct lineages. By contrast, phages Lsmp1 and STM06 are specialists, and each can only lyse one distinct Smc isolate. All tested Pittsburgh phages share high average nucleotide identity (94-98%) and have

genomes of ~60-61 kilobases. Lsmp1 is genomically distinct, and its genome is ~42 kilobases.

Conclusion: Here, we observed that Smc phages can be specialists or generalists that can lyse genetically diverse isolates. Our findings could inform the development of new therapies designed to target multidrug-resistant Smc infections that are difficult to treat.

Abstract ID: 3090 / Poster Number: 300.15P

Genotype-by-Genotype Interkingdom Interactions: Molecular Insights into the Cross-Talk Between Sinorhizobium meliloti and Trichoderma Species for Rhizomicrobiota Assembly

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Microbial interactions are key drivers of microbiota functionality and ecosystem dynamics, profoundly influencing plant health, growth, and productivity. Among bacterial and fungal communities inhabiting the rhizosphere, *Trichoderma* species and rhizobia are of particular importance due to their well-established roles in promoting plant growth and providing protection against pathogens.

In this work, we present an integrated analysis of the interactions between the symbiotic nitrogen-fixing bacterium *Sinorhizobium meliloti* and soil fungi of the genus *Trichoderma*. The studies presented aim to test the hypothesis of a genotype × genotype interaction between *S. meliloti* and *Trichoderma* species, shedding light on the molecular determinants underlying these interactions. By investigating strain-specific responses within the rhizomicrobiota, we seek to provide insights into their potential impact on plant-microbe symbioses, as well as those factors shaping the success of microbial consortia, with the ultimate goal to guide the development of more effective microbial combinations, optimizing their application for sustainable agricultural practices.

By designing experiments where four different *S. meliloti* strains and four different *Trichoderma* species were used, we combined microbiological observation, physiological, metabolic, and transcriptomic analyses, to investigate the effects of fungal spent media from four *Trichoderma* species (*T. velutinum, T. tomentosum, T. gamsii,* and *T. harzianum*) on four strains of *S. meliloti*. We then focused on the strain-specific molecular recognition by transcriptomic analyses of *T. velutinum* in presence of different *S. meliloti*.

We show the existence of rhizobial strain-specific response to *Trichoderma* species, with the presence of either synergistic, neutral, or antagonistic interactions, depending on the rhizobium-*Trichoderma* combination. Results revealed strain- and species-specific

effects on rhizobial physiology and transcriptomics, with up to 25% of rhizobial genes differentially expressed. These changes significantly influenced the symbiotic performance of *S. meliloti* with its host legume (*Medicago sativa*), demonstrating complex genotype × genotype interactions between fungi and bacteria. On the fungal side, the transcriptome varied with bacterial strain, emphasizing the specificity of these interactions. Overall, we identified between 139 and 254 differentially expressed genes, indicating a significant remodelling of fungal metabolism and growth. Strikingly, ~90% of these genes lacked functional annotation, pointing to an uncharacterized genetic space critical for fungal-bacterial communication.

These findings highlight the complexity and specificity of microbial interactions in the rhizosphere, shedding light on the molecular basis of plant-microbe symbioses, thus underscoring the potential of leveraging strain-specific interactions to optimize bioinoculant development for sustainable agriculture.

Abstract ID: 2775 / Poster Number: 145.17P

Genotypic and Phenotypic Analysis of Multidrug Resistant Escherichia coli isolates from a poultry farm in Kaduna, Nigeria

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Introduction: Antimicrobial resistance (AMR) poses a significant threat to public health. The misuse of antimicrobials in livestock production is a key driver of AMR emergence in agriculture, where prophylactic use and as growth promoters is employed to limit economical losses.

Objective: By isolating *E. coli* from poultry farms we aim to illustrate the importance of antimicrobial stewardship in agriculture.

Methods: Litter, feed and surface environmental samples were collected from poultry farms. *E. coli* strains were isolated using standard microbiological culture, detecting species diversity using Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR. Antimicrobial susceptibility was assessed through broth dilution minimum inhibitory concentration screening against 108 antimicrobials used in veterinary medicine, while gDNA extraction and long-read sequencing allows genotypic characterisation.

Findings: 214 *E. coli* isolates were recovered, where typing evidenced intraspecies diversity. AMR were determined for 43 antimicrobial agents, with highest rates shown for enrofloxacin (96.6%), sarafloxacin (95.6%), ofloxacin (94.7%), oxolinic acid (91.3%) and tetracycline (90.7%). In addition, several MDR *E. coli* strains were isolated from these environmental sources, with *TetA, qnrB19, sul1, dfrA1, aac* and bla_{TEM} frequently detected. Heterogenous resistance is reported between sample types with surface samples showing high rates of ceftriaxone (68.4%) resistance. *E. coli* genomic epidemiology emphasises large diversity within the collection detecting dozens of sequence types (ST) including ST10, ST206 and ST540.

Conclusion: These results stress the need for 'One Health' surveillance to detect these AMR reservoirs, while contributing to our understanding of emerging AMR from intensive livestock production, where transmission to humans poses a significant global health risk.

Abstract ID: 3624 / Poster Number: 268.16S

Genotypic and Phenotypic Characterization of a Four-Bacteriophage Cocktail for Targeting Multidrug-Resistant Klebsiella pneumoniae

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The risk of acquiring carbapenemase-resistant *Klebsiella pneumoniae* is directly related to invasive procedures and exposure to the hospital environment and cause difficult to treat infection. Bacteriophages, viruses that specifically kill bacteria, are used in phage therapy to target antibiotic-resistant infections. The aim of the research is to develop a phage cocktail active against the most widely circulating *K. pneumoniae* strains in hospital setting for therapeutic applications. To achieve this, the study concentrated on the genotypic and phenotypic characterization of four selected phages to support its formulation.

Method: Phages were isolated from water samples using *K. pneumoniae* ATCC BAA 2146 and clinical strains as hosts. Genomes were sequenced (Illumina method) and analyzed and morphology was confirmed with TEM. The pH sensitivity (2–9, 11) at 37°C, adsorption rates and one-step growth curves were assessed by spot assay. The phage host range was assessed by efficiency of plating (EOP) determination on 91 *K. pneumoniae* clinical strains including carbapenemase-producing (KPC), New-Delhi beta-metallolactamase (NDM-1) and Verona integron-encoded metallo-beta-lactamase (VIM) strains. The killing kinetics of phages at Multiplicity of infection (MOI) 1 and 10 was tested agaist its host and a panel of selected clinical isolates for 24H by optical density measurement. The antibiofilm activity was assess by treating *in vitro K. pneumoniae* ATCC BAA 2146 strain biofilm pre-formed on porous beads, and determining the reduction of CFU/mL, by colony counting, at 3h and 24h after phages treatment.

Findings: Seven phages (Scott, Totoro, Olmo, Kilian, Boris, Mononoke, and Merlino) were identified. Genomic analysis confirmed their virulent nature, with no antibiotic-resistance genes detected, and four were classified as a new species. Phages showed stable titers at pH 3–11. EOP analysis indicated a narrow host range with strong activity against KPC strains. The study focused on Kilian, Totoro, Scott, and Olmo for cocktail formulation. Adsorption assays showed rapid attachment within 5 minutes, except for

Olmo (2.5 minutes). One-step growth curves revealed short latency (5–10 minutes) and robust propagation. In vitro, Kilian, Totoro, Scott, and the cocktail (MOI 1, 10) significantly reduced bacterial load up to 24 hours for 2 KPC strains, with the cocktail outperforming single phages in one case. Anti-biofilm assays showed a ~2-log reduction after 3 hours and a 1-log reduction after 24 hours of cocktail treatment.

Conclusion: This study highlights the potential of a phage cocktail against multidrug-resistant *K. pneumoniae*. The phages exhibited suitable traits for therapy, reduced bacterial loads in vitro, outperformed individual phages against KPC isolates, and demonstrated strong anti-biofilm activity. These findings support phage-based treatments as a strategy to combat antibiotic resistance.

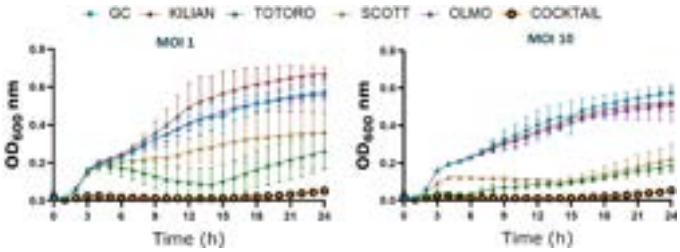


Figure 1: Optical density at 600nm measurements of K. pneumoniae- KPC clinical isolates tKp30 treated with single phages and phage cocktail composed of these phages at MOI 1 and 10. GC=growth control. Multiple unpaired Student's t-test were performed *p ≤0.05, The phage cocktail significantly controls bacterial load up to 24 hours at both MOI 1 and MOI 10, showing better performance compared to single phages: Only phages Kilian and Totoro are able to control bacterial growth at MOI 10 up to 6 hours. N=4.

Abstract ID: 2515 / Poster Number: 067.16P

Genotypic characterization and antimicrobial resistance of Campylobacter jejuni isolated from poultry products and farms

Presenting author: Dr. Eun sook An, National Institute of Food and Drug Safety Evaluation, Cheongju-si, South Korea

Co-authors: *Su-hyeon Joung, Yusi Lee, Yongchjun Park, Insun Joo*

Campylobacter spp. are the most common bacterial cause of human gastroenteritis worldwide. Poultry is a major source of Campylobacter infections in humans. In this study, we analyzed 150 Campylobacter jejuni strains isolated from poultry meat and poultry feces distributed in Korea. Antimicrobial susceptibility testing was performed on eight antimicrobial agents, and whole-genome sequencing (WGS) was used to analyze seven virulence genes, whole-genome multilocus sequence typing (wgMLST), and single nucleotide polymorphism (SNP). Among 150 C. jejuni isolates, the major virulence gene cdtA was detected in 86% of the isolates. Antimicrobial susceptibility testing showed that over 85% of the isolates were resistant to nalidixic acid and ciprofloxacin, with 65 strains classified as multidrug-resistant (MDR) to three or more antimicrobial classes. wgMLST analysis results were classified into 42 Sequence Types (ST), with 97% belonging to clonal complexes (CC) 21 and 464. Phylogenetic relationships using SNP analysis divided the isolates into 68 clusters. Notably, 29 isolates collected from poultry meat and feces in each of the three farms exhibited high genetic homology, suggesting potential cross-contamination or genetic spread within farms. This suggests that C. jejuni isolated from poultry meat and feces could be a potential source of human Campylobacter infections, and therefore public health and food safety policies may be needed to protect consumers from exposure to C. jejuni.

Abstract ID: 3614 / Poster Number: 124.16P

Geographic Variations in Antimicrobial Resistance: Insights from the Poultry Industry in South Asia

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Antimicrobial resistance (AMR) poses a significant challenge to the poultry industry in Asia due to the widespread use of antibiotics. Previously, region-specific correlations between caecal enterotypes (based on 16S rRNA amplicons) and resistomes (based on AMR AmpliSeq) of chickens in India, Bangladesh and Vietnam were found. The strong influence of geographic region suggests significant differences in the reservoir of antimicrobial resistance genes (ARGs) in these countries. This study therefore investigates regional differences in the distribution of ARGs and their relationship to antimicrobial selection pressures.

Deep Illumina shotgun sequencing (n=120) and long-read Oxford Nanopore sequencing (n=56) were performed on a subset of caecal DNA samples. Assembly of individual samples as well as regional and combined co-assemblies were performed. Plasmids were identified and screened for ARGs, while metagenome-assembled genomes (MAGs) were used to map chromosomally integrated ARGs. Antibiotic residues from chicken meat and feathers were quantified to evaluate selection pressure. The MAGs of individual samples and regions were then compared in pan-genome analysis to identify region-specific genes linked to adaptation or resistance.

Enterobacteria, particularly *Escherichia coli*, were dominant ARG reservoirs across all regions, while acquired resistance in *Campylobacter jejuni* was notable. Regional resistome profiles were shaped by the differing prevalence of plasmid-borne and chromosomally integrated ARGs in abundant species. Antibiotic residues correlated with plasmid-borne ARG abundance, suggesting a significant role of selection pressure in regional variations.

Geographical variation in ARG reservoirs highlights the importance of tailored AMR surveillance strategies and calls for region-specific interventions to mitigate AMR in the poultry industry.

Abstract ID: 3261 / Poster Number: 276.15P

Global diversity and ecological functions of viruses in BTEX-contaminated environment

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BTEX compounds (benzene, toluene, ethylbenzene, and xylene) in groundwater pose significant environmental and public health risks. While microbial communities capable of BTEX degradation are well established, the role of viruses in shaping these communities remains poorly understood. This study aims to comprehensively analyze viral diversity and ecological functions in BTEX-contaminated environments. We conducted a global survey of 12,501 viral operational taxonomic units (vOTUs) from 210 samples collected across eight countries. Our findings reveal distinct viral community patterns that correlate with BTEX contamination. Most vOTUs exhibited high site specificity, indicating strong environmental filtering. Using metagenomic approaches, we demonstrate that viruses can influence the abundance and activity of key BTEX-degrading bacteria. We identified novel auxiliary metabolic genes (AMGs) in viral genomes potentially involved in aromatic compound degradation and validated their protein structures using AlphaFold predictions and Foldseek structural comparisons. These AMGs were then compiled into a curated database, providing a valuable resource for future research. This study provides the first global atlas of viral diversity in BTEX-contaminated environments, highlighting the crucial role of viruses in modulating microbially mediated BTEX degradation and offering new insights for bioremediation strategies.

Abstract ID: 3279 / Poster Number: 278.15P

Global rewiring of prokaryotic cellular networks induced by mero-oligoploidy

Presenting author: Mrs. Francesca Vaccaro, University of Florence, Florence, Italy

Co-authors: *Michele Giovannini, Arooba Arshad, Michael Mederer, Antonio Frandi, Silvia Buroni, Matteo Brilli, Marco Fondi*

Introduction: While bacteria are commonly referred to as monoploid organisms, i.e. possessing a single copy of each chromosome, many species are polyploid and possess up to more than ten copies of their chromosome(s). Additionally, in specific physiological conditions (when cell cycle duration is shorter than chromosome replication time), some species contain several origins of replications per chromosome copy because of overlapping round of replications (mero-oligoploidy). While monoploid species can have, at most, two copies of the same gene due to chromosome replication, mero-oligoploid can have up to 8-10 copies of the same locus. Despite being known since the 60s, the effect(s) of mero-oligoploidy on cellular physiology have never been quantified. How cells integrate/buffer multiple signals (e.g. interaction of copy number variation induced by changes in division time and transcription factor mediated regulation) is still unclear.

Results: Here, we propose a systems biology approach that quantifies the effects of mero-oligoploidy in bacteria on all their main biological networks, with a specific focus on metabolism. We combine theoretical modelling with -omics technology obtained from cells grown in strictly controlled conditions to unravel how cells deal with altered loci abundance (gene multiplicity) induced by the presence of multiple active replication forks within the same cytoplasm and how the gene regulatory network compensate for this effect.

Conclusions: Results obtained revealed the role extra players (growth rate and thus mero-oligoploidy) to the overall description of cellular homeostasis regulation.

Abstract ID: 770

Global soil prokaryotic communities' exposure to climate change: an ecological niche perspective

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Co-authors: *Olivier Broennimann, Antoine Guisan*

Global change, particularly climate change, with its associated shifts in temperature, precipitation patterns, and increased frequency of extreme weather events, poses a significant threat to soil ecosystems. A previous study focused on the Swiss Alps revealed that niche breadth for soil microorganisms narrows toward environmental extremes, highlighting the prevalence of specialized taxa in harsh conditions. However, due to the localised scope and limited environmental gradients studied, the influence of niche truncation could not be fully excluded from the observed trends.

In this study, we expand the scope by using a global database of soil prokaryotes to analyse the environmental niches occupied by soil bacteria and archaea worldwide. Our goal is to identify specialist and generalist microorganisms and to evaluate their vulnerability to climate change using the niche marginality index under both current and projected future climatic conditions. By examining different climate scenarios, we aim to assess how soil prokaryotic communities will respond to climatic shifts in the coming years.

This research provides critical insights into the ecological implications of climate change on soil microbial communities. By understanding the responses of these communities, we can better anticipate changes in ecosystem functioning and resilience, particularly in terms of nutrient cycling and soil health, under future climatic conditions.

Abstract ID: 2155 / Poster Number: 227.15P

Glyphosate-based herbicide inhibits while the organic alternative pelargonic acid stimulates methane production in anoxic soil microcosms

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Co-authors: *Chiara Lesuis, Daniel Straub, Carolin Huhn, Esteban Góngora, Katharine J Thompson, Sara Kleindienst*

Soil plays a critical role in biogeochemical cycles that regulate the transformation of essential elements such as carbon (C). However, the functionality of the soil microbiome is vulnerable to anthropogenic influences (1). Therefore, human activity, such as the application of agricultural glyphosate-based herbicides (GBHs), can lead to direct and indirect impacts on climate change through the production of greenhouse gases (2). GBHs contain glyphosate (GLY) as the active ingredient and various co-formulants, such as surfactants. Although they are often referred to as "inert", surfactants are among the most commonly used co-formulants as they reduce surface tension, therefore enhancing the herbicide's efficiency (3). Contrary to this, previous research has shown that surfactants alter microbial community composition and inhibit or stimulate the activity of specific microbial taxa, such as some involved in oil degradation (4). This raises significant concerns about the broader ecological impacts of surfactants, primarily when used alongside other potentially harmful substances such as glyphosate in agricultural settings (5). Here, we used agricultural soil microcosms to investigate whether surfactants influence the activity of key microbial players involved in the carbon cycle and the subsequent impact on the formation of greenhouse gases.

Oxic and anoxic soil microcosm experiments were amended with either GLY, GBH, or the organic alternative to GLY, pelargonic acid (PA), which is a surfactant. The microcosms were established in triplicate using EU application rates of GLY, GBH and PA (i.e., 15 mg/kg) and a 'hotspot' concentration (i.e., 150 mg/kg). GLY, surfactants, anions, and gases were analysed separately using one or a combination of liquid chromatography, ion chromatography, gas chromatography, and mass spectrometry. DNA and RNA were extracted and sequenced to produce metagenomes and metatranscriptomes to determine community composition, gene profiling, and activity. The planned flow of analysis for chemistry and omics is visualised in Figure 1.

The PA impacted the carbon cycle in the anoxic microcosms through an increased methane production in both the low (PA, 15 mg/kg) and high amendments (PA, 150

mg/kg) compared to the biotic controls. Indeed, in the high PA bottles, exponential methane production was observed from as early as day 25, compared to slow production of methane in the biotic control (from day 45) and glyphosate (from day 50) bottles. This suggests that PA stimulated directly or indirectly methanogenesis. However, methane was observed only after day 70 in the GBH bottles, indicating there are likely inhibitors or toxic compounds in formulations that can slow anaerobic respiration. Collectively, these data indicate changes in microbial community activity in the presence of surfactants or GBHs and that surfactants may directly or indirectly impact C-cycling microbes. Current work is being undertaken to confirm our predictions by analysing the metagenomic and metatranscriptomic data from both microcosms. The chemistry data suggests that GBHs could inhibit methane production, which could lead to lower greenhouse gas emissions and/or a large-scale disruption to the soil microbiome, raising further concerns for the agricultural sector.

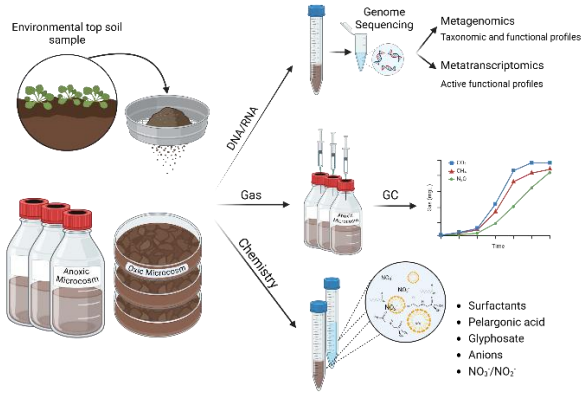


Figure 1. Flow scheme of oxic and anoxic microcosm analysis

Abstract ID: 296 / Poster Number: 061.17P

Green Synthesized Silver Nanoparticles Induce Membrane Permeability and Morphological Damage in Staphylococcus aureus

Presenting author: Mrs. Canan Sevinc Sasmaz, Necmettin Erbakan university, Konya, Turkey

Co-authors: *Fatih Erci, Emrah Torlak*

Introduction: Staphylococcus aureus has emerged as a significant public health issue due to its increasing virulence and resistance to a growing number of antibiotics. Therefore, understanding the effects of antimicrobial substances, such as silver nanoparticles (AgNPs) synthesized using green methods, is essential for controlling S. aureus infections.

Objective: The aim of this study was to synthesize silver AgNPs from Hypericum perforatum L. and evaluate their antibacterial activity against S. aureus. Furthermore, the effects of AgNPs on morphological damage and membrane permeability in S. aureus cells were examined.

Method: The characterization of the AgNPs was conducted using techniques such as UV-Vis spectroscopy, X-ray diffraction, Transmission Electron Microscopy, and Fourier Transform Infrared spectroscopy. The antibacterial activity of AgNPs were evaluated using a standard microdilution method. β-galactosidase assay was used to assess the integrity and permeability of the cell membrane. The morphological changes in bacterial cells were examined using scanning electron microscopy.

Findings: TEM analysis revealed that the AgNPs were spherical with an average particle size of approximately 35 nm. FT-IR analysis confirmed the presence of various functional groups on the surface of the AgNPs. The AgNPs demonstrated significant antibacterial activity against S. aureus with a minimum inhibitory concentration (MIC) of 75 µg/mL. The findings indicate that the nanoparticles significantly disrupted bacterial membrane permeability. Furthermore, SEM revealed structural impairments in the S. aureus bacterial cells treated with green-synthesized AgNPs.

Conclusion: Our findings emphasize the potential of AgNPs to effectively combat bacteria, disrupt vital bacterial processes, and exhibit promising antimicrobial properties.

Abstract ID: 1767 / Poster Number: 057.16P

Grouping of clinical isolates of Pseudomonas aeruginosa in Japan

Presenting author: Takashige Kashimoto, PhD, Veterinary Public Health, Kitasato University., Towada, Japan

Co-authors: *Takayuki Sano, Kohei Yamazaki*

Pseudomonas aeruginosa can be classified into three groups based on the presence of specific virulence genes: Group 1 (pore-forming toxin *exlA* [-], T3SS machinery *pscL* [+], T3SS effector *exoU* [-]), Group 2 (*exlA* [-], *pscL* [+], *exoU* [+]), and Group 3 (*exlA* [+], *pscL* [-], *exoU* [-]). Group 1 strains, which represent the typical phenotype of *P. aeruginosa*, are associated with less pronounced clinical symptoms compared to strains in other groups. Group 2 strains, characterized by the presence of ExoU with phospholipase A2 activity, are linked to an increased risk of early mortality. Group 3 strains, although rarely detected, have been shown in mouse models to cause acute hemorrhagic pneumonia.

In this study, the above grouping system was applied to 125 *P. aeruginosa* strains isolated from patients' blood at university hospitals. The analysis revealed that 82.5% of the strains belonged to Group 1, 17.5% to Group 2, and none to Group 3. Notably, close monitoring and attention are necessary for Group 2 strains due to their association with severe outcomes. The absence of Group 3 strains suggests a low infection rate for this group in Japan.

Future research will aim to expand the collection of strains from additional sources, increase the sample size, and integrate patient clinical data to evaluate potential differences in disease severity across the groups.

Abstract ID: 1987 / Poster Number: 421.15P

Growth with a pollutant can be a challenge even for bacteria: strategies to respond to genotoxic metabolites of dichloromethane utilization by Methylobacterium extorquens DM4

Presenting author: Dr. Françoise Bringel, GMGM, Université de Strasbourg, Strasbourg, France

Co-authors: *Maria Osipenko, Domitille Jarrige, Ethan Rodrigues, Catherine Badel, Emilie Muller, Stéphane Vuilleumier, Raphaël Delépée*

Dichloromethane (DCM, CH_2Cl_2) is a toxic organohalogenated compound massively used in industry. Its accidental release is deleterious on human health and the environment[1]. DCM biodegradation involves DcmA, an enzyme of the glutathione S-transferase (GST) family that catalyses dehalogenation by breaking the C-Cl bonds of DCM. In methylotrophic bacteria, the dehalogenase DcmA is essential for aerobic growth with DCM as carbon and energy source[2]. DcmA activity on DCM generates toxic short-lived reactive metabolites that form covalent bonds with nucleotide bases[3]. DNA adducts, if left unrepaired, may lead to mutations and cell death. A *polA* mutant of *Methylobacterium extorquens* DM4 has long been known to be unable to grow with DCM due to its DNA polymerase I with a non-functional DNA repair domain[4]. Such repair processes are essential for DCM degraders but remain largely uncharacterized.

To identify new functions involved in the bacterial response to DCM genotoxicity, a lab evolution experiment was used to evolve the DM4 *polA* strain to regain its ability to grow using DCM. After approximately 100 generations in presence of 8 mM DCM, isolated and genome sequenced evolved strains able to grow with DCM, displayed suppressor mutations in the α SU of pol III encoded by the *dnaE* gene. Complementation assays providing the evolved *dnaE* variant allele was sufficient to restore DCM growth in strain DM4 *polA*. These results suggest that the amino acid substitution mutations detected in the evolved DnaE slow down the passage of DNA polymerase III on DNA, increasing replication fidelity and ensuring cell survival during DCM growth and minimizing the impact of DNA adducts associated with the DCM dehalogenation genotoxicity.

The effects of genetic context (taxon of DCM-degrader or genotype, i.e. wild-type or mutant impaired in DNA repair & maintenance processes) on DNA adduct profiles and abundance associated with GST-mediated DCM conversion is currently also investigated using mass spectrometry.

Taken together, the results will contribute to evaluate whether DNA adducts may be useful as biomarkers of environmental DCM contamination.

Abstract ID: 2843 / Poster Number: 022.16P

Gut bacteria degrade the danger signal extracellular ATP to hypoxanthine

Presenting author: Mr. Carlos Hernández-Ortego, Umeå University, Umeå, Sweden

Co-authors: *Valeria Salazar Sanchez, Sophie Tronnet, Per Rogne, Lalitha Tadala, Julie Klein, Dorothee Langenbach, Johanna Urzua, Koen Askamp, Sun Nyunt Wai, Magnus Wolf-Watz, Andrea Puhar*

Extracellular ATP (eATP) acts as a Danger Associated Molecular Pattern. In mammalian cells eATP binds to purinergic receptors located on the cell surface, starting signalling cascades and modulating inflammation. We have previously shown that intestinal epithelial cells secrete ATP through connexin hemichannels during infection with bacterial pathogens, triggering inflammation. Additionally, gut bacteria release ATP during growth. Whether intestinal bacteria can regulate the eATP in the gut to dampen inflammation, and how, is poorly studied. Here, we show that *Escherichia coli* is able to enzymatically degrade eATP to hypoxanthine. To identify the factors involved, we screened a genome-wide mutant library of non-pathogenic *E. coli* BW25113. This revealed the key enzymes in the eATP degradation pathway, and identified potential transporters and regulators. The roles of the factors were confirmed using biochemical and genetic techniques, with special emphasis on the eATP degrading factor, which was purified and biochemically characterized. Degradation of eATP occurs in the bacterial envelope, and the responsible enzyme is not secreted. However, outer membrane vesicles carry the eATP-degrading enzyme, which could thus act remotely. We further tested eATP degradation in various strains of pathogenic Gammaproteobacteria. Finally, we confirmed the role of the eATP-degrading factor in modulating eATP concentrations in bacterial cultures and in an enterocyte infection model. This work expands our mechanistic knowledge of how bacteria regulate the eATP concentration in the gut, and the ensuing impact on the development of inflammation.

Abstract ID: 3353 / Poster Number: 086.16P

Gut bacterial fermentation of green, black and selected herbal teas in vitro

Presenting author: Ms. Blathine Donnelly, University of Reading, Reading, United Kingdom

Functional gut disorders affect approximately 40% of the global population, posing significant health concerns. Recent trends focus on functional foods and a proactive approach to prevention over cure. Tea, a widely consumed beverage, presents as a promising functional food candidate if it exhibits prebiotic properties. This study evaluated the prebiotic potential of Lipton (Signature Blend Green and Black) and Pukka teas (Matcha Green, Three Liquorice, Three Fennel, Three Mint, Three Cinnamon, Three Ginger, After Dinner and Feel New) through assessing the gut microbiome in vitro.

Faecal samples from healthy volunteers (aged 18–45) were inoculated into anaerobic batch cultures for 48 hours, along with tea substrates comparable to a supplement containing 2 g of plant material (0.004% w/v) or 2 cups of tea (0.001% w/v). Short-chain fatty acid (SCFA) levels were quantified using gas chromatography-flame ionisation detection (GC-FID), and bacterial populations were assessed by fluorescence in situ hybridisation coupled to flow cytometry. Fermentation resulted in increased SCFA levels between baseline and 48 hours. Matcha Green (0.004% w/v) significantly increased acetate and propionate levels after 48 hours compared to the control. Three Cinnamon at the same dose significantly increased acetate levels at 24 and 48 hours. Black Tea (0.001% w/v) significantly increased acetate levels after 48 hours compared to the control. Bacterial enumeration is ongoing. Three Cinnamon, Three Ginger, and Black Tea show potential to positively impact the gut microbiome. Prebiotic properties of the ingredient that shows the best potential will be further evaluated in an in vivo intervention study.

Abstract ID: 3867

Gut microbial production of imidazole propionate drives Parkinson's pathologies

Presenting author: Jihyun Kim, POSTECH, Pohang, South Korea

Co-authors: *Ara Koh, Hyunji Park, Eunkyung Bong*

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the selective loss of midbrain dopaminergic neurons and α-synuclein aggregation, leading to motor dysfunction. Increasing evidence suggests a link between gut microbiota and PD, but the **specific microbial species and metabolites involved remain unclear**.

Objectives: This study aimed to identify gut microbiota-derived metabolites contributing to PD pathogenesis. Specifically, we investigated the role of ***Streptococcus mutans***, a bacterium enriched in PD patients, and its metabolite **imidazole propionate (ImP)** in promoting neurotoxicity, motor deficits, and exacerbating α-synuclein pathology.

Methods: We analyzed gut microbiome data from PD patients to assess the abundance of **urocanate reductase (UrdA)**, an enzyme responsible for ImP production. Using germ-free and antibiotic-treated mice, we examined the effects of gut-colonized ***S. mutans*** and **ImP** on **dopaminergic neurodegeneration, neuroinflammation, and motor dysfunction**. We further investigated **mTORC1 activation**, a key signaling pathway linked to ImP-induced neurotoxicity. Additionally, we measured **plasma ImP levels in PD patients** to validate its potential role in human disease.

Findings: Our study revealed that **PD patients exhibit elevated UrdA levels** in their gut microbiome and increased plasma ImP concentrations. Colonization of germ-free mice with ***S. mutans*** resulted in increased systemic and brain ImP levels, leading to **dopaminergic neuron loss, neuroinflammation, and motor deficits**. Additionally, **gut colonized *S. mutans* exacerbated α-synuclein aggregation** in a preformed fibril-induced PD mouse model, indicating its potential to accelerate PD progression. Furthermore, **ImP administration alone was sufficient to induce key PD features**, suggesting its direct neurotoxic role. Mechanistically, we identified **mTORC1 activation** as a crucial mediator of *S. mutans*-induced neurotoxicity.

Conclusion: This study identifies a novel **gut-brain axis** mechanism in PD, demonstrating that gut-derived **ImP** from ***S. mutans*** penetrates the brain and contributes to **dopaminergic neurodegeneration via mTORC1 activation**. Elevated plasma ImP levels in PD patients suggest its potential as **a biomarker and therapeutic target**. These

findings emphasize the role of the gut microbiota in PD pathogenesis and pave the way for **microbiome-targeted interventions** in neurodegenerative diseases.

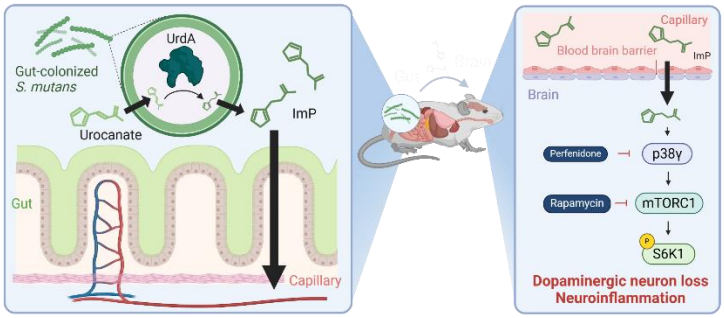


Figure 1. Graphical summary. Gut microbial production of imidazole propionate (ImP) via urocanate reductase (UrdA) from *Streptococcus mutans* can penetrate the brain, where it selectively induces dopaminergic neurodegeneration, neuroinflammation, and motor deficits through mTORC1-dependent mechanisms**.**

Abstract ID: 3538 / Poster Number: 091.16P

Gut Microbiome Divergence and Opportunistic Pathogens in Wild vs. Captive Turquoise Killifish

Presenting author: Dr. Flávio Costa, Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany

Co-authors: *Samuel Kean, Daniel Davila, Dario Valenzano*

The turquoise killifish (*Nothobranchius furzeri*) is known for its short lifespan, making it an excellent model for ageing research. Previous studies have highlighted that the gut microbiome undergoes compositional shifts during ageing and plays a crucial role in modulating lifespan. Remarkably, heterochronic microbiome transplantation (from young to old killifish) has been shown to extend lifespan and improve general motility, establishing killifish as the first organisms to demonstrate the microbiome's ability to influence host ageing. We investigated the gut microbiome of turquoise killifish using both cultivation-dependent and -independent methods. Metagenomes of captive and wild populations were sequenced using nanopore technology, while culturomics was employed to isolate and characterize bacteria from captive fish. Our findings revealed that the gut microbiome composition of captive fish differs significantly from wild counterparts. Captive killifish gut microbiota was dominated by *Vibrio* and *Aeromonas* species, whereas wild fish harbored *Aeromonas* and *Klebsiella* as dominant taxa. Dietary differences were also evident: wild fish consumed diverse prey such as mosquitoes and dragonflies, contributing to a more diverse gut microbiome. In contrast, captive fish fed primarily on bloodworms displayed reduced microbial diversity. Cultivation methods retrieved dominant gut bacteria from captive fish, revealing pathogenic potential, including antimicrobial resistance genes and virulence factors. These findings suggest that certain bacterial strains may act as opportunistic pathogens under conditions of microbial imbalance, such as ageing. Our study highlights the critical role of diet and microbial homeostasis in shaping the gut microbiome and influencing age-associated health and longevity.

Abstract ID: 2846

Gut Microbiomes in Freshwater Fish: Unveiling Microbial Diversity, Host Health Dynamics, Ecological Functions, and Conservation Implications

Presenting author: Prof. Ram Krishan Negi, Department of Zoology, University of Delhi, Delhi, India

Co-authors: *Tarana Negi*

The gut microbiota of fish is crucial for maintaining host health, immunity, metabolism and nutrient cycling. Despite growing interest, little is known about the structural and functional diversity of fish gut microbiomes and impact of environmental factors on it. This research seeks to fill these gaps by investigating the gut microbiota of various freshwater fish species, including *Cyprinus carpio* var. *specularis, Tor putitora* and *Hypophthalmichthys molitrix* from Gobind Sagar reservoir, *Cyprinus carpio and Oreochromis niloticus* from Yamuna River. The research focuses on functional traits like nitrogen cycling, pathogen resistance, and adaptation to environmental pollution which are crucial for their survival and health.

The gut content was isolated in aseptic conditions and DNA extracted was analysed with the combination of 16S rRNA gene amplicon and shotgun metagenomic sequencing. Taxonomic diversity and functional profiles of the microbiota were determined for multiple gut compartments (foregut, midgut, and hindgut) across species. The data were analyzed for microbial composition, ecological network analysis, and functional prediction. The presence of antibiotic resistance genes, virulence factors, and heavy metal degrading microbes was also examined.

Our findings show difference in microbial communities across different species. In omnivorous fish species, *Cyprinus carpio* var. *specularis*, the hindgut microbiota is richer than foregut and has higher abundance of nitrogen-fixing, ammonia-oxidizing, and denitrifying bacteria like *Rhodospirillaceae*, *Oxalobacteraceae*, *Nitrosomonadaceae*, and *Nitrospiraceae* which have an important role in nitrogen assimilation and ammonia detoxification. The abundant phyla in the foregut *were Euryarchaeota, Fusobacteria, Cyanobacteria, Verrucomicrobia*, and *Firmicutes* important for providing immunity and nutrients to the host. In the case of *Tor putitora* (Mahseer), an endangered species, *Proteobacteria* was the dominant phylum. *Aeromonas* and *Caulobacter* were prevalent, along with antibiotic-resistant *Klebsiella* and *E. coli* strains. Genome analysis identified *Aeromonads* as primary fish pathogens that highlights potential health risks. The invasive *Silver Carp* (*Hypophthalmichthys molitrix*) showed specialized microbial assemblages in

different gut regions, according to its dietary habits that facilitates its invasion. *Proteobacteria* was the most abundant phylum, with varying percentages across the foregut (31.2%), midgut (46.59%), and hindgut (36.58%). *Actinobacteria*, prominent in the foregut (13.24%), decreased in the midgut and hindgut, where *Fusobacteria* and *Firmicutes* became more prevalent. *Fusobacteria* dominated the midgut and hindgut, while *Firmicutes* and *Bacteroidetes* were abundant in the foregut. The gut microbiomes of *Cyprinus carpio* and *Oreochromis niloticus* from the polluted River Yamuna was also dominated by *Proteobacteria* like other fish species. The gut microbiome of *Cyprinus carpio* and *Oreochromis niloticus* contained microbial communities like *Pseudomonas fluorescens* and *Klebsiella pneumoniae* capable of heavy metal degradation and *Nitrosomonas sp.*, *Methanosaeta harundinacea for* organic matter decomposition that indicate their adaptability to environmental stressors.

The study shows how different species of fish and various compartments of their guts have diverse and adaptable microbial communities and responds to environmental pollution. Our study offer valuable insights for understanding microbial ecology and fish health. These findings are valuable for improving bioremediation efforts, managing pollutants, and supporting sustainable fish farming.

Abstract ID: 2715 / Poster Number: 197.16P

Gut Microbiota Synergize Acupuncture Treatment Responses in Atopic Dermatitis

Presenting author: Yerin Seo, Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, South Korea

Co-authors: *Sujin Lee, Kyuseok Kim, Hi-Joon Park, Soon-Kyeong Kwon*

Acupuncture (Acc) therapy alleviates symptoms of atopic dermatitis (AD), including itch severity and SCORAD (Scoring AD) index, by stimulating the autonomic nervous system through nerve bundles such as cells, skin, muscles, and peripheral nervous systems. Interestingly, significant alterations in gut microbiota composition have been observed in AD patients following Acc treatment, particularly among those with gastrointestinal symptoms. This study investigates gut microbiome traits that influence the therapeutic effectiveness of Acc in AD patients. To achieve this, we analyzed gut microbiome profiles of AD patients categorized into responder (R) and non-responder (NR) groups based on changes in SCORAD. The R group displayed greater gut microbiome stability throughout treatment, suggesting that maintaining microbial homeostasis may facilitate AD improvement. Furthermore, distinct microbial signatures were identified in the R group, distinguishing them from the NR group. Prior to Acc therapy, the genera *Alistipes* and *Odoribacter* were significantly more abundant in responders. Co-occurrence analysis revealed that these genera formed part of the same co-abundance network. Additionally, metagenome-assembled genomes derived from whole-metagenome sequencing (WMS) data provided insights into the metabolic features associated with enhanced responsiveness to Acc treatment. These findings offer valuable potential gut microbiome biomarkers for personalized Acc therapy to individual patients, particularly those undergoing long-term AD management.

Abstract ID: 4143

Gut Motility-Microbiome Interregulation

Presenting author: Prof. Dr. Sahar El Aidy, Department of Microbiome Engineering, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands; Amsterdam Microbiome Expert Centre, University of Amsterdam, Amsterdam, The Netherlands

Gut motility is one of the most tightly regulated physiological functions, essential for the transit, mixing, and breakdown of luminal content. Its coordination with transport processes, such as fluid flow, molecular absorption, and microbial dispersal, is critical to gastrointestinal function. Emerging evidence highlights a bidirectional interregulation between gut motility and the microbiota: not only does motility shape microbial distribution, but microbial metabolism of food components and medications, in turn, modulates motility.

For example, we showed how microbial conversion of serotonin precursors can accelerate intestinal transit, whereas transformation of polyphenols like mangiferin into norathyrol slows it down. In Parkinson's disease, both the drugs themselves and their bacterial metabolites slow small intestinal motility, while residual medication transformed in the colon further contributes to constipation, a common non-motor symptom. These complex interactions underscore the need for more integrative models of microbiome–motility dynamics.

To address this, we recently developed IntestiFlow, a platform for high-resolution measurement and manipulation of gut motility in the presence of defined microbial communities. This system enables precise investigation of how microbial metabolism feeds back on host physiology, offering new opportunities to dissect the intertwined roles of motility, microbial activity, and host health.

Abstract ID: 3855

Gut Mycobiota Dysbiosis is Associated with Melanoma and Response to Immunotherapy

Presenting author: Dr. Natalia Szóstak, Institute of Bioorganic Chemistry of Polish Academy of Sciences, Poznan, Poland

Co-authors: *Anna Philips*

Introduction: Emerging evidence highlights the role of gut microbiota, including the fungal community (mycobiota), in cancer progression and response to immunotherapy. While bacterial impact has been extensively studied, the influence of gut fungi on melanoma and immunotherapy efficiency remains largely underexplored.

Objective(s): This study aimed to investigate gut mycobiota composition in melanoma patients and to assess its correlation with disease progression and clinical outcome during anti–PD-1 immunotherapy.

Method: We conducted metagenomic sequencing to characterize gut fungal communities in fecal samples from stage III and IV melanoma patients (n=86; 164 samples in total) and healthy participants (n=134). Of the 86 melanoma patients, 62 were undergoing anti–PD-1 therapy with nivolumab or pembrolizumab, while 24 clinically stable patients were receiving monthly injections of the whole-cell genetic therapeutic melanoma vaccine AGI-101H as part of the ETAM2-5 Trial. Each melanoma patient was assessed according to RECIST v.1.1 criteria (SD, CR, PR, PD). Fungal profiles were monitored longitudinally at three time points during anti–PD-1 therapy: before treatment, at 3 months, and after 12 months. Fungal profiling was performed using Kraken2/Bracken, followed by extensive statistical analyses, including survival analysis and modeling with Cox proportional hazards (CPH).

Findings: Melanoma patients exhibited a distinct gut mycobiota composition compared to healthy controls, characterized by an increased abundance of *Candida albicans*, *Candida dubliniensis*, and *Neurospora crassa*, alongside decreased levels of *Saccharomyces cerevisiae* and *Debaryomyces hansenii*. During anti–PD-1 therapy, the relative abundance of *Malassezia restricta* and *C. albicans* increased. Higher levels of *Saccharomyces paradoxus* were linked to positive clinical responses, whereas *Tetrapisispora blattae* was associated with non-beneficial outcomes. Importantly, elevated *M. restricta* and *C. albicans* levels, coupled with high serum LDH and overweight status, were predictors of disease progression and reduced therapy efficacy. Patients with high *M. restricta* levels were 2.2 times more likely to experience disease progression

compared to those with lower levels, while patients with elevated *C. albicans* levels faced a twofold increased risk of progression. Median progression time was notably shorter for patients with higher *M. restricta* levels (~5 months) compared to those with lower levels (~13 months). An even greater disparity was observed for *C. albicans*, with median progression times of ~3 months versus ~14.5 months for high and low abundance groups, respectively.

Conclusion: This study identifies melanoma-associated mycobiome dysbiosis and highlights specific fungal species linked to treatment outcomes. The findings suggest that gut fungi may modulate the immune response to anti–PD-1 therapy, offering potential biomarkers for patient stratification and therapeutic interventions aimed at modulating the gut mycobiota for improved cancer care.

Abstract ID: 449 / Poster Number: 123.15F

Gut phageome dynamics of the relapse/remission cycle in ulcerative colitis

Presenting author: Dr. Maria Kulecka, University College Cork, Cork, Ireland

Co-authors: *Ana Velikonja, Sara Pereira, Chloe Huseyin, Andrey Shkoporov, Marcus Claesson*

Introduction: The course of ulcerative colitis (UC) is characterized by intertwining periods of remission and relapse. Most microbiome UC studies focus on the gut bacteriome. However, recent discoveries suggest that phage-mediated bacterial cell lysis may play a role in sustaining gut inflammation.

Objective: Our aim was to explore the gut phageome of patients with UC who suffer from frequent relapses and find associations between gut viral composition and disease state.

Methods: 148 stool samples were collected monthly from 25 patients. Faecal calprotectin measures (FCal) and Simple Colitis Activity Index (SCAI) were used to assess levels of intestinal inflammation and severity of symptoms, respectively. 3 patients remained in remission, 5 in relapse and the rest was switching between the two. Shotgun sequencing was used to assess metagenome composition.

Findings: Phages were detected in 132 samples from 24 patients. They belonged almost exclusively to the bacteriophage order *Crassvirales,* infecting mainly family *Bacteroidaceae*. While bacterial diversity was negatively correlated with present and future UC symptoms severity (Spearman's coefficient<-0.2, p-value<0.05), phage diversity and richness were both positively correlated with them (Spearman's coefficient>0.2, p-value<0.05). *Diorhovirus intestinalis* and *Kingevirus communis* were positively associated with symptom severity (padj<0.05, LINDA) and were central hubs in the phage interactions network in patients with active intestinal inflammation.

Conclusion: Gut phage load is positively associated with symptom severity in adult patients with UC. Our analysis was limited to known DNA viruses so it will be complemented by whole genome sequencing of phageome, including RNA viruses and poorly characterized viral species.

Abstract ID: 1249 / Poster Number: 412.17P

Harnessing Fusarium spp. for Green Nanoparticle Synthesis and Their Encapsulation in Calcium Carbonate Microsphere for Controlled Release

Presenting author: Dr. Aniketkumar Gade, Nicolaus Copernicus University, Torun, Poland

Co-authors: *Julia Nadrowska, Joanna Trzcińska-Wencel, Patrycja Golinska, Mahendra Rai*

Introduction: The use of fungi in general and Fusarium spp in particular for catalysing specific reactions leading to inorganic nanoparticles is a new and rational biosynthesis strategy. Extracellular synthesis of nanoparticles by fungi offers an advantage of obtaining large quantities in a relatively pure and at a rapid rate. The use of water as an environmentally benign solvent, biomolecules as reducing and stabilizing/capping agents making it the green process.

The encapsulation of these nanoparticles into calcium carbonate microspheres, which act as carriers for controlled release. The calcium carbonate matrix not only provides a biocompatible environment but also allows for a sustained and precise release of the nanoparticles, improving efficiency and reducing waste.

Objective: In the present study Fusarium spp were used for the synthesis of iron oxide, zinc oxide (ZnO), magnesium oxide(MgO) and copper oxide(CuO) nanoparticles. Antibacterial evaluation against plant pathogenic bacteria. Loading of synthesized nanoparticles into casein micelle and encapsulation of casein loaded nanoparticles in calcium carbonate microspheres for controlled release of nanoparticles.

Methods: Fungal secretions were used for the reduction of precursor salts and formation of the nanoparticles. The CuO, ZnO, MgO and iron oxide nanoparticle synthesis was detected by taking a UV-Vis spectrum. Further nanoparticles were characterized using different techniques like FTIR, XRD, NTA, TEM and zeta potential measurements.

MIC and MBC were determined by broth dilution method against *Agrobacterium tumefaciens* IOR 911, *Pectobacterium carotovorum* PCM 2056, *Pseudomonas syringae* IOR 2188 and *Xanthomonas campestris* IOR 512.

Synthesized nanoparticles (NPs) were loaded into casein micelle for the formation of Casein-NP complex. Formation of optimum casein-NP complex was based on the analysis done by UV-visible spectroscopy, SEM-EDX and ICP-MS analysis. The casein-Np

complex was encapsulated in calcium carbonate microspheres and analysed by ICP-MS, SEM and TEM-EDX.

Findings: *Fusarium graminearum* D was found to be suitable for the synthesis of ZnO, CuO and iron oxide nanoparticles while *F. tricinctum* was found to be suitable for the synthesis of MgO nanoparticles. Inhibitory potential of synthesized NPs against plant pathogenic bacteria was found to be of order CuO> ZnO > MgO > iron oxide. Ammonium carbonate was found to be suitable for the formation of calcium carbonate microspheres.

Conclusion: Synthesis of iron oxide, ZnO, CuO and MgO NPs is a green process. Demonstrated significant activity against bacterial plant pathogens. Synthesized NPs-casein complex can be encapsulated in calcium carbonate microsphere by precipitation method. This encapsulation enhances the stability and functionality of the nanoparticles, making them suitable for various applications, including drug delivery, agriculture, and environmental remediation.

Abstract ID: 3094 / Poster Number: 317.16F

Harnessing Microbial Diversity in PIWI Grapevines for Sustainable Winemaking

Presenting author: Mr. Mattia Cesana, University of Milan, Milan, Italy

Co-authors: *Nicola Mangieri, Alessio Altomare, Marta Baviera, Giulio Staffieri, Diego Mora, Daniela Fracassetti, Giorgio Gargari*

PIWI grapevines represent a promising alternative to traditional *Vitis vinifera* varieties. These hybrids exhibit resistance to certain fungal and oomycete diseases, offering a potential solution for reducing the use of phytosanitary products and promoting sustainable farming practices. The METAPIWI project aims to investigate the microbiological differences between PIWI and conventional grapevines, focusing on the influence of the microbiome associated with these two varieties on spontaneous fermentation. This highlights their crucial role in producing distinctive aromas and metabolites that enhance wine quality.

The project seeks to identify microbial metabolic pathways responsible for the production of aromatic compounds in wines fermented with native microorganisms. This will be achieved through a multidisciplinary approach integrating chemical analyses, metagenomics, metabolomics, and culturomics. Culturomics will enable the isolation and detailed analysis of cultivable microbes, providing valuable insights into their metabolic functions. Additionally, predictive biomarkers will be developed to enhance wine quality and mitigate fermentation challenges.

Preliminary results indicate that PIWI grapevines host a broader diversity of non-*Saccharomyces* yeasts, such as *Starmerella bacillaris*, *Metschnikowia pulcherrima*, and *Hanseniaspora uvarum*, compared to their conventional counterparts. This increased diversity has been linked to improved fermentation in PIWI musts, underscoring their potential to support sustainable viticulture and winemaking.

In conclusion, the METAPIWI project pioneers the study of interactions between microbial communities and grapevine varieties, particularly in relation to fermentation and aroma production. By leveraging advanced techniques like metagenomics and metabolomics, this research stands at the forefront of viticulture, offering groundbreaking insights for sustainable winemaking practices.

Abstract ID: 1265 / Poster Number: 413.16S

Harnessing Microbial Metabolites to Reduce Dependence on Synthetic Phosphorus and Potassium Fertilizers

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Introduction: The 20th century witnessed a dramatic increase in agricultural productivity due to innovations introduced during the Green Revolution. However, the widespread reliance on synthetic fertilizers, particularly phosphorus (P) and potassium (K), has led to significant environmental challenges, including soil degradation, eutrophication, and greenhouse gas emissions. This necessitates the development of sustainable agricultural practices to reduce dependence on synthetic fertilizers. One promising solution is leveraging microbial metabolites to enhance nutrient bioavailability and promote plant growth.

Objective: This study aimed to evaluate the potential of microbial metabolites, specifically siderophores and accompanying metabolites (SAM), in reducing the reliance on synthetic P and K fertilizers. The research objectives were to assess changes in nutrient bioavailability, analyze soil microbiome structure and activity, and evaluate plant growth and fitness under varying fertilization conditions.

Method: A biofertilizer developed in previous work, based on SAM produced by the psychrotolerant strain Pseudomonas sp. ANT_H12B, was used in greenhouse experiments. Radish plants were grown under both optimal and suboptimal P/K fertilization conditions, with and without the addition of SAM. Nutrient bioavailability (iron, P, and K) was quantified, while changes in the soil microbiome were analyzed using sequencing-based approaches. Plant growth parameters, including biomass and root development, were measured to evaluate the impact of the biofertilizer.

Findings: The addition of SAM significantly increased the bioavailability of P and K, particularly under suboptimal fertilization conditions. This effect was attributed to the solubilization of nutrients through microbial metabolites such as siderophores and organic acids. Enhanced nutrient availability stimulated native soil microbiota, promoting nutrient cycling and soil health. Plants treated with SAM exhibited improved growth and fitness compared to controls, highlighting the potential of microbial metabolites as an effective alternative to synthetic fertilizers.

Conclusion: Microbial metabolites offer a promising solution to reduce reliance on synthetic fertilizers by enhancing the bioavailability of essential nutrients. The results demonstrate the potential of metabolite-based biofertilizers to support sustainable agricultural practices, contributing to environmental conservation and reducing the negative impacts associated with traditional fertilization methods. Further research is needed to optimize formulations for diverse environmental conditions and crops.

Abstract ID: 3241 / Poster Number: 318.16F

Harnessing Nature: Xanthomonas sp. Synthesise Yellow Pigments of Significant Antioxidant Potential

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The yellow pigment extracted from *Xanthomonas* sp. is known to potentially exhibit antioxidant activity and prevent lipid peroxidation. However, its application as a novel antioxidant remains underexplored due to its incomplete purification and characterization. This study aimed to purify the yellow pigment from *Xanthomonas oryzae* pv. *oryzae* (XOO) and evaluate its antioxidant potential. Crude pigment (CP) was extracted from XOO pellet using solvent extraction, followed by saponification and subsequent washing by solvents to derive the enriched pigment (EP). EP was characterized via spectroscopic techniques such as UV-spectroscopy, FTIR, and HPLC. The antioxidant activity was evaluated through DPPH R_f-Shift assay (developed by us) using TLC Explorer and the conventional DPPH assay. Cytotoxicity was assessed via cell viability assays using NIH/3T3 fibroblast cells, while DCFDA and MDA assays quantified ROS and lipid peroxidation. Spectral analysis showed an absorption peak at 440 nm, with HPLC results revealing peaks at 2.5 and 15.7 minutes. The DPPH R_f-Shift assay confirmed antioxidant activity in EP, comparable to β-carotene standard. EP demonstrated 1.8-fold higher activity than β-carotene in the DPPH assay. Both CP and EP maintained 80% cell viability at doses 20 µg/mL and reduced ROS levels by 40%. EP significantly decreased lipid peroxidation, comparable to β-carotene, while CP showed minimal effects. The observed reduction in ROS levels, coupled with low cytotoxicity, underscores the antioxidant activity of EP following its successful purification. Given *Xanthomonas'* industrial approval, this pigment offers a sustainable alternative to synthetic antioxidants for pharmaceutical and nutraceutical applications.

Abstract ID: 684

Harnessing nature's hidden alliances: oil degradation potential of procaryotes and algae

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Crude oil can flow naturally to the earth's surface without human intervention. Such natural oil seeps occur around the world, leaking into marine, freshwater or terrestrial environments through geological processes and fractures in underground rocks. While oil is toxic to most living organisms, many bacteria and some algae can use it as a carbon source and degrade it. From a microbiological perspective, these natural seeps are relatively understudied compared to anthropogenic spills, despite significant biological, chemical and physical differences.

In our research, we study the native microorganisms and species interactions, including prokaryotes and eukaryotic microalgae, isolated from natural oil seeps. We hypothesize that these native organisms are better adapted to oil and can degrade anthropogenic oil spills faster than microorganisms from freshly contaminated sites. In addition, algae harbor oil-degrading prokaryotes in their phycosphere, which helps them survive in harsh, toxic conditions while providing CO_2 from oil mineralization.

We sampled microorganisms and isolated oil-tolerant algae from two natural oil seeps in Germany and set up microcosm experiments to test our hypotheses. Oil degradation was assessed by monitoring visual and microscopic changes, pH, cell numbers (DAPI/flow cytometry), determining microbial community composition via 16/18S rRNA gene sequencing, and measuring CO_2 evolution using reverse stable isotope labelling (RSIL) method. RSIL allows us to measure the mineralization of complex oils without the need to know their exact chemical composition.

Our results show that adding microbes from natural oil seeps to an oil spill can accelerate oil degradation rates by up to 60%. In addition, prokaryotic algal mixed-cultures degrade oil effectively.

Understudied natural oil seeps provide a diverse habitat with a large potential of well-adapted oil-degrading microorganisms and consortia that could accelerate the degradation of anthropogenic oil spills and contribute to carbon cycling. Prokaryotic-

algal interactions appear to be important in creating more stable conditions for both to thrive in toxic oil environments.

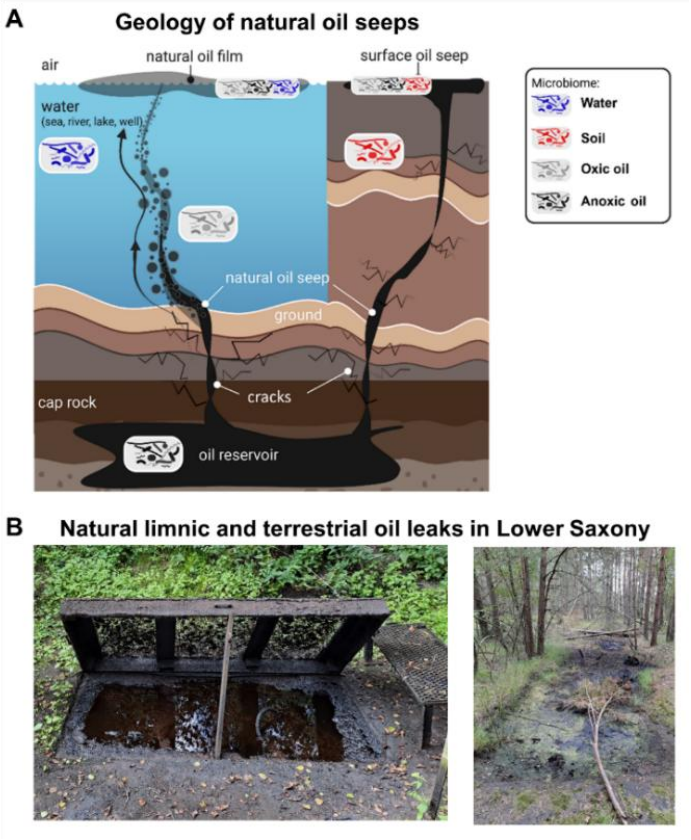


Figure 1: **A)** Illustration of natural oil leaks and films: Crude oil from underground oil reservoirs emerges to the surface through small cracks in the rock and can occur in the sea, in lakes and rivers (left) as well as in terrestrial areas (right). The composition of the microbes varies depending on the location (created with BioRender.com). **B)** Natural oil leaks in Germany. Left: Tar pit, oil reaches the surface of the groundwater basin and forms an oil film. Right: Oil leak in a forest, heavy oil (black area) reaches the earth's surface without human intervention. Despite toxicity, grass, moss, lichen, algae and trees grow directly adjacent or on top of the oil.

Abstract ID: 483 / Poster Number: 264.17P

Harnessing synthetic microbial biotechnology for efficient plastic waste upcycling

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Plastics have become indispensable in various industries today, but their extensive use has resulted in a severe environmental crisis—plastic waste accumulation. Traditional methods of plastic waste management are insufficient, facing persistent pitfalls, such as overall material downgrading and release of hazardous microplastics and chemicals into the environment. Plastic upcycling, on the contrary, is gaining prominence in converting plastic waste into valuable chemicals and materials in an eco-friendly manner. The current project presents an innovative approach to plastic waste upcycling: employing green bio/mechano/chemical technologies for the hydrolytic degradation of plastic polymers into oligomers and their constituent building blocks, is followed by the creation of microbial cell factories capable of producing industrially relevant compounds from these plastic-derived feedstocks. Successful microbial upcycling hinges on efficient carbon assimilation from plastic feedstocks and the presence of functional intracellular biosynthetic pathways to generate valuable bioproducts. To achieve this, a wide variety of microorganisms with natural capabilities for these processes are identified through bioprospecting, and synthetic biology is employed to engineer or enhance the other necessary functions. Alternatively, some well-established microbial hosts including Bacillus spp., Pseudomonas spp. or Yarrowia spp. serve as suitable chassis. By integrating these advanced methodologies, we aim to create sustainable and efficient microbial systems for plastic waste valorization. Our ultimate goal is to establish a research and innovation hub in Synthetic Microbial Biotechnology that would advance scientific knowledge, raise public awareness, promote environmental protection, and boost economic development, all the while minimizing humanity's reliance on finite fossil fuel resources.

Abstract ID: 3265 / Poster Number: 231.16P

Harnessing the gut microbiome for personalized treatment of lymphoma and leukemia

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Allogeneic hematopoietic cell transplantation (allo-HCT) is one of the few curative treatments for hematologic malignancies such as lymphoma and leukemia. While life-saving, it is also a dangerous procedure associated with significant adverse effects, such as graft-versus-host disease (GVHD), increased infection susceptibility, disease relapse, and many other complications that reduce the patients' quality of life. Several factors can influence the effectiveness of allo-HCT, including the microbiome composition and its impact on metabolism as a strong modulator of immune function. Yet, critical knowledge gaps remain in understanding how the microbiota and its interplay with the host can influence the development of GVHD and the severity of adverse outcomes. To address these gaps, we aim to characterize a cohort of 100 allo-HCT patients enrolled in a single-center prospective cohort study at the University Hospital Basel with an immediate 180-day follow-up after allo-HCT. Using a multi-omic approach, we aim to characterize the temporal dynamics of the microbiome and metabolome, integrating these data with clinical and laboratory measurements to identify associations that could be used to predict risks for GHVD and other complications. This will also enable the discovery of potential biomarkers, an important step toward improving the personalized management and care of patients with hematological malignancies. By elucidating the complex interactions between the microbiota, immune system, and the host, this research has the potential to translate into novel interventions, ultimately improving the quality of care and reducing the risk of complications in patients following allo-HCT.

Abstract ID: 3179 / Poster Number: 344.15S

Harnessing the potential of natural antimicrobials: oregano and cinnamon essential oils vs. Listeria monocytogenes

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The growing consumer demand for safe, minimally processed foods free from chemical additives has driven increased research into green and innovative solutions utilizing natural antimicrobials. Plants serve as a valuable source of bioactive compounds able to prevent or reduce the growth of food-borne pathogens and spoilage microorganisms. Among these, essential oils (EOs), characterized by a promising antimicrobial potential, can ensure the safety and quality of fresh products, reducing the environmental impacts of agro-food sector. In this context, different EOs obtained from Mediterranean and medicinal plants were investigated for their chemical composition through chromatographic techniques and tested in vitro against food-borne pathogens to assess their Minimum Inhibitory Concentration (MIC) in different conditions. Based on these findings, oregano and cinnamon EOs, characterized by the higher antimicrobial activity, were selected to assess their effect when used at MIC, halved MIC and double MIC on Listeria monocytogenes Scott A. In particular, both culture-dependent (plate counting) and culture-independent (flow cytometry) methods were used to evaluate the physiological state of the target microorganism during EO exposure and the cell recovery potential after EO removal. When cells of L. monocytogenes were exposed to a concentration of 0.25 mg/ml (MIC value) of oregano EO, mainly constituted of carvacrol, a loss of culturability, already after 30 min of exposure, was observed. However, after 24 h from EO removal, culturability was restored. On the other hand, in the presence of a double MIC concentration (0.50 mg/ml), culturability was always below the detection limit (< 1 log CFU/ml), even after the removal of the natural preservative. The study of the relative frequency of the different cell sub-populations (live, injured and dead) assessed with a dual staining (SYBR Green I and propidium iodide) flow cytometric procedure, showed a slight increase in viable cells already after 2 h of recovery in the sample exposed to 0.25 mg/ml of oregano EO. On the contrary, 0.50 mg/ml of oregano EO exerted a bactericidal effect under all conditions considered (more than 99% of the total cells were recognized as dead). Cinnamon EO exhibited a different behavior. In particular, its activity exerted a bacteriostatic effect, resulting in an inhibition of L. monocytogenes growth detected with culture-dependent methods. In this case, in the sample treated

with the double MIC concentration (0.50 mg/ml), it was possible to observe a good level of viability recovery after the EO removal (about 25 % of the total population).

The results confirmed that the tested EOs, especially oregano, showed good potential in controlling L. monocytogenes in vitro. The use of cytofluorimetric assays allowed to increase the knowledge of the EO mechanism of action against this pathogen. These data highlighted the promising perspective of the EO application in the food industry as an alternative to synthetic additives to obtain safe and natural products.

This research is a part of InnoSol4Med PRIMA-Section 2 project, supported by the European Union, aiming to increase the quality, safety, and nutritional value of traditional Mediterranean foods using sustainable methods and innovative ingredients.

Abstract ID: 2268 / Poster Number: 075.15P

Harnessing the power of bacteria to fight recalcitrant post-consumer PET-waste burden

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Co-authors: *Patricia Concórdio-Reis, Buket Tas, Marija Nicevic, Margaret Brennan-Fournet, Jasmina Nikodinovic-Runic, Cristiana Torres, Maria Reis, Filomena Freitas*

Introduction: Polyethylene terephthalate (PET), one of the most widely used petrochemical-based thermoplastics, has significant harmful environmental and health impacts due to its improper disposal given its recalcitrancy to degradation under environmental conditions. Despite the available recycling technologies, many wasted PET products comprise impurities of different nature (e.g., oils, pigments, etc.), additives or other polymers, that impair their recycling. Therefore, novel approaches are still required to complement the existing ones.

Objectives: This work aimed at the valorisation of post-consumer PET waste into value-added bacterial biomass.

Method: Post-consumer PET waste, comprising 95% PET, 5% polyethylene and traces of carbon black additives, was depolymerized via reactive extrusion, resulting in a material named REX-PET. This material was processed into an aqueous solution containing sodium terephthalate (TPA) that was used as the sole carbon source for the cultivation of *Glutamicibacter nicotianae* strain 24A, isolated from soil contaminated with plastic waste collected at a municipal solid waste management facility.

Findings: The 62-h fed-batch bioreactor (3 L) cultivation of *G. nicotianae* strain 24A, under a controlled temperature of 30 ºC and a pH value of 7.0, yielded 9.4 g/L of dry biomass, with a protein content of 48wt%. A TPA consumption rate of 20 g/(L.day) was displayed by the culture that reached a TPA removal efficiency of 89% and a biomass yield of 0.4 g/g. Additionally, the biomass presented a bright yellow coloration indicative of the presence of a pigment that was extracted and characterized.

Conclusion: This study demonstrated the potential of the newly isolated *G. nicotianae* strain 24A for the upcycling of post-consumer PET waste into bacterial biomass and pigments of high value, thus contributing for tackling the problems associated with the management of wastes containing this recalcitrant petrochemical-derived plastic

contaminated with other chemicals, whose current recycling through other technologies in impracticable.

Abstract ID: 3439

HcpE and DsbK contribute to the host inflammatory response to Helicobacter pylori

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Co-authors: *Noor Salloum, Jeffrey Lester, Charles Bodet, Christophe Burucoa*

Introduction: The bacterium *Helicobacter pylori* (HP) colonizes chronically 50% of the world population, leading to gastric ulcers or cancers in 2-10 % of infections. Due to rising antibiotic resistance, curtailing HP-induced diseases requires identifying new factors that contribute to its virulence, including its ability to elicit inflammation. Amongst *HP*'s virulence factors are the Helicobacter Cysteine-rich Proteins (Hcp). The presence of up to 7 Hcps (depending on *HP* strain) despite *HP*'s small genome underscores their importance in *HP*'s persistence within its natural niche: the human stomach. The Hcps contain protein / protein interaction motifs that suggest a role in host / bacteria interactions. While anti-Hcp antibodies are detected in infected patients, they are unable to clear the infection and thus Hcps were long considered as immune decoys. However, HcpA/C were shown to interact with host components and may modulate the function of immune cells. The effects of Hcps on gastric cells remain unknown and there may be functional differences between Hcps that remain to be explored.

Objectives: We focus on HcpE (HP0235), the largest family member that may interact with many host proteins through its 9 interaction motifs, and on the DiSulfide Bond forming protein DsbK, a folding factor essential for HcpE production. We aim to determine their role in *H. pylori* virulence.

Methods: We used Western blot with in-house anti-HcpE antibodies to assess secretion of HcpE by laboratory or clinical specimens, and used ELISA done with recombinant HcpE to assess anti-HcpE antibody titers in patient sera. We used knockout mutants of HcpE and DsbK and recombinant HcpE to assess the role of HcpE and DsbK in gastric cell activation measuring the cytokines released by qRT-PCR or ELISA. Also, mice were orally gavaged with *HP* wild-type or mutants and we assessed the *HP* load in their stomach 4 and 6 weeks post gavage, and used their spleens to assess the inflammatory potential of HcpE on splenocytes by ELISA.

Findings: We showed that HcpE is secreted outside HP in 2 laboratory strains NCTC 11167 (human) and SS1 (mouse adapted) and that ~30% of secreted HcpE was comprised in outer membrane vesicles that may allow entry of HcpE in eukaryotic cells

by membrane fusion**.** We also showed that DsbK is essential for HcpE production and secretion. We further demonstrated the production and secretion of HcpE by clinical isolates of *H. pylori in vitro*, and its production *in situ* in infected patients. Using our HcpE and DsbK knockout mutants, we showed the role of HcpE and other Hcps in in interactions with human gastric cells *in vitro* and in *ex vivo* gastric explants, and in triggering inflammation in both models. Finally, we demonstrated the role of HcpE and DsbK in murine gastric colonization and splenocytes activation.

Conclusion: Our data show a role of Hcps in host / pathogen interactions largely due to immuno-modulatory functions and a role in gastric colonization, with a predominant role of HcpE over all other Hcps. This opens up possibilities to use Hcps or the gate keeper DsbK that controls their production as novel therapeutic targets to alleviate the burden of *HP*-associated disease. This could benefit the ~6 million patients who develop gastric ulcers or cancers each year worldwide due to *HP* infection, also saving billions of annual health care costs.

Abstract ID: 3395 / Poster Number: 328.16F

Headspace Volatile Organic Compounds (VOCs) as Indicators of Microbial Spoilage in Refrigerated Cooked Food/Leftovers

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Introduction: Households contribute over half of total food waste in the European Union. Indeed, refrigerated cooked food leftovers are often discarded earlier due to suspected spoilage despite a lack of clear indicators. To provide accurate feedback on food status, detecting microbial volatile organic compounds-(VOCs) could provide reliable information, enabling sensor technologies to reduce food waste.

Objectives: To investigate the microbiological and VOCs changes in refrigerated cooked food and to correlate them with food spoilage phases.

Methods: Meatballs and paella (n=5/food), each with 18 (90g/container) hermetic containers, were stored at 6°C for 15 days. Microbial (Mesophiles-MES, Psychrotrophs-PSY, Enterobacteriaceae-ENB, Pseudomonas-PSM, Lactic Acid Bacteria-LAB, Yeast, and Moulds-YnM) changes were monitored. Bidimensional gas chromatography with flame ionization and mass spectrometry detectors were used for VOCs analyses.

Results: Microbial growth increased from 3 to 15 days in both food. In meatballs, on day 6, MES, PSY, and LAB growth surpassed 6 log CFU/g; hence, consumption is not advisable. In paella, MES and PSY exceeded 6 log CFU/g after day 6, while ENB growth already reached 4 log CFU/g on day 7 and continued to grow; thus, it might not be safe for consumption. Coincidently with these events, various VOCs in both foods exhibited moderate to significant positive/negative correlations with microbial growth; alcohols, sulfur, terpenes, hydrocarbons were positively correlated, while aldehydes and amide were negatively correlated. Distinctively, alkanes in paella; alkenes, and ethers in meatballs showed positive correlations with microbial growth.

Conclusion: The experiment demonstrated that monitoring VOCs profiles can effectively track microbial food spoilage stages.

Abstract ID: 1170 / Poster Number: 049.16P

Heat-treated L. plantarum strains show Anti-Obesity Effects by Modulating Lipid Metabolism

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Co-authors: *Jong-Woo Kim, Kee-Tae Kim, Na-Kyoung Lee, Hyun-Dong Paik*

Obesity is a significant global health challenge associated with metabolic disorders, including type 2 diabetes, cardiovascular diseases, and non-alcoholic fatty liver disease (NAFLD). This study investigated the anti-obesity effects of heat-treated *Lactiplantibacillus plantarum* strains (WB4201, WB4202, and WB4203) isolated from kimchi using a 3T3-L1 preadipocytes.

Cells were cultured in BCS-supplemented DMEM until they reached confluency over 3–4 days, then differentiated for 8 days. Differentiation was induced with MDI medium on day -2, and insulin-containing medium was replaced every two days from day 0. *L. plantarum* strains were added at the start of differentiation and during subsequent medium changes. Differentiated and undifferentiated cells were assigned as the positive and negative control, and LGG, a well-characterized probiotic strain, served as the commercial control.

Heat-treated *L. plantarum* strains demonstrated significant regulatory effects on the expression of genes and proteins involved in lipid metabolism. Adipogenic (C/EBP-α, PPAR-γ, Akt2), lipogenic (FAS, SREBP-1c, ACC1, GPAT), and fatty acid β-oxidation (CPT1, CD36) related genes were analyzed by RT-PCR. *L. plantarum* strains exhibited a 30–40% reduction in adipogenic and lipogenic gene expression compared to the positive control. CPT1 expression modestly increased, indicating enhanced fatty acid β-oxidation. Western blot analysis confirmed the downregulation of SREBP-1, PPAR-γ, C/EBP-α, and FAS, with *L. plantarum*-treated groups exhibiting stronger inhibition of lipid metabolism than LGG.

These findings highlight the potential of heat-treated *L. plantarum* WB4201, WB4202, and WB4203 as candidates for modulating lipid metabolism and developing health-functional foods to combat obesity and related disorders.

Abstract ID: 1763 / Poster Number: 136.15P

Heavy Metal Contamination Alters Soil Bacterial Community Structure Around Cement Factories in Korea

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Co-authors: *Sang-Ah Lee, Dongguk Song, Mehwish Taj*

The cement industry has played a crucial role in economic development since the Industrial Revolution. However, cement production releases heavy metals, which can disrupt the balance and functioning of microbial communities. Despite this, few studies have explored the impact of cement production on nearby environmental microbiomes. In this study, we collected topsoil samples (0-60 cm) from four cement factories in Korea to evaluate the levels of heavy metals (arsenic, cadmium, lead, nickel, mercury, and chromium) and analyse bacterial communities. Using the pollution load index, 15 samples were classified as highly contaminated, 3 as moderately polluted, and 6 as unpolluted. Heavy metal contamination significantly influenced bacterial community structure. Linear discriminant analysis revealed that, at the family level, *Micromonosporaceae*, *Azospirillaceae*, *Steroidobacteraceae*, and *Genodermatophilaceae* were enriched in polluted samples, while *Devosiaceae* was more abundant in unpolluted samples. Bacterial communities clustered into modules corresponding to contamination levels. Taxonomically, the heavy metal-tolerant module was primarily composed of *Gaiella*, *Bacillus*, *Nitrospira*, and *Vicinamibacteraceae*. Arsenic and cadmium emerged as key metals contributing to the formation of the heavy metal-tolerant module. Overall, our findings suggested that heavy metal contamination is linked to distinct bacterial communities, which may play a role in bioremediation processes.

Abstract ID: 1542 / Poster Number: 282.17P

Heterologous expression of aminoglycoside biosynthesis gene clusters

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The spread of antimicrobial resistance (AMR) is a global medical challenge, driving the search for new antibacterial agents. Aminoglycosides are a small class of broad-spectrum antibiotics synthesized by genera Micromonospora and Streptomyces. Discovered in the 1940s, they were widely used during the early stages of antimicrobial chemotherapy. However, the absence of new representatives and the emergence of alternative antibiotic classes have led to a significant decline in their use. The rising prevalence of AMR problem and the synergistic activity of aminoglycosides with other antibiotics have renewed interest in this class. The complexity of working with Micromonospora and the lack of effective heterologous hosts for aminocyclitol type natural products biosynthetic gene clusters (BGCs) limit the discovery of new aminoglycosides.

The Streptomyces kanamyceticus LV-54 strain, derived from S. kanamyceticus DSM40500 through mutagenesis, has lost its ability to produce kanamycin. Cloning of kanamycin BGC (kan BGC) in this strain restores antibiotic production, while its expression in commonly used heterologous hosts (S. albus Del14 and S. lividans ΔYA9) was not successful. This prompted an idea of utilizing S. kanamyceticus LV-54 as a universal host for aminoglycoside BGC expression. 13 aminocyclitol BGCs were selected from the in-house collection and introduced into S. kanamyceticus LV-54. The production of metabolites was evaluated by biological activity against Gram-positive and Gram-negative test cultures. Optimization of cultivation conditions enabled the activation of 5 out of 13 studied BGCs. Notably, one of these five expressed BGCs encode biosynthesis of previously unknown aminocyclitols.

Abstract ID: 649 / Poster Number: 266.16P

Heterologous production of hyaluronidase by Kluyveromyces lactis

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Co-authors: *Leticia Minervino Da Silva, Antonio Milton Vieira Gomes, Carlos Alexandre Breyer, Renata Bannitz*

Hyaluronidases (HAses) are enzymes that cleave the chains of the biopolymer hyaluronic acid (HA). They have various applications and can be used as an adjuvant in anesthetic pharmaceutical formulations, reversing problems caused by HA fillers and also have applications in bioproduction processes of HA with different molecular masses. Even today, HAses are mainly obtained by extraction from animal tissues, especially cattle and sheep, in an unsustainable and expensive process. As an alternative, various heterologous expression systems have been developed using bacterial, yeast and mammalian cells. This study aims to clone leech HAse (class II) using the GRAS-status yeast Kluyveromyces lactis. Initially, the Lhyal gene from Hirudo nipponia was inserted into the Pklac2 cloning cassette for integration into the yeast genome. A strain was then constructed using the electroporation protocol. The results showed that the gene was integrated and were confirmed by PCR. Protein expression was verified and confirmed by SDS-PAGE; and the presence of enzymatic activity was proven by the visualization of halos on agarose plates containing AH. This is the first study to report the production of HAse in K. lactis. The study contributes to advances in the development of HAse production platforms. The next steps will focus on optimizing the culture medium, as well as validating a specific activity measurement protocol, with the aim of achieving a more attractive and efficient production process, capable of meeting the demand for HAse in a safe and sustainable way.

Abstract ID: 2771 / Poster Number: 144.17P

Heterotrophic bacteria in drinking water: evaluating antibiotic resistance and the presence of virulence genes

Presenting author: Assoc. Prof. Lesego Molale-Tom, North-West University, Potchefstroom, South Africa

Heterotrophic bacteria, particularly those resistant to antibiotics, pose significant health risks to individuals with infections or compromised immunity. The prevalence of these bacteria in water sources underscores the need for targeted surveillance and mitigation strategies. This study investigates the prevalence, enzyme activity, and antibiotic resistance profiles of heterotrophic plate count bacteria from two South African water treatment facilities, North West-C (NWC) and North West-G (NWG) Water samples from NWC and NWG were analyzed for β-hemolysis, enzyme activity (hemolysin, lipase, DNase), and antibiotic resistance profiles. Multiple antibiotic resistance (MAR) indices were calculated, and whole genome sequencing was performed to identify resistance genes. β-hemolysis was prevalent (NWC: 82.5%, NWG: 86.7%), and 98% of isolates exhibited enzyme production, with significant differences in hemolysin ($P = 0.013$), lipase ($P = 0.009$), and DNase ($P = 0.006$) activity between sites. Ciprofloxacin residues persisted throughout water treatment, and isolates demonstrated high resistance to β-lactams and trimethoprim (47%–100%), particularly in NWC. MAR index analysis revealed that 90% of isolates exceeded 0.20, suggesting exposure to high antibiotic usage environments. Whole genome sequencing identified diverse antibiotic resistance genes, underscoring the adaptability of these bacteria. Heterotrophic bacteria in water sources harbour diverse antibiotic resistance genes, high resistance levels, and enzyme activity, highlighting significant public health risks. Effective water treatment and surveillance strategies are critical to mitigating these threats.

Abstract ID: 3687 / Poster Number: 344.16P

Hexyl gallate as green alternative against citrus canker: Biofilm eradication potential and fate in the environment

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Introduction: Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (Xac), is one of the main bacterial diseases affecting the citrus industry. Our group investigates bio-based phenolics as green alternatives to currently used copper sprays. Hexyl gallate proved to be efficient in both *in vitro* studies and preliminary greenhouse trials. However, some aspects of its use on a broader scale are still unknown. In a real-case scenario, it should be able to eradicate Xac biofilms formed on the leaf surfaces and degrade safely in the environment. We present our recent findings on its biofilm eradication efficacy and its environmental fate.

Methods: The biofilm eradication potential of hexyl gallate was evaluated using confocal laser scanning microscopy. Biofilm composition was characterized to identify factors that limit its activity. The degradation was evaluated through a hydrolysis assay.

Findings: Hexyl gallate eradicated fragile Xac biofilms at concentrations only four times higher than those needed for planktonic cells. However, prolonged exposure to lower concentrations did not improve its effectiveness. Xanthan, the primary biofilm component, was identified as the possible limiting factor in the diffusion of hexyl gallate. Under abiotic alkaline conditions, hexyl gallate has a half-life of 1 to 2 days and degrades mainly into ellagic acid.

Conclusion: Due to its relatively fast degradation to a non-toxic product, hexyl gallate could represent a safer option for citrus canker control and sustainable agriculture than copper sprays. However, an optimized formulation needs to be developed to improve its biofilm eradication potential.

Abstract ID: 3068 / Poster Number: 268.15P

Hidden in plain soil: biogeography and ecology of the Genus Palsa-504

Presenting author: Dr. Francesco Delogu, University of Aalborg, Aalborg, Denmark

Co-authors: *Thomas Jensen, Kalinka Knudsen, Sofie Vestergaard, Per Nielsen, Mads Albertsen*

Soil is one of the pillars of life on our planet, allowing plants to prosper and to support the human civilization with agriculture. Identifying important microbial players in the soil-plant interface has been a major conquest, such as cracking the composition of the Nitrogen-fixing rhizobium complex associated with legumes. In 1986, Acidothermus cellolyticus, a thermophilic, acido-tolerant and carbohydrate-degrading bacterium, was isolated from a geyser in Yellowstone. Since its discovery nearly 40 years ago, no genome has been recovered from soils, but *A. cellolyticus* has been reported as ubiquitous and dominant in soils and rhizospheres worldwide using 16S data.

In a Danish set of 10,000+ samples, we observed again the ubiquity and dominance of the genus Acidothermus. However, we could match the 16S observations with a large metagenome-assembled genome collection. Here, we present how up to ten genera of the family Streptosporangiaceae had been conflated with the genus Acidothermus, hiding some of the most common soil and rhizosphere-associated genera in plain sight.

We focused on the most prevalent genus Palsa-504 and, re-analysing the microbeatlas.org data, showed it occurred in peatlands, forests and shrubs (15%, 14% and 13% prevalence, respectively) worldwide. Moreover, using the Harmonized World Soil Dataset, we showed that its distribution does not depend on large-scale soil properties. We also explored the genetic potential of the Palsa-504 pangenome to demonstrate a distinct ecological role from Acidothermus.

In conclusion, we rectified a common misconception and re-introduced multiple genera as common in soil, shedding light on their potential ecological role.

Abstract ID: 2761 / Poster Number: 131.15F

Hidden Marine Temperate Mesophotic Microbiota

Presenting author: Ms. Rafaela Roque, Centre of Molecular and Environmental Biology (CBMA), University of Minho, Braga, Portugal

Co-authors: *Vanessa Rodriguez, Filipa Soares, Diego Flores, Marcos Rubal, Pedro Gomes, Pedro Santos*

Marine microbial communities are essential drivers of global biogeochemical cycles, ocean ecology, and planetary health. Mesophotic ecosystems, found at intermediate depths (30–150 m) represent unique transitional zones between shallow and deep waters, yet they face increasing threats by environmental pressures such as climate change and anthropogenic activity. These vulnerable ecosystems support critical ecological processes, including nutrient cycling, and waste bioremediation, while providing essential biodiversity refuges. Despite their importance for marine life, temperate mesophotic ecosystems (TMEs) remain understudied, especially concerning the diversity, dynamics, and adaptative potential of their associated microbiota.

To address this gap, multiple sediment samples were collected from 16 distinct mesophotic zones (30–90 m depth), using remotely operated vehicles. DNA from all sediment samples was retrieved and analysed through 16S rRNA metabarcoding. Results revealed a significant number of shared ASVs across depths, suggesting the presence of a microbial core in TMEs. This evidence may serve as a baseline for assessing ecosystem health and improving biomonitoring strategies in mesophotic environments, based on microbiome profiling.

Additionally, over 60 different bacterial species were selectively isolated and identified from TME sediments, based on their biotech potential. These isolates are being further analysed through phenomics and genomics, to establish a well characterized TME-derived microbial biobank. Overall, this study provides new insights into TME microbiota composition, dynamics and traits, paving the way for biotechnological research supporting blue economy innovations, while the sustainable use and conservation of marine bioresources for future generations.

Abstract ID: 1911

High diversity of species and resistance genes associated with microplastics in urbanised aquatic ecosystems

Presenting author: Dr. Elsa Gadoin, UGent, Gent, Belgium

Co-authors: *Méril Massot, Martijn Callens, Pauline Arnout, Stéphanie Bedhomme, Andreja Rajkovic*

Microplastics (MP) are ubiquitous in aquatic ecosystems, where they are rapidly colonized by microbial communities, referred to as the plastisphere. Of great concern is the detection of potential pathogens and antimicrobial resistance genes in plastisphere, that might be transported through MP drifting. Therefore, better characterization of plastisphere composition and variability is essential to finer evaluate the impact of MP pollution on aquatic ecosystems and its consequences, at both sanitary and ecological level.

We used metagenomic tools to assess taxa diversity, antimicrobial resistance and virulence genes in the plastisphere of polypropylene (PP) particles incubated *in situ* in freshwater and marine locations, following a salinity and anthropic gradient around Ostend (Belgium).

Our results demonstrated significant variability of the plastisphere across incubation sites, regarding both prokaryote and eukaryote composition. Potential pathogenic bacteria (PPB) represented on average 7% of total bacterial reads within the plastisphere while detected virulence genes were mainly involved in nutrition and adherence. Normalizing metagenomic reads with digital qPCR, results demonstrated a selective enrichment of various antimicrobial resistance and virulence genes in biofilm samples, while these were less abundant but more diverse in surrounding water samples. Wide diversity of PPB, antimicrobial resistance and virulence genes was observed in all studied sites, which might be explained by high anthropogenic activities and heavy urbanisation. Enrichment of PPB, antimicrobial resistance and virulence genes in biofilms compared to planktonic communities underscore MP capacity to act as a reservoir for these elements, calling for better understanding of the risks associated with their dispersion in aquatic environments.

Abstract ID: 1915 / Poster Number: 304.17P

High Solid Enzymatic Saccharification of Pretreated Lignocellulosic Biomass using the Cellulolytic Enzyme Cocktail

Presenting author: Jeonghee Yun, PhD, Kookmin University, Seoul, South Korea

Co-authors: *Seungjun Kim, Iksu Ha, Hee Wook Ryu, Kyung-Suk Cho*

Enzymatic saccharification is a core step in the biorefinery process for production of free sugar from pretreated lignocellulosic biomass. There is required to run the process at high solids loadings to consider economical practical for large-scale use, but solid loading increase can cause low operability by a high viscosity slurry. In this work, enzymatic saccharification at high biomass loadings (15%, w/v) of pretreated woody biomass using the cellulolytic enzyme cocktail was examined in a working volume of test tube and bioreactor scale. The unused woody biomass (bark residue-containing Quercus sp.) was pretreated under steam-explosion condition by severity factor 2.8 (225℃, 3 min), and cellulose was used as control substrate. The optimized enzymatic cocktail composed by 20% of Trichoderma sp. KMF006 crude enzyme and 80% the commercial enzyme of Cellic CTec3 (Novozymes) was used for effective sugar release. The quantification analysis of sugar as saccharification products was conducted by HPLC analysis. The chemical composition of the bark-containing Quercus sp. after pretreatment showed 42.22% glucan, 5.44% xylan, 1.75% arabinan, and 40.61% lignin. The saccharification rate for cellulose at a 15% substrate loading using each enzyme alone was 92.5% and 96.5% for the commercial enzyme and the developed crude enzyme, respectively. The saccharification rate for steam-exploded woody biomass with 15% of high substrate concentration was 101.7% in a test tube scale, and it showed 119.8% in a 6 L bioreactor scale-up. The findings could help enhance the economic feasibility of the high solids enzymatic saccharification process in subsequent cellulosic sugar utilization process.

Abstract ID: 3755 / Poster Number: 446.15P

High-throughput comparative analysis of Kluyveromyces marxianus strains to optimise industrial applications

Presenting author: Mr. Roberto Romano, University College Cork, Cork, Ireland; SUSFERM Centre for Sustainable Fermentation and Bioprocess, Cork, Ireland

Co-authors: *John Morrissey, Gaurav Rajauria*

Kluyveromyces marxianus is a non-conventional yeast whose industrial potential has been recently exploited. The wide range of substrates on which can grow, the fastest growth rates among eukaryotic organisms, the thermotolerance and the General Regarded As Safe status make it a perfect candidate for precision fermentations. Even if many strains *of K. marxianus* have been discovered and differences in growth conditions have been observed among them, systemic studies to compare those strains and optimise their growth conditions are still lacking.

For this reason, our project has two main goals: to compare the growth of different *K. marxianus* strains on raw materials to select the most promising strains for industrial application; to optimising the growing conditions of those strains to enhance biomass production.

The study fits in a wider project to create an infrastructure that connect multiple University and Industry realities in Ireland involved in Industrial Biotechnology. The analyses have been performed with the help of facilities that allow to run different yeasts growth curves in parallel. The first screening has been conducted in Clariostar using as substrates cheese whey and ligno-cellulosic raw substrates. The condition optimisation of the best strains has been studied using Biolector.

Among the pool of strains, nine have been selected to have the highest biomass production in the first screening. Following-up optimisation has included pH, temperature, vitamins and nitrogen source.

In this way, it has been possible to exploit the potential of *K. marxianus* for fermentation from raw material, allowing it to express its industrial potential.

Abstract ID: 1342 / Poster Number: 090.17P

High-throughput screening for evolution of antibiotic susceptibility in longitudinal uropathogenic isolates

Presenting author: Ms. Lieze Agten, KU Leuven, Leuven, Belgium; VIB – KU Leuven, Leuven, Belgium

Co-authors: *Lise Caljon, Lotte Vanmeerbeek, Laura Schillebeeckx, Noémie Luyts, Wouter Everaerts, Bram Van den Bergh, Natalie Verstraeten, Jan Michiels*

Since the discovery and implementation of antibiotics, the rapid emergence and spread of bacterial antibiotic survival mechanisms have been posing major threats to healthcare systems worldwide. Among these mechanisms, antibiotic resistance, which enables bacteria to grow and survive during antibiotic treatment, is the most notorious, raising public concern amid the global antimicrobial resistance crisis. Another survival strategy complicating treatment is antibiotic persistence, a phenotype attributed to a small subset of cells in an isogenic bacterial population that survive high antibiotic concentrations by entering a transiently multi-drug tolerant state. Post-treatment, these persister cells can revert to their antibiotic-sensitive state, allowing them to establish a new infection-causing population. Interestingly, recent *in vitro* studies have shown that persistence contributes to the development of resistance, and that frequent exposure to antibiotics stimulates evolution towards high persister levels.

To assess whether these *in vitro* evolutionary patterns are mirrored *in vivo*, over 2000 longitudinal bacterial isolates from urine samples of patients with suprapubic catheters were collected and analyzed. Initial identification on CHROMagar™ Orientation medium was complemented by 16S rRNA sequencing. Urine culture revealed high microbial loads of both common and less common uropathogens, often exceeding 10^5 colony forming units per milliliter, which is indicative of catheter-associated asymptomatic bacteriuria.

To simultaneously assess antibiotic resistance of 384 isolates, a high-throughput agar dilution minimum inhibitory concentration (MIC) assay was developed, making convenient use of an Echo 525 acoustic liquid handler. Preliminary results using amikacin and ciprofloxacin reveal a double-peaked MIC distribution among the isolates, with peaks around 1 µg/ml and 64 µg/ml for both antibiotics (Figure 1). Notably, the distribution for amikacin is generally shifted towards higher MICs compared to ciprofloxacin. Additionally, a subset of isolates display temporal variation in resistance levels, suggesting evolution of antibiotic resistance in this clinically relevant model.

Ongoing research focusses on the optimization of a high-throughput persistence assay to assess persister levels across all isolates. Combined, these novel experimental setups will provide valuable insights into the evolution of antibiotic susceptibility in a clinical context, greatly improving our understanding of the dynamics of antibiotic resistance and persistence.

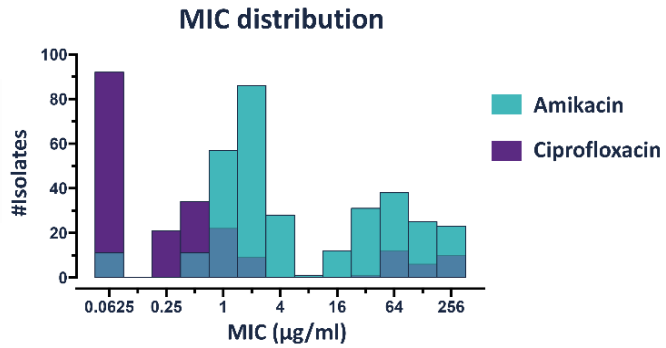


Figure 1: Distribution of minimum inhibitory concentration (MIC) values for antibiotics amikacin and ciprofloxacin across a subset of uropathogenic isolates. A double-peaked MIC distribution is evident, with peaks around 1 µg/ml and 64 µg/ml for both antibiotics.

Abstract ID: 1917 / Poster Number: 381.15P

High-throughput screening to find genes related to mutational robustness in Escherichia coli

Presenting author: Ms. Lore Devriendt, KU Leuven, Leuven, Belgium; VIB, Leuven, Belgium

Co-authors: *Louise Boelens, Philip Ruelens, Natalie Verstraeten, Jan Michiels*

Mutations fuel evolution, yet the majority are deleterious, presenting a fundamental paradox in biology: how do organisms survive the largely deleterious effect of random mutations while still exploiting rare beneficial mutations to adapt? Mechanisms of mutational robustness, which reduce the phenotypic consequences of mutations, remain poorly understood. In bacteria, chaperones like GroEL and DnaK play a key role in this process by stabilizing protein function despite mutations. Notably, GroEL overexpression enhances the fitness of mutated bacteria and correlates with higher rates of non-synonymous mutations, suggesting its role in accelerating evolution. However, systematically identifying genes that buffer mutations and elucidating their mechanisms remains challenging, due to the need to evaluate many genes against a broad spectrum of mutations.

To address this, we propose an innovative approach which integrates two high-throughput techniques in *Escherichia coli*. First, pooled CRISPR interference to methodically silence genes in mutation accumulation lines and identify those genes critical to mutational robustness, as their loss reveals otherwise hidden deleterious effects of mutations. Second, MAGESTIC, a novel method that permits introducing tens of thousands of unique mutations across cells, each linked to a barcode, enabling precise mapping of mutations that can be buffered by identified buffer genes. This integrated strategy promises novel insights into mutational robustness.

By combining these complementary strategies, we aim to uncover previously uncharacterized buffer genes and ultimately provide fundamental insight into the biological mechanisms underlying mutational robustness.

Abstract ID: 2580

High-throughput single-cell genomics for association of bacteria with their mobile genetic elements

Presenting author: Dr. Mária Džunková, University of Valencia, Valencia, Spain

Co-authors: *Inés Alberola Mora, Vicente Arnau, Pilar Domingo-Calap, Lucas Mora, Rokaia Lejri, Alicia Ortiz Maiques, Marta Pola, Dafne Porcel Sanchis, Juan Valero Tebar*

Single-cell genomics provides large advantages over bulk metagenomics, especially in its ability to detect active infections by phages in bacterial cells, as well as to associate bacteria with their mobile genetic elements (MGEs), many of which may harbour auxiliary metabolic genes essential for microbial survival.

Nevertheless, the broader application of single-cell genomics has been hindered by the challenges in handling small reagent volumes which used to be applied to individual cells sorted into 96- or 384-well plates, as well as the high costs of processing each cell as a separate sequencing library.

Our laboratory has recently experienced the transition from the laborious single-cell genomics performed in plates to the high-throughput single-cell genomics by Atrandi microfluidics platform.

We contributed to the further development of this platform by testing its ability to distinguish closely related strains and detect active phage infection using long read sequencing. Furthermore, we developed a software for demultiplexing of the reads from split-and-pool barcoding of 10,000 cells in a single run. We also employed the Atrandi semipermeable capsules in custom capsule-PCR assays targeting specific genes to specifically sort cells containing connections to MGEs of our interest in natural ecosystems. The presentation provides an overview of our results in searching for MGEs in single-cells in various ecosystems, ranging from industrial extreme environments to symbiotic microbes of soft-bodied marine animals.

The platform provides a throughput comparable with classical bulk metagenomics, but with the advantage of single-cell barcoding, providing links between MGEs and their host bacteria.

Abstract ID: 3236 / Poster Number: 360.16P

Hmu heme uptake mechanism of pathogenic members of the Bacteroidota phylum – heme binding and release of HmuY hemophore-like proteins

Presenting author: Teresa Olczak, PhD, University of Wroclaw, Wroclaw, Poland

Co-authors: *Michał Śmiga, Francesco Malatesta*

Introduction: Dysbiosis in the oral microbiome may disrupt the mutualism between microbiota, resulting in periodontal diseases and concomitant systemic diseases. Among periodontopathogens are Bacteroidota members, e.g., *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, and *Porphyromonas endodontalis*. Being heme auxotrophs, they uptake heme from the host as an iron and protoporphyrin IX source using the Hmu system, with the leading role played by HmuY hemophore-like proteins.

Objective: HmuY proteins gain heme in the environment where heme levels are tightly restricted by host heme-sequestering proteins and facilitate heme transport into the bacterial cell. This study aimed to compare the heme-binding modes of HmuY proteins and to identify a potential mechanism of heme release from HmuY proteins for subsequent transport to outer membrane transporters.

Method: Overexpressed and purified native and modified by site-directed mutagenesis protein variants were characterized using UV-visible spectroscopic methods.

Findings: $HmuY^{Pg}$ is the first representative of the HmuY family that coordinates heme-iron using two histidines, which is in contrast to two methionines used by $HmuY^{Tf}$ and $HmuY^{Pi}$, and a histidine-methionine pair used by $HmuY^{Pe}$. Analysis of native protein structures and heme-binding properties of variants lacking one of the heme-iron coordinating ligands suggests a stepwise release of heme.

Conclusion: Differences exist between HmuY proteins in heme-iron coordinating ligands and heme-binding modes. The release of heme from HmuY proteins may be a multi-step process that requires a one-by-one sequential dissociation of amino acids coordinating heme-iron. This process can be influenced by factors found under altered conditions of periodontal diseases.

Abstract ID: 281 / Poster Number: 131.16S

Homemade versus commercial foods explain gut microbiome diversity and composition among one-year-old infants

Presenting author: Dr. Maryam Kebbe, University of New Brunswick, Fredericton, Canada

Co-authors: *Ben Perrett*

Introduction: Nutrition during the early months of life has long term implications on human health. Certain diets have been shown to alter the gut microbiome, however, the relationship between food source and the infant gut microbiome has not yet been investigated.

Objective: This secondary data analysis aimed to explore the relationship between food source (commercial, homemade, or both homemade and commercial diets) and the diversity and composition of the infant gut microbiome.

Method: This study was a secondary data analysis of a subset of the CHILD study cohort. Infant solid feeding data were collected via questionnaires at 6 months of age, while stool samples were collected at 1-year-of-age. Infants with missing solid feeding data at 6 months of age or missing stool samples at 1 year of age were excluded. The infant gut microbiome was profiled using 16S rRNA sequencing. Analyses were performed in R. 4.1.17. employing linear models with Box-Cox transformation for microbial diversity differentials and Multivariate Association with Linear Models ('MaAsLin2') for taxon abundance differentials across dietary source using CLR and TSS normalization. Models were adjusted for maternal pre-pregnancy body mass index, infant sex, infant ethnicity, hospital length of stay at birth, antibiotics at birth, mode of delivery, feeding mode at 1 year/time of stool collection, pet ownership, and study center location. A sensitivity analysis in weaned infants was performed. Benjamini–Hochberg correction was applied, and $p < 0.05$ and $q < 0.25$ were considered significant.

Findings: A total of 368 participants (unweaned cohort) and 82 participants (weaned cohort) were included in our analyses. In the unweaned cohort, inverse Simpson diversity index was significantly higher in infants fed homemade diets compared to infants fed commercial diets in the raw and adjusted models (0.031 ± 0.014, $p = 0.025$). In the weaned cohort, *Ruminococcus* was significantly higher in the homemade group as well as homemade and commercial feeding group, compared to the commercial feeding group in adjusted models (CLR homemade: 0.067 ± 0.0019, $p = 0.00082$, $q = 0.16$; CLR

homemade and commercial: 0.079 ± 0.0022, p = 0.00076, q = 0.16; TSS homemade: 5.40 ± 0.97, p = 0.031, q = 0.24). CLR and TSS normalization revealed unique differences between dietary source and microbial abundance in the weaned cohort.

Conclusion: Homemade diets may contribute to richness, evenness, and composition of the infant gut microbiome, particularly when solid food is the primary source of dietary intake. Further research is needed to fully understand the long-term health implications of different early-life feeding practices.

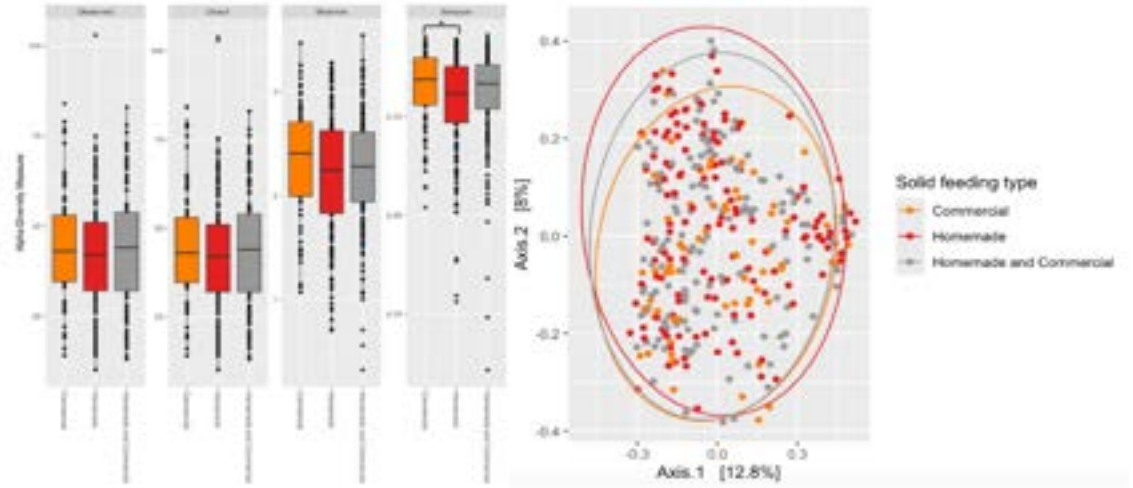


Figure 1: α- and β-diversity distributions by dietary source among infants (n=368). Left: Dotplots of richness, Chao1, Shannon index, and Simpson index. Right: Principal coordinates analysis based on unweighted UniFrac distances.

Abstract ID: 2722 / Poster Number: 140.17P

Horizontal transfer of an antibiotic resistance integron between two Pseudomonas species and genomic evolution of Pseudomonas aeruginosa strains in a Cystic Fibrosis patient's lungs

Presenting author: Dr. Alice Patry, Université de Limoges, Inserm UMR1092, CHU de Limoges, Limoges, France ; Laboratoire de Bactériologie-Virologie-Hygiène, CHU Limoges, Limoges, France

Co-authors: *Géofroy Kinhoégbè, Jane Languepin, Magalie Dupuis-Grasset, Fabien Garnier, Christophe Bordi, Sandra Da Re*

Pseudomonas aeruginosa is an opportunistic pathogen responsible of high morbidity and mortality in cystic fibrosis (CF) patients with chronic infection. These patients are subject to strong antibiotic selection pressure and eradication of *P. aeruginosa* is difficult due to its great adaptability to its environment. We present here the horizontal transfer of an antibiotic resistance integron from *Pseudomonas putida* to *P. aeruginosa* isolated from a CF patient through a conjugative element (ICE) and the evolutionary structural genomic analysis of *P. aeruginosa* strains isolated from the same patient's lungs over 12-years.

We carried out whole genome sequencing of our strains by combining two technologies: long-read sequencing (Oxford Nanopore) and short-read sequencing (Illumina). The resulting hybrid assemblies were annotated and thoroughly analysed.

Our results show that in *P. putida*, the integron is carried by a transposon, which is integrated into an ICE. We also show that this ICE was horizontally transferred between *P. putida* and *P. aeruginosa*. All subsequent *P. aeruginosa* strains were clonal and evolved into two distinct populations over time, indicating sub-clonal diversity in the patient's lung. These two *P. aeruginosa* populations displayed different genomic rearrangement patterns. In one population, there were major genomic changes, including inversions, segment losses, and translocations, which could affect genetic diversity and virulence. In the other population, the genomic alterations were more limited, suggesting greater genetic stability.

To our knowledge, this is the first study to show the transfer of an antibiotic resistance integron between two different bacterial species in the lung of a CF patient.

Abstract ID: 1564 / Poster Number: 382.15P

Horizontal transfer of ICEclc-like elements in Pseudomonas aeruginosa clinical isolates

Presenting author: Ms. Valentina Benigno, University of Lausanne, Lausanne, Switzerland

Co-authors: *Hanna Budny, Jan Roelof Van der Meer*

Integrative and conjugative elements (ICEs) are widespread autonomous mobile DNA within bacterial chromosomes. ICEs contain the genes necessary for excision from the chromosome, conjugative transfer to a new recipient cell, and chromosomal reintegration. They can also carry accessory genes that, while not essential for transfer, confer adaptive phenotypes to the host, contributing to bacterial survival under stressful or changing conditions.

Here, we characterized the presence, distribution, and variation of ICEs related to ICE*clc* among *Pseudomonas aeruginosa* clinical isolates within a geographically restrained environment to understand the factors shaping their evolution. We examined 181 *P. aeruginosa* genomes from patient and hospital environment isolates, mostly obtained from a single hospital over 20 years. Nearly 90% of the isolates carried one or more ICE*clc*-like elements with varying degrees of conservation of ICE*clc* lifestyle and transfer genes. ICE clones largely matched host clonal phylogeny but not exclusively, indicating both clonal evolution and horizontal transfer in the hospital environment. Variable gene regions were notably enriched for heavy metal resistance genes, potential efflux systems, and multidrug resistance proteins.

We studied the activation and transfer of a representative subset of ICE*clc*-type elements and found that ICE excision could be induced in *P. aeruginosa* by ectopic expression of BisDC, the known master regulator of ICE*clc* activation, pointing to a similar regulatory cascade. Some elements were successfully transferred to *P. putida*, where they conferred increased tolerance to specific heavy metals. These findings highlight the role of ICEs in shaping the genetic plasticity and adaptation of *P. aeruginosa* in clinical settings.

Abstract ID: 3119 / Poster Number: 027.17F

Host genetics shape microbiome response to antibiotics: The role of B4galnt2 in microbiome recovery

Presenting author: Dr. Aleksa Čepić, Max Planck Institute for Evolutionary Biology, Plön, Germany

Co-authors: *John Baines*

Introduction: The intestinal microbiota plays an important role in host health, contributing to nutrient metabolism, immune system development, and protection against invading pathogens. Antibiotics, while essential for combating infections, disrupt microbial homeostasis, leading to dysbiosis and increased susceptibility to intestinal infections by opportunistic and pathogenic bacteria. Host genetics, including glycosyltransferase gene *B4galnt2*, shape microbiota composition and recovery dynamics, but the underlying mechanisms remain unclear.

Objectives: This study investigates the role of *B4galnt2* expression in modulating microbiota recovery following antibiotic treatment, focusing on its impact on microbial composition, activity, and functional adaptations.

Methods: Using *B4galnt2*$^{+/-}$ and *B4galnt2*$^{-/-}$ mice, we employed a multi-omic approach, including 16S rRNA profiling, metagenomics, and metatranscriptomics, to characterize microbiota dynamics after treatment with streptomycin, kanamycin, or vancomycin. Recovery was assessed via microbial diversity, taxonomic shifts, and functional profiling.

Findings: Results revealed that streptomycin-induced dysbiosis was characterized by faster recovery in *B4galnt2*$^{-/-}$ mice, with early enrichment of beneficial taxa such as *Akkermansia muciniphila*, *Blautia*, and *Roseburia intestinalis*. These mice also exhibited increased expression of motility-related genes and distinct antibiotic resistance profiles. Kanamycin and vancomycin treatments showed similar but less pronounced genotype-specific effects. Functional analyses highlighted metabolic and motility pathways as key contributors to resilience.

Conclusion: Our findings reveal that *B4galnt2* expression plays an important role in shaping microbiota recovery dynamics after antibiotic-induced dysbiosis. This highlights the importance of incorporating host genetics into microbiota-targeted therapeutic strategies and provides valuable insights to support the development of personalized interventions tailored to genetic and microbiome profiles.

Abstract ID: 1916 / Poster Number: 107.15F

Host Lifestyle Shapes Gut Bacterial Growth Dynamics

Presenting author: Mrs. Zaira Zafroon, Institute of Clinical Molecular Biology, Kiel, Germany

Co-authors: *Malte Rühlemann, Ana Paula Schaan, Mathieu Groussin, John Banines, Mathilde Poyet*

Background: Differences in growth rates between bacteria shape microbiome compositions. In humans, gut bacterial compositions vary across worldwide geographic locations, primarily due to lifestyle differences. However, whether host lifestyle is associated with differences in bacterial growth rates is unknown. This study aimed to identify genomic and ecological signatures of gut bacterial adaptation to host lifestyle by analyzing replication rates of gut bacterial species across host populations with varying industrialization levels.

Methods: We applied high-resolution metagenomic sequencing and growth rate estimation algorithms to analyze bacterial replication in the Global Microbiome Conservancy cohort, a cross-sectional cohort of 1,000+ healthy individuals from 12 countries representing diverse lifestyles.

Results: We found that gut bacterial growth rates are correlated with many host factors, including industrialization, Bristol score, age, sex and BMI. Growth rates are also associated with bacterial factors, including phylogeny and relative abundance. Using variance partitioning, we further reveal that host lifestyle and industrialization are the strongest contributors to variations in growth rates for many bacterial species. For instance, key members of the gut microbiome, such as Agathobacter and Ruminococcus species have elevated growth rates in industrialized and non-industrialized populations, respectively. We also found that coating of bacteria by secreted immunoglobulin A is negatively correlated with bacterial growth rate for a set of bacterial species.

Conclusion: Our results provide evidence that variation in growth rate contribute to the ecological adaptation of the gut microbiome to industrialized lifestyles. This underscores the importance of studying bacterial growth rate dynamics in shaping gut bacterial community and host-microbiome interactions.

Abstract ID: 2729 / Poster Number: 141.17P

Host virulence gene expression control exercised by Staphylococcus aureus pathogenicity islands

Presenting author: Dr. María Ángeles Tormo-Mas, Hospital Universitario y Politécnico La Fe. IIS La Fe, Valencia, Spain

Co-authors: *Marina Costa Lacuesta, Mercedes Cervera-Alamar, Patricia Bernabé-Quispe, Samara Sabsabi Soriano, Irene Cruz López, Guillermo García-Laínez,*

The opportunistic pathogen *Staphylococcus aureus* can persist and proliferate in different environments thanks to the production of a wide variety of virulence factors such as toxins, biofilm formation or antibiotic resistance genes. Some of these are encoded in mobile genetic elements (MGE) such as the pathogenicity islands (SaPI) representing a mechanism of virulence gene dissemination thought bacterial population. Bacteriophages, another type of MGE, can influence regulators of the host in addition to virulence dissemination. Taking this into account, we hypothesised that SaPIs could have additional unexplored roles in bacterial physiology affecting host gene regulation as bacteriophages do.

SaPI induction in *S. aureus* RN450 resulted in golden pigment production, the carotenoid staphyloxanthin (STX) known for its virulent antioxidant activity. Through gene deletion, we found out that PtiA and PtiM proteins from SaPIs, which are implicated in bacteriophage interference, are responsible for host STX synthesis through crtOPQMN operon expression. By RNA-seq and retrotranscribed PCR we observed the generation of a co-transcript of genes with the operon, comprising genes related to aminoglycoside antibiotic resistance (GNAT) and cell wall remodelling (isaA, oatA, ssaA) whose regulation is exercised by an antitermination mechanism. Several other genes were regulated by PtiA-M, including different virulence factors such as staphylocoagulase and nitrogen and phosphate metabolism operons for host adaptation, which overall resulted in increased phagocytosis resistance in a macrophage survival assay.

PtiA-M are then the first moonlighting proteins discovered in SaPIs highlighting the impact of these MGE on chromosomal virulence regulation as a new discovered function of SaPIs.

Abstract ID: 723 / Poster Number: 037.16P

Host-derived electron acceptor enables propionate-utilization of Salmonella Typhimurium through interaction with a nitrogen metabolism

Presenting author: Ms. Chaeeun Lee, Pohang University of Science and Technology (POSTECH), Pohang 37673, South Korea

Co-authors: *Wonho Jeon, Serin Lee, Bookyung Park, Woongjae Yoo*

Introduction: The human gastrointestinal tract hosts a complex microbial community known as the gut microbiota. Gut microbiota plays a critical role in digesting complex carbohydrates (fiber), producing a variety of metabolites such as short-chain fatty acids (SCFAs), including propionate. These SCFAs mediate colonization resistance against pathogen infections. However, our previous research demonstrated that Salmonella can overcome propionate-mediated colonization resistance by utilizing propionate as a carbon source to perform anaerobic respiration in the inflamed gut. We identified that nitrate serves not only as an alternative electron acceptor but also as a key factor in activating propionate utilization by upregulating prp gene expression, facilitating Salmonella expansion in the colon. Despite this discovery, the detailed regulatory mechanisms remain unclear.

Objective: This study aims to elucidate novel regulatory mechanisms governing Salmonella gene expression involved in propionate-utilization under dynamic host gut conditions.

Findings: Our study revealed that a Salmonella mutant lacking the ptsN gene (which encodes EIIANtr, a component of the nitrogen-metabolic phosphotransferase system) was unable to grow on propionate. The data demonstrated that reduced glutathione (GSH) production in the ptsN mutant kept Fnr (Fumarate and Nitrate Reductase Regulatory Protein) in its inactive form, resulting in a colonization defect in the gut.

Conclusion: Collectively, our findings suggest that intestinal inflammation-derived nitrate provides a fitness advantage to Salmonella by enhancing propionate catabolism through its interaction with nitrogen metabolism. This mechanism allows Salmonella to outcompete commensal bacteria for gut colonization.

Abstract ID: 3484

Host-pathogen interactions on Adherent-invasive Escherichia coli using human organoid-derived monolayers

Presenting author: Ms. Maria Núria Ramos Corominas, University of Girona, Girona, Spain

Co-authors: *Llorenç Fernández Coll, Isabella Dotti, Aida Mayorgas, Azucena Salas, Margarita Martínez Medina*

Adherent-invasive *Escherichia coli* (AIEC) are capable to adhere and to invade intestinal epithelial cells and to survive and replicate within macrophages; however, the mechanisms associated to its pathogenicity remain not fully understood. We have infected primary differentiated organoid-derived monolayers (d-ODMs) from human intestinal epithelium to better understand AIEC pathogenicity and host response.

We established a protocol to assess the adhesion and invasion capabilities of the AIEC LF82 strain, the probiotic *E. coli* Nissle1917 and the commensal *E. coli* K12 in the d-ODMs. Bacterial genes involved in adhesion, amino acid metabolism, colanic acid biosynthesis and copper homeostasis, as well as eukaryotic genes for bacterial sensing, tight junctions, cytokines, and autophagy were quantified by qPCR at 6, 12 and 24 hours post-infection (p.i.). Additionally, at 6 hours p.i., we performed Dual-RNA sequencing of the cellular and supernatant fractions of the infected cultures.

AIEC LF82 was invasive, whereas *E. coli* Nissle 1917 and *E. coli* K12 were unable to invade intestinal epithelial cells of d-ODMs. At early infection time (6 hours p.i.), both AIEC LF82 and non-AIEC (*E. coli* K12) strains promoted a response in eukaryotic cells, but it was not strain-specific. Interestingly, after longer incubation (24 hours p.i.), LF82 specifically induced the expression of genes associated with barrier function, autophagy and cell receptors. Additionally, bacterial genes related to amino acid metabolism, colanic acid biosynthesis, and fimbriae were upregulated in LF82 at 24 hours. Transcriptomic analysis revealed LF82-specific differentially expressed genes (DEGs) when compared to non-AIEC strains at 6 hours p. i.. Up to 209 genes were upregulated in LF82 in both cellular and supernatant fractions, highlighting their potential as molecular markers for AIEC detection. Furthermore, 342 DEGs were specifically in LF82 when the cellular and supernatant fractions were compared, providing insights into the pathogenic mechanisms of this AIEC strain.

Overall, d-ODM is a viable model to investigate the molecular mechanisms underlying AIEC pathogenicity and its impact on epithelial cell function.

Abstract ID: 1966

Host-Symbiont Interactions in the Mammalian Lung

Presenting author: Dr. Abdulgawaad Saboukh, Institute for Experimental Medicine, Kiel University, Kiel, Germany; Max Planck Institute for Evolutionary Biology, Ploen, Germany

Co-authors: *John Baines*

Introduction: The human body hosts diverse microbial communities colonizing every surface. The commensal microbes that populate the gut, skin, and lungs are now recognized for their role in maintaining organ homeostasis. While the lungs were previously considered sterile in healthy individuals, they are now understood to harbor a microbial community essential for lung development and immunity. Although external factors influencing lung microbiota have been studied extensively, the role of host genetics remains less understood. Previous research identified a strong association between the abundance of the *Lactobacillus* genus and the expression of mitogen-activated protein kinase-activated protein kinase 2 (*Mk2*) and interleukin 10 (*Il10*) in murine lungs. These genes play critical roles in regulating inflammatory responses, and *Lactobacillus* species have been shown to act as probiotics with immunomodulatory effects in lung inflammation. This study utilized an in vitro cell culture system to investigate the relationship between *Lactobacillus* abundance and the expression of *Mk2* and *Il10*.

Objectives: This study aimed to deepen our understanding of how host genetics shape lung microbiota composition. Specifically, we sought to investigate the association between the abundance of *Lactobacillus* and the expression of the human and mouse host genes *Mk2* and *Il10*. Additionally, by simulating an inflammatory environment in cell culture, we aimed to identify potential probiotic *Lactobacillus* taxa and elucidate their interactions with host cells at the transcriptional level.

Methods: An in vitro cell culture system was employed to explore this association. Four selected *Lactobacillus* species (*Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus murinus*) were co-cultured with three different mammalian cell lines (human and mouse lung epithelial cells and mouse macrophages). Lipopolysaccharides were used to mimic an inflammatory environment. Cellular cytotoxicity, viability, and reactive oxygen species (ROS) production were assessed using lactate dehydrogenase, MTS Tetrazolium, and dihydroethidium assays, respectively. Candidate gene expression was measured using RT-qPCR, while RNA-seq

was utilized to identify additional genes and biological processes affected by *Lactobacillus* presence.

Findings: Mammalian cells co-cultured with the selected *Lactobacillus* taxa exhibited cellular viability and cytotoxicity levels comparable to untreated cells. *Lactobacillus reuteri* and *Lactobacillus murinus* notably reduced ROS production when co-cultured with human lung epithelial cells and mouse macrophages. RNA-seq analysis and candidate gene expression profiling revealed that these *Lactobacillus* species mitigate inflammatory responses via the P38-MAPK and JNK1 pathways. *Lactobacillus rhamnosus* and *Lactobacillus murinus* emerged as promising candidates for further research aimed at developing anti-inflammatory interventions.

Conclusion: *Lactobacillus rhamnosus* and *Lactobacillus murinus* modulate host inflammatory responses by regulating the expression of pro- and anti-inflammatory cytokines. This indicates that the immunomodulatory effects of both species may constitute a proxy fitness-related trait that is influenced by host genetic variation. These species were selected for further evaluation of their probiotic potential in an acute lung injury mouse model. Future studies will focus on identifying the specific compounds, such as proteins or metabolites, responsible for these observed probiotic effects.

Abstract ID: 4051 / Poster Number: 201.17P

How Antibiotic Resistance in Salmonella Typhimurium Shapes Probiotic Competition and Therapeutic Efficacy

Presenting author: Ms. Ines Collin, KU Leuven, Leuven, Belgium

Co-authors: *Gitta De Wit, Bram Lories, Hans Steenackers*

As the antibiotic resistance crisis persists, reducing the number of treatment options, the search for effective alternatives has never been more urgent. Probiotics have emerged as a promising solution. However, given the similarities in the modes of action of antibiotics and antimicrobial compounds secreted by probiotics, we hypothesized that established antibiotic resistance mechanisms could alter susceptibility to probiotics, possibly modulating their efficacy.

First, competition assays were conducted between various *S.* Typhimurium lineages, either with experimentally-evolved or plasmid-encoded resistance to different clinical antibiotics, and known probiotic strains. The goal was to identify combinations that alter the pathogen's competitive abilities. Collateral sensitivity events abundantly occurred within our dataset, whereas significant indications for cross-resistance were not found. Although monospecies growth curve assays indicated that some antibiotic-resistant strains exhibited reduced growth, this did not appear to be a prerequisite for reduced fitness in competition with probiotics. Whole genome sequencing already pinpointed the mutated genes that may be responsible for the resistance phenotype. Next, a probiotic supernatant assay will determine whether the developed resistance mechanisms directly increase susceptibility to probiotic-secreted compounds or whether subtle growth effects, not detected via growth rate experiments, underlie the reduced fitness in co-culture.

Hence, this approach provides further clarity on the exact genetic basis underlying the social interactions. The prevalence of collateral sensitivity over cross-resistance reinforces probiotics as viable alternatives in the antimicrobial therapeutic landscape.

Abstract ID: 653 / Poster Number: 309.16F

How do bacteria performing dissimilatory nitrate reduction to ammonium respond to organic carbon in anammox reactors?

Presenting author: Prof. Hee-Deung Park, Korea University, Seoul, South Korea

Co-authors: *Hoang Phuc Trinh, Sang-Hoon Lee*

Bacteria fulfilling dissimilatory nitrate reduction to ammonium (DNRA) are commonly observed in the anaerobic ammonium oxidation (anammox) reactors and reported to positively influence nitrogen removal efficiency. However, how DNRA bacteria interact with other nitrogen-metabolizing bacteria (e.g., anammox, denitrifying, ammonia-oxidizing, and nitrite-oxidizing bacteria) by operational conditions remains to be elucidated. This study utilized two upflow anaerobic sludge blanket reactors to investigate those bacterial interactions in association with DNRA activity as well as nitrogen removal performance by different C/N ratios for 220 days. Reactor performance coupled with ^{15}N isotope pairing technique demonstrated DNRA activity even when organic carbon was not supplied. DNRA activity increased further by supplying organic carbon, but the reactors exhibited abrupt decrease when C/N ratio increased from 0.3 to 1.0. DNRA activity was partly recovered by decreasing C/N ratio to 0.5 but a complete recovery was not achieved. Bacterial community compositions fluctuated during the reactor operation. Figure 1(a) shows that DNRA bacteria, including anammox bacteria possessing DNRA activity (*Anaerolineaceae*, *Fimbriimonadales*, *Ca*. *Jettenia*), were most abundant when the C/N ratio was 0.2. However, heterotrophic denitrifying bacteria dominated over other bacteria at C/N ratios of 1.0 and 0.5. Network analysis for different functional bacteria revealed that an anammox bacterium (*Ca*. *Jettenia*) and a DNRA bacterium (*Fimbriimonadales*) formed a module and were positively correlated between them, and these bacteria were negatively correlated with some heterotrophic denitrifying bacteria (*Massilia*, *Terrimonas*, *Ferruginibacter*, *Mycobacterium*, and *Pedosphaeraceae*). A PCA analysis further revealed that the C/N ratio was a significant factor influencing the relative abundance of these three functional bacteria in the anammox reactors. Consequently, it can be concluded that achieving optimal C/N ratios is pivotal in fostering a symbiotic relationship between anammox and DNRA bacteria, thereby facilitating enhanced nitrogen removal in the anammox process.

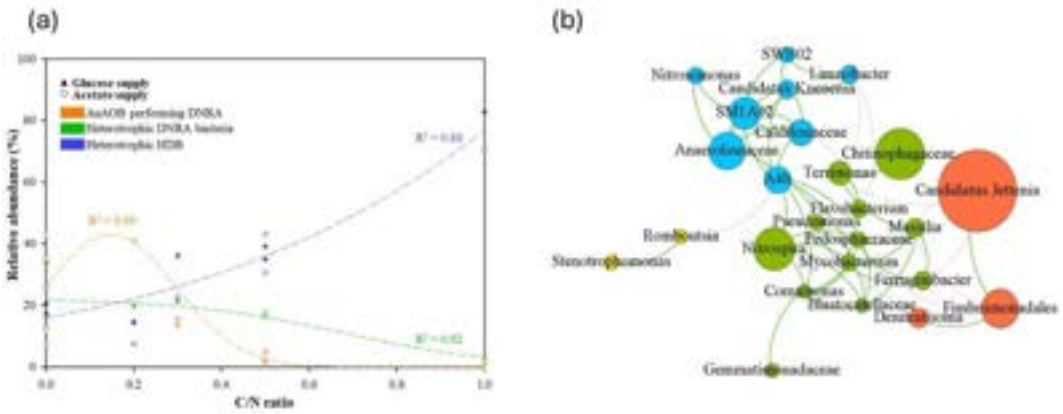


Figure 1: Functional bacteria observed in an anammox reactor (a) Relative abundance of three functional groups (anammox bacteria performing DNRA, heterotrophic bacteria performing DNRA, heterotrophic denitrifying bacteria (HDB)) as a function of C/N ratio. (b) Co-occurrence network between nitrogen-metabolizing bacteria genera belonging to five groups (anammox bacteria, ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, HDB, and DNRA bacteria). The orange, green, blue, and yellow colour of the dots indicate the modularity 1, 2, 3, and 4, respectively. The size of the dots is proportional to their relative abundance. Edges represent connections with $|\rho| > 0.6$ and the p-value < 0.05. The green and pink colour of the connections represent positive and negative Spearman's correlation coefficients, respectively.

Abstract ID: 3646

How do biotic interactions influence cyanobacteria populations dynamics? A SynCom approach

Presenting author: Beatriz Baselga Cevera, PhD, University of Minnesota, St. Paul, United States

Co-authors: *Paloma Martínez-Alesón, Nahui Olin Medina-Chávez, Michael Travisano*

Introduction: Cyanobacteria blooms present remarkable physiological, biochemical, and genetic heterogeneity. There has been much research aimed at understanding cyanobacteria bloom capabilities and impacts on the environment. As dynamic microbial communities, cyanobacteria blooms are determined by biotic interactions, affecting the toxicity, strain composition, and dynamics of cyanobacteria blooms. However, temporal and functional interactions between cyanobacteria and co-occurring bacteria are largely underexplored.

Objective: To decipher microbial dynamics within Cyanobacteria bloom by studying simplified communities in laboratory settings.

Methods: To address this knowledge gap, we carried out a synthetic microbial communities (SynCom) experiment in laboratory conditions. Microcystin-LR-producing and non-producing unicellular cyanobacteria xenic environmental isolates were investigated independently and in combination. Through the propagation of replicate communities, we studied the population's dynamics of the cultivable host bacteria, cyanobacteria species growth, and microcystin-LR production. Cyanobacteria and host bacteria were identified via sequencing, and the communities' composition was determined through metagenomics.

Findings: Results suggest a succession of cyanobacteria strains on the combined SynCom toxigenic, and non-toxic experimental group. Cyanobacteria strains were identified as an MC-LR-producing *Microcystis* sp. strain and a *Synechococcus* sp. strain. Host bacteria dynamics fluctuated throughout the experiment, but the community composition was stable within experimental groups. Host bacteria identified are aerobic, predominantly nitrogen-fixing, and anoxygenic phototrophic bacteria. Toxicity assays suggest that some host bacteria are affected by microcystin-LR.

Conclusion: These results enable us to assess community-intrinsic properties in correlation with cyanobacteria growth and toxicity and evaluate temporal and functional SynCom dynamics.

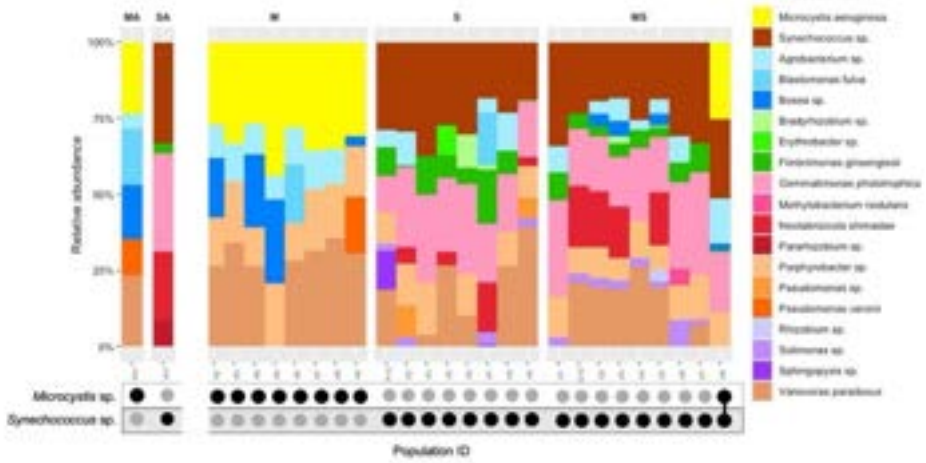


Figure 1: Metagenome-based relative abundance species plot. Representation of the initial xenic communities at the beginning of the experiment (Microcystis sp. ancestral xenic strain (MA) and Synechococcus sp. ancestral xenic strain (SA)), and after 4 transfer cycles per experimental treatment (Microcystis sp. (M), Synechococcus sp. (S) and both xenic trains in combination (MS)).

Abstract ID: 4083 / Poster Number: 005.17S

How do climate extremes impact soil microbiomes across seasons?

Presenting author: Dr. Zachary Bailey, University of Bern, Bern, Switzerland

Co-authors: *Itzel Lopez Montoya, Madhav Thakur*

Extreme climate events are increasing in frequency and intensity, posing significant threats to terrestrial ecosystems. Soil microbial communities, which play a pivotal role in regulating soil biogeochemical processes, are particularly vulnerable to these changes. This study investigates how bacterial and fungal communities respond to climate extremes, focusing on shifts in taxonomic, functional and genetic diversity. Using a grassland mesocosm experiment, we subjected the mesocosms to simulated climate extremes, including heatwaves and severe drought. Soil samples were collected and analyzed using 16S rRNA gene sequencing, ITS sequencing, and shotgun metagenomics at three time points across the growing season for two years to track changes in microbial community composition and functional potential. Our results reveal that, microbial communities generally follow a seasonal pattern, and these communities exhibit convergent responses of taxonomic diversity to drought and heat. In the first year, Shannon diversity initially increased immediately following drought, likely due to both the influx of nutrients from dead plants and seasonality (Figure 1). In post-drought communities, we see an increase heat and drought resistance genes such as sporulation. Therefore, the legacy of drought and heat was evident not in bacterial diversity alone, but instead in functional genes, highlighting shifts in microbial functional diversity under extreme climatic conditions. This study underscores the profound impact of climate change on soil microbial communities, emphasizing the study of functional adaptations across seasons, which could alter microbiome-associated functions in terrestrial ecosystems.

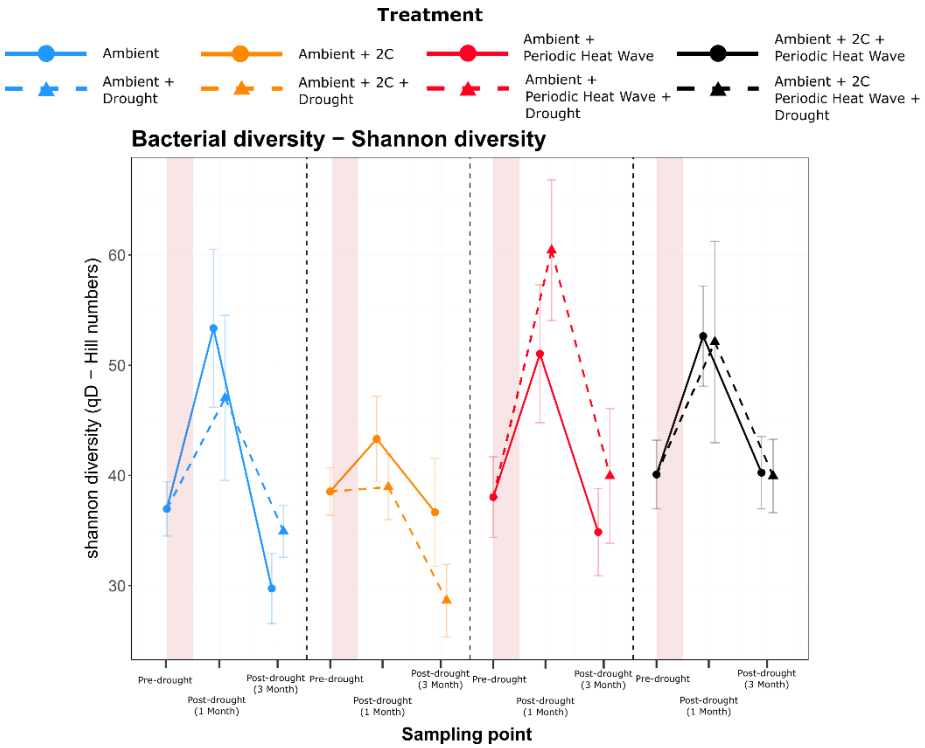


Figure 1: Seasonal changes in Shannon diversity indices for four bacterial types across four distinct soil mesocosm treatments throughout 2023. Pre-drought corresponding to spring, post-drought by 1 month being in summer and the post-drought by 3 months being collected in the fall. The treatments included conditions with and without a drought period, indicated by the red band. The data illustrate the impact of drought and heat on bacterial diversity over time.

Abstract ID: 2382 / Poster Number: 056.15P

How does heavy metal pollution affect microbial diversity in the Arctics?

Presenting author: Dr. Suni Mathew, University of Turku, Turku, Finland

Co-authors: *Marjo Helander*

Introduction: Heavy metal pollution has accelerated due to anthropogenic activities such as mining, agrochemicals and coal combustion. Despite the risks to biodiversity, the effects of long-term heavy metal pollution on microbial diversity in these regions remain poorly understood. Microbes in these Arctic and subarctic regions are endemic, and the extreme seasonal variations of microbial activities are globally unique compared to other climatic zones. Low winter temperatures inhibit the transformation and mobility of heavy metals, causing contaminants to persist longer in cold environments and amplifying risks to biodiversity.

Objective: The present study looks into the long-term effects of heavy metal pollution on microbial community composition associated with Arctic plants and identify metal-tolerant bacteria for enhancing plant tolerance to metals.

Method: Bacterial culturing techniques and Sanger sequencing to identify bacterial diversity and taxa and metagenomics to study the changes in bacterial community composition in pine and lingonberry. Samples were collected from 30-year experimental plot simulating metal emissions near smelters, at Kevo Research Station in northern Finland.

Findings: Bacterial diversity and abundance were reduced in samples from metal polluted plots compared to the controls. Metagenomics also showed significant changes in bacterial community composition of treated plots compared to controls. Two of the most dominant taxa were Frigoribacterium and Frondihabitans, which are potentially metal-tolerant bacteria.

Conclusion: The findings highlight the potential of Frigoribacterium and Frondihabitans in phytoremediation. With growing demand for green transition that requires increased mining activities, it is crucial to protect biodiversity and prioritize restoration of polluted ecosystems.

Abstract ID: 3347

How microbiology can support agriculture against climate change: plant growth promoting microorganisms and regenerative agriculture practices in the pursuit of soil restoration

Presenting author: Maria Elena Antinori, PhD, Catholic University of the Sacred Heart, Piacenza, Italy; Innovations for Sustainable Agricolture R&D srl, Piacenza, Italy

Co-authors: *Federico Ardenti, Gabriele Bellotti, Andrea Fiorini, Francesc Domingo, Marc Vinas Canals, Luigi Lucini, Edoardo Puglisi*

Agriculture should be a key, target sector in the effort to reduce GHG emissions and restore ecosystem health and diversity, two challenges European Union and supranational institutions as FAO are considering urgent. SIRAM (Sustainable Innovations in Regenerative Agriculture in the Mediterranean Area), a PRIMA project which has involved ten partners from eight countries in the Mediterranean area, has tried to address these issues by studying the effectiveness of different practices belonging to four pillars: the application of microorganisms for plant nutrition and defense; the reuse of waste biomasses; the application of regenerative agriculture practices; the use of resistant varieties. Going together with the investigation of the molecular and microbiological mechanisms linked to these practices, the project aimed at promoting sustainable practices in agriculture, tailored to different environmental scenarios and scientifically coherent.

In particular we aimed to demonstrate that i) regenerative agriculture practices restore soil health and biodiversity and some measurements are especially useful to determine soil health and quality ii) the application of selected microorganisms together with regenerative agriculture practices increase yield and quality of production, in comparison with conventional agriculture.

To pursue these objectives, i) we measured different agronomical (SOC, POM, P and K) and microbiological parameters (main biogeochemical cycles through qPCR and enzyme extraction, alfa and beta diversity through amplicon sequencing) in two case studies in two different countries (Italy and Spain), managed according to different regenerative agriculture practices (no-tillage, cover crops); ii) we isolated more than 100 bacteria and fungi from maize rhizosphere and screened them for Plant Growth Promoting (PGP) activities *in vitro (*IAA and siderophores production, ACC deaminase activity, phosphate

solubilization, nitrogen fixation, drought resistance, impact on seed germination) and in pot. Moreover, the combined action of PGP microorganisms and vermicompost fertilization was measured in pot in comparison with standard chemical fertilization. We applied the best microorganism selected in previous steps in corn production in field, together with vermicompost and in conventional versus no till farming.

We observed that regenerative agriculture practices affect soil health and biodiversity, especially influencing nutrient cycling; common measurements, even among different environments and treatments, can establish this conclusion. From PGP in vitro selection, bacteria of genera *Pseudomonas*, *Nesterenkonia*, *Burk*ho*lderia* and *Bacill*us and fungi of genus *Trichoderma* were found to be the best and combined in three distinct consortia, tested in the pot experiment. *Trichoderma asperelloides* PV51 was finally applied in field together with vermicompost and found able to increase yield of corn production, especially when combined to no till farming, in comparison to conventional agriculture and no vermicompost application. *Trichoderma asperelloides* PV51 genome was analyzed and possible molecular mechanisms involved in plant growth considered. These mechanisms could be further studied thanks to metabolomics on corn roots.

Regenerative agriculture practices and the application of microrganisms able to enhance plant growth and nutrition represent a great opportunity for supporting agriculture in the challenge against climate change. Science and microbiology should uphold these practices by determining ways to measure their impact on soil and environment health and quality and by validating them through the understanding of the mechanisms they act through. Experimenting different combinations of these practices in different environmental scenarios as attempted by SIRAM project can represent the opportunity to collect data and observations in this sense. The recognition of the best practices in microorganisms selection and application and in the measurement of effects on crop production and environment is also important, as attempted in this work.

Abstract ID: 3701 / Poster Number: 191.17P

How Reliable Are the Antibiotic Susceptibility Results from Automated Systems for Pseudomonas aeruginosa?

Presenting author: Prof. Arzu İLKİ, Marmara University School Of Medicine, Department of Microbiology, İstanbul, Turkey; Marmara University Pendik research and Training Hospital, İstanbul, Turkey

Co-authors: *Dilara TEZER, Özlem Gizem NİĞDELİOĞLU SEZER, Öykü TÜRKOĞLU, Sevim ÖZSOY*

Background: *P. aeruginosa* is an important bacterium causing life threatening infections and exhibiting extensive antibiotic resistance.

Objective: We assessed the performance of the VITEK®2/AST N420 (bioMerieux,France) cards, valued for its rapid results and less manual workload, with the standard disc diffusion method in determining the antibiotic susceptibility testing of *P. aeruginosa* isolates.

Methods: The study included 134 *P. aeruginosa* blood culture isolates. Piperacillin-tazobactam (TZP), Ceftazidime (CAZ), Amikacin (AK), Cefepime (FEP), Imipenem (IMI), Meropenem (MEM) and Ciprofloxacin (CIP) susceptibilities were determined by disc diffusion method and the VITEK®2/AST-N420 card. The results were evaluated according to EUCAST (V15.0) breakpoints. Isolates were evaluated for categorical agreement (CA), very major error (VME) and major error (ME) rates.

Results: TZP, CAZ, AK, FEP, IMI, MEM, CIP resistance rates through standard disc diffusion testing were 43.4%, 44.9%, 13.6%, 31.98%, 37.3%, 30.4%, 30.9% respectively. The highest CA was detected for AK with 85.6%, VME rate was 3.2% and ME rate was 10.4%. For MEM, CA, VME and ME were found to be 73.8%, 7.1%, and 11.9% and For IMI, these rates were 84.12%, 7.9% and 7.9% respectively. For CIP, these values were 80.43%, 7.3%, 12.2% and for FEP, 78.81%, 10%, and 11.7% respectively. CA for CAZ was 75.19% and TZP was 72.64%. VME and ME rates were 14.7%, 10% and 17.3%, 12.7% respectively.

While the system offers rapid and practical results, its high error rates, particularly for some antibiotics, highlight the need for continuous performance evaluation and validation against standard methods.

Abstract ID: 2997 / Poster Number: 203.16P

HPV detection and genotyping as an earlier aproach in cervical cancer screening of the female genital tract in Kochani, North Macedonia

Presenting author: Dr. Monika Karova, Center of Pubic Helath, Kochani, Macedonia

Co-authors: *Natalia Miloradovska, Elena Tortevska Danilov, Radomir Jovchevski*

Introduction: Human papilloma virus (HPV) is a small, non-enveloped deoxyribonucleic acid (DNA) virus that infects skin or mucosal cells. The circular double stranded viral genome is approximately 8-kb in length.

Objective: To define the most common genotypes of HPV in our region.

Materials and Methods: We have retrospectively evaluated the results of HPV detection and typing in the cervical samples of 321 women, ages between 21 and 65 years, attending gynecological clinics in our city from January 2023 to December 2024. The examinations materials were cervical swabs taken by a gynecologist and sent to our laboratory in a medium. The materials were processed using PCR methodology on the CFX96 Touch Real Time PCR Detection System (Bio-Rad, USA) which detects 28 genotypes of HPV.

Results: Out of 321 samples, 211(65,7%) were negative and 110 were positive (34,3%). Regarding the prevalence of high-risk types, the most common were: 16 (20/18%), 53 (16/14%), 31 and 51 (13/11%), 45 and 66 (10/9%). Most prevalent low-risk types were: 42 (19/17%), 54 (13/11%), 61 (9/8%) and 6 (8/7%). The youngest patient with a positive result from our examinations was born in 2002, with a result showing two genotypes: 53 and 54.

Conclusion: For the general population of women, HPV-DNA detection is recommended as the primary screening method - starting at the age of 30 years, with regular testing every 5-10 years. HPV vaccine has the potential to prevent more than 90 % of cancers caused by HPV.

Abstract ID: 1769 / Poster Number: 251.16P

Human commensal Adlercreutzia equolifaciens localize in intestinal mucosa and attenuates colitis

Presenting author: Jiayue Yang, PhD, Keio University, Tsuruoka, Japan

Co-authors: *Nozomu Obana, Gaku Nakato, Nobuhiko Nomura, Shinji Fukuda*

Various commensal bacteria inhabit the human intestine[1]. In particular, intestinal mucosal bacteria are reported to affect host health through modulation of host immune system and relate to human disease such as inflammatory bowel disease (IBD) [1-3]. Therefore, it is important to further investigate their function. However, knowledge of mucosa-associated bacteria is limited due to the invasiveness of conventional sampling methods. In this study, we have developed a non-invasive sampling method for mucosa-associated bacteria from stool samples. Through a murine experiment, we found that swab samples of fecal surfaces display a similar microbiome profile as mucosa-associated bacteria. With this method, we found that *Adlercreutzia*, which has never been reported as mucosa-associated bacteria before, inhabits the colon mucosal layer. To examine the function of this bacteria in the host, we orally gavaged the type strain of *Adlercreutzia equolifaciens* to germ-free mice. Dextran sulfate sodium (DSS)-induced colitis is a murine experimental model of IBD. The colonization of *A. equolifaciens* greatly improved the survival rate and attenuated the DSS-induced colitis in gnotobiotic mice.

To elucidate the mechanism, we performed metabolome analysis of the cecum content of the gnotobiotic mice. We found that taurine was significantly accumulated in *A. equolifaciens*–colonized gnotobiotic mice. Taurine supplementation significantly attenuated DSS-induced colitis in germ-free mice. We also investigated the mechanism of taurine accumulation. Deconjugation of taurine from conjugated bile acids is the major pathway of taurine production by gut microbes. Indeed, metabolome analysis revealed that taurocholate was reduced in the cecum content of *A. equolifaciens*–colonized gnotobiotic mice. We also showed that A. equolifaciens deconjugation enzyme bile salt hydrolase (BSH) has very high deconjugation ability in vitro. These results suggest that the high deconjugation ability of *A. equolifaciens* leads to the accumulation of taurine and the attenuation of colitis (Fig. 1). Our finding might contribute to the development of the gut microbiome–based therapeutics in IBD.

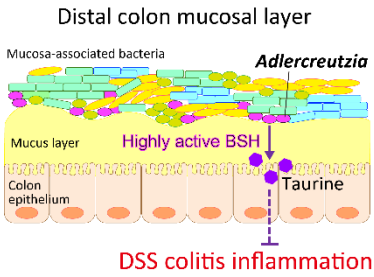


Figure 1: Summary of this study

Abstract ID: 581 / Poster Number: 033.16P

Human milk oligosaccharides differentially modulate infant gut microbiota: a synthetic community approach

Presenting author: Mr. Yangwei Shan, University of Amsterdam, Amsterdam, Netherlands

Co-authors: *Myrthe Beijnvoort, Stanley Brul, Jianbo Zhang*

Human milk oligosaccharides (HMOs) play an important role in the development of the gut microbiota in early life. The use of HMOs by individual infant gut species has been extensively studied, but how HMOs influence infant gut microflora at the community level and the underlying mechanisms are less known. Here, we constructed an infant bacterial community (SynCom1) consisting of 8 representative infant gut species belonging to Bacillota, Bacteroidota, and Actinomycetota. SynCom1 was cultivated in media supplied with individual HMOs. As expected, we found that the total cell density of the community significantly increased and each individual HMO was completely consumed after 24h. Nevertheless, we observed divergent effects of HMOs in the bacterial composition and diversity, with sialylactoses dictate the dominance of Bacteroidota in the community. In contrast to sialyllactoses, neutral tetraoses maintained relatively higher diversity. The growth patterns of individual species in individual HMOs demonstrated that Bacteroides were the only one growing in sialyllactoses, supporting the dominance of Bacteroidota. Correspondingly, HMOs showed structure-dependent effects on the production of short chain fatty acids and organic acids such as acetate. Interestingly, butyrate was detected when SynCom1 grew in both sialyllactoses and tetraoses, despite the fact that the butyrate producer does not grow in all HMOs in monoculture, suggesting a potential cross-feeding. In summary, HMOs showed distinct effects on the infant gut community composition and metabolic function. The synthetic community could serve as a simplified model infant gut microbiota to test nutrients in the context of precision nutrition.

Abstract ID: 3033 / Poster Number: 164.17P

Human placental extract demonstrates in vitro antibiofilm activity against Pseudomonas aeruginosa and Escherichia coli

Presenting author: Bayomi Biju, PhD, Indian Institute of Technology (IIT)Kharagpur, Kharagpur, India

Co-authors: *DIAMOND JAIN, PIYALI DATTA CHAKRABORTY, TRIDIB NEOGI, ANINDYA SUNDAR GHOSH*

Background: Biofilm-associated infections pose a critical global health challenge, especially with rising antimicrobial resistance. *Pseudomonas aeruginosa* and *Escherichia coli* are notorious for their persistent and recalcitrant infections in healthcare settings. Biofilms create a protective environment, dramatically reducing the efficacy of conventional antibiotics and host immunity. The stubbornness of biofilms to traditional treatments necessitates the exploration of novel antibiofilm agents.

Objective: Assessing antibiofilm properties of human placental extract (Placentrex) against P. aeruginosa and E. coli.

Methodology: The minimum inhibitory concentration (MIC) of Placentrex was determined via broth micro-dilution. Antibiofilm activity at inhibitory and sub-inhibitory concentrations was assessed using crystal violet staining and bright field microscopy. Effects on pre-formed biofilms were analyzed using TTC assay and BacLight Live/Dead staining. Placentrex's interactions with antibiotics were tested via checkerboard assays, selecting synergistic and additive inhibition concentrations for further antibiofilm studies.

Results: Placentrex at 50 mg/mL reduced biofilm formation by ~87% for *P. aeruginosa* and ~61% for *E. coli*. It decreased pre-formed biofilms by ~93% and ~90%, respectively, after 24 hours. Biofilm viability was reduced by ~93% for *P. aeruginosa* and ~92% for *E. coli*, confirmed via fluorescence microscopy. Checkerboard assays revealed synergistic effects between Placentrex and azithromycin, ciprofloxacin, doxycycline, and levofloxacin against *P. aeruginosa*, with additive effects observed for amikacin and gentamycin. Against *E. coli*, additive effects were noted with ciprofloxacin and doxycycline. These combinations enhanced antibiofilm efficacy compared to Placentrex alone.

Conclusion: This study highlights the potential of Placentrex as an effective antibiofilm agent, for developing innovative strategies to combat biofilm-associated infections

Abstract ID: 2674

Human spleen macrophages clear the pneumococcus by apoptosis

Presenting author: Dr. Francesco Flandi, University of Bologna, Bologna, Italy

Co-authors: *Neama Alnabati, Giulia Cattabriga, Talia Richardson, Daniele Ghezzi, Tareq Alsaoudi, Luisa Martinez-Pomares, Ashley Dennison, Marco Rinaldo Oggioni*

Introduction: The bacterium *Streptococcus pneumoniae* is one of the major causes of serious diseases and mortality, causing pneumonia, meningitis, and sepsis. Despite extensive research, the mechanisms by which the human immune system clears *S. pneumoniae* remain only partially understood. In particular, while *S. pneumoniae* is known to be targeted by phagocyte-mediated clearance in the spleen, the role of splenic macrophages has not been fully elucidated.

Objectives: This study investigates the functional roles of splenic macrophages during *S. pneumoniae* infections. By exploring the dynamics of macrophage activation, the research aims to elucidate key immune pathways involved in the clearance of encapsulated bacteria.

Method: To investigate how splenic macrophages respond to *S. pneumoniae*, we propose an innovative *ex vivo* human spleen perfusion model. This model allowed us to examine the functional role of the human spleen during pneumococcal infections. Tissue biopsies were analyzed using immunohistochemistry to detect specific markers associated with macrophage activation, including activated caspase-3 (cas3), its target M30 and the lysosome-associated membrane protein 1 (lamp1). These markers provide critical insights into the pathways involved in macrophage-mediated bacterial killing and tissue response.

Findings: Our findings demonstrate that the human spleen plays a crucial role in capturing and removing *S. pneumoniae*. Within the first hour of perfusion, 90% of the bacteria was cleared from the system, highlighting the spleen efficiency in capsulated bacteria removal. Immunohistochemistry analysis revealed a ten-fold increase in cleaved cas3 levels within macrophages at one hour post-infection, suggesting that macrophage apoptosis may play a role in the bacterial clearance. Over time, both the number of bacteria and the levels of activated cas3 decreased, indicating a dynamic correlation between bacterial killing and macrophage cell-death. Moreover, this process was followed by the increase in cleaved M30 levels within macrophages at late time

points of perfusion. This reflects prolonged cas3 activation, suggesting sustained apoptotic activity in macrophages during the immune response to pneumococci. Despite these changes, the percentage of lamp1-positive macrophages did not increases significantly, suggesting that lysosomal activity might not be the only mechanism for pneumococcal killing. Our results indicate that macrophage cas3 mediated cell-death may complement other immune responses to effectively control *S. pneumoniae* infections.

Finally, we observed that CD169+ macrophages harbor 10 times more cas3 than CD163+, suggesting that these cells may serve as a critical reservoir for the initial stages of invasive pneumococcal infections. In addition, out of 10^{10} macrophages, 10^{8} are positive for activated cas3. Considering an infection dose of 10^{8} bacteria and assuming that each macrophage takes one bacterium, each cell that internalizes a bacterium undergoes apoptosis.

Conclusion: This study demonstrated the crucial role of the *ex vivo* human spleen perfusion model in improving our understanding of the human immune responses. It provides new evidence that splenic macrophages play a critical role in the clearance of pneumococci, involving caspase-3 mediated cell-death. These findings could contribute to the development of more effective treatments to prevent the transition of *S. pneumoniae* from localized infections to systemic disease, and enhance our understanding of the spleen-mediated immunity.

Abstract ID: 2867 / Poster Number: 098.15S

Hydrocarbon levels and input frequency shape microbial hydrocarbon-degrader assemblages in Helgoland seawater microcosms

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Co-authors: *Constantin App, Daniel Straub, Rafael Tarozo, Christian Hallmann, Sajjad Abdighahroudi, Esteban Góngora, Katharine J Thompson, Sara Kleindienst*

The dynamic activities of oil exploration, production, and transportation significantly increase the vulnerability of marine environments to oil pollution. Anthropogenic oil inputs to marine environments range from large-scale spill events to smaller, chronic inputs such as land-based runoff or ship discharges. While extensive research has focused on hydrocarbon biodegradation in response to large-scale pollution events, microbial community dynamics under smaller-scale pollution scenarios remain poorly understood.

To address this knowledge gap, we established a series of Helgoland seawater microcosms amended with different hydrocarbon loads (low, medium and high) in the form of water-accommodated fractions (WAFs) over a 20 days incubation period (Fig. 1). Low-WAF was introduced through pulsed feeding, whereas medium- and high-WAF loads were applied as a single dose. We employed a combination of chemical and meta-omics approaches, including genome-resolved metagenomics and metatranscriptomics, to: i) quantify hydrocarbon biodegradation capabilities under low-, medium- and high- WAF loads, (ii) identify key hydrocarbon-degrading microorganisms, and iii) assess the expression profiles of hydrocarbon-degradation genes under different WAF loads.

Our results showed that 35% and 11% of total petroleum hydrocarbons were degraded in the medium- and high-WAF setups, respectively. Under pulsed feeding with a low-WAF load, an average of up to 9% of hydrocarbons was degraded following each feeding event (i.e., every 2 days). Gas chromatography-mass spectrometry (GC-MS) analysis confirmed rapid degradation of alkanes within 2 days of incubation, while polycyclic aromatic hydrocarbons (PAHs) exhibited slower degradation rates. Additionally, up to 10 medium- to high-quality metagenome-assembled genomes (MAGs) were identified, which encoded key genes involved in hydrocarbon degradation. Mapping of transcripts to these MAGs suggested that *Cycloclasticus* sp. exhibited the highest expression of the ring-hydroxylating beta subunit, a key gene involved in initiating PAH degradation, across all WAF setups. *Oleispira antarctica* was identified as the key alkane degrader in the low-

and high-WAF setups, encoding the alkane monooxygenase gene. In contrast, putatively "novel species" within the order *Gammaproteobacteria*, which encodes cytochrome P450, was identified as the key alkane degrader in the medium-WAF load.

The findings suggest that *Cycloclasticus* sp. and *Oleispira antarctica* are key players in hydrocarbon degradation under low- and high-WAF loads in Helgoland seawater. Additionally, our results imply that pulsed feeding helps sustaining high activity levels of hydrocarbon degradation genes, thereby facilitating the adaptation of hydrocarbon degraders to larger pollution events. Furthermore, the identification of a novel *Gammaproteobacteria* species as a key alkane degrader under medium-WAF loads highlights the diversity of alkane degraders, with the key players likely being influenced by the pollution level and frequency of oil input.

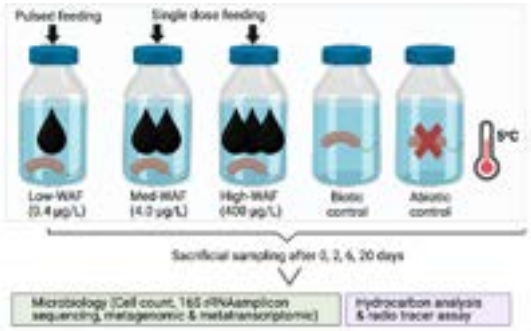


Figure 1: Experimental setup of Helgoland seawater microcosms simulating low-, medium- and high-oil contamination scenarios indicated as low-WAF, med-WAF and high-WAF setups, respectively. Biotic and abiotic controls were also included.

Abstract ID: 3526 / Poster Number: 330.16F

Hydrophobic Bacterial Carriers: An Innovative Strategy for Enhanced Soil Bioremediation Efficiency

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The expansion of crude oil manufacturing, processing and transportation contributes to oil pollution, posing socio-economic and environmental risks. Light and bioavailable fraction of petroleum oil, composed of short chain hydrocarbons, is often remediated by the native microbial consortia, however, hydrophobic recalcitrant fraction has a strong tendency to bind to organic matter and soil particles. Low bioavailability of long chain and aromatic hydrocarbons, therefore, results in their persistence in the environment.

To promote the degradation of hydrocarbons in soil, a novel concept was established by (i) minimizing the distance of pollutant to bacteria cell, (ii) concentrating the pollutant at the cell nearby distance, (iii) promoting cross-metabolic processes between pollutant degrading and auxiliary bacteria and (v) providing a protective matrix against grazing and predation.

Novel hydrophobic biodegradable microbial carriers were developed with embedded bacterial biomass in the hydrophobically modified alginate gel, coating a solid support organic (hay, cellulose, biochar and sawdust) material providing a high surface area.

Prepared carriers were incubated in the polluted soil and after 40 days of incubation, 92 ±19 % reduction of hydrocarbons in the soil was observed using hydrophobic microbial carriers, compared to the 19 ± 2% in the control treatment relying on natural attenuation and 50 ±19 % reduction in sterile hydrophobic carriers. Recalcitrant hydrocarbons, i.e. tetracosane (C40), were reduced up to 60 %.

The proposed solution offers a cost-effective, biodegradable and effective novel concept of bacterial bioremediation, creating a hydrophobic surface facilitating the adsorption of the hydrocarbons and simultaneously providing the necessary metabolic pathways for their degradation.

Abstract ID: 3514

I want to revise microbiology: Let's play MicrobEscape Room!

Presenting author: Dr. Isabel Murillo, University of Bristol, Bristol, United Kingdom

Co-authors: *Laura Wright, Lydia Mason, Emma Stevenson*

Introduction: The use and play of games are an invaluable educational tool. It is widely used in primary and secondary education and has proven to be a pedagogical success. There is an increasing interest in using playful learning in higher education and to achieve this goal, many UK universities are embedding games as part of their curricula. Elements of games can be added to any discipline. Still, for this to be successful and sustainable, it has to provide students with additional knowledge on their studies and opportunities to create memorable moments with their peers. We design and use games as a revision tool in the discipline of microbiology.

Objective: The creation and use of an escape room game as a microbiology revision tool co-created with students and played by students. This game is called MicrobEscape Room.

Method: Tailored games to enhance students' participation and engagement with the discipline of microbiology have been used to teach first-year students. This game has been co-created with students from our programme who have already taken the target unit. The material is provided by the academic, and some guidance is given to tailor the activity to the intended learning outcomes. The game is played in small groups (5-6 students) within a large group (around 100 students). We use a game based on the escape rooms principle where students have to solve different problems to progress to the next stage. The session is run for 1 hour 30 minutes and a feedback form is provided to students to give feedback.

Findings: By making students participating in the creation of the games, we ensure that the game's content is attractive to students and pitched to the right level of knowledge. Co-creation has a dual benefit: the learning occurs during the creation and the playing.

Sessions with elements of games create a relaxed environment where students can interact with peers and play without the fear of failing (Figure 1). Games are designed to be inclusive and to consider non-native English speaker students, who might feel the language is a barrier. The social element of the games is especially important for 1st-year students as this type of session creates the appropriate environment to build new

relationships with other students. The success is achieved by providing flexible rules that can be adapted to the diversity of the individuals in the groups and by giving them a common challenge.

Conclusion: The use of tailored games helps students revise the unit and improves student participation in classroom tasks and their engagement with microbiology.



Figure 1: First year students playing MicrobEscape Room in the classroom.

Abstract ID: 3260 / Poster Number: 425.17P

Identification and Characterisation of Biological Nitrification Inhibition in Barley for Sustainable Nitrogen Management

Presenting author: Mr. Jack Henderson, University of Aberdeen, Aberdeen, United Kingdom

Co-authors: *Cecile Gubry-Rangin, Marcel Jaspars, Tim George*

Excessive nitrification in agricultural systems leads to nitrogen (N) loss through nitrous oxide emissions – a potent greenhouse gas – and nitrate leaching, which contributes to eutrophication in water bodies. This process undermines nitrogen use efficiency (NUE) in modern agriculture, resulting in significant economic and environmental costs.

Biological Nitrification Inhibition (BNI) is a plant-mediated mechanism that inhibits nitrifying microorganisms through the exudation of bioactive metabolites. BNI offers a promising strategy for enhancing sustainability in agriculture by reducing N loss and nitrous oxide emissions. While BNI has been observed in major crops such as rice, wheat, and maize, it has yet to be documented in barley. Barley, the fourth most widely cultivated cereal crop globally, holds particular economic significance in Scotland through its role in the Scotch whisky industry. Identifying high-BNI barley cultivars is critical for developing varieties with improved NUE.

This study aimed to identify barley lines with high-BNI activity and explore the underlying chemical mechanisms of BNI. Several barley cultivars were screened for BNI activity by analyzing rhizosphere nitrifier communities and assessing nitrification inhibition.

Our results confirm BNI activity in barley and identify a cultivar with high-BNI potential. We show that high-BNI barley influences rhizosphere microbial community composition, and ongoing work is focused on determining the chemical structure of the BNI compounds exuded by barley roots. These findings open the door for the development of high-NUE barley cultivars, contributing to more sustainable farming practices that reduce inputs while boosting productivity, with positive environmental and economic impacts.

Abstract ID: 3433 / Poster Number: 302.16S

Identification and characterization of the sugar transporters involved in cellulase induction in Neurospora sp

Presenting author: Shriya Singh, PhD, Regional Centre for Biotechnology, Faridabad, India

Introduction: Plant biomass is the most abundant and cost-effective renewable feedstock. The polysaccharides in lignocellulosic biomass are tightly bound through covalent and non-covalent interactions, requiring high enzyme loading for complete hydrolysis. Fungi have been known producers of cellulase secretion. Our previous study has shown that there are small inducers(oligosaccharides) that lead to the induction of cellulases. Studies have also indicated the participation of sugar transporters (STs), which are crucial for the secretion of cellulases. However, the true identity of many of these STs is yet to be unfolded.

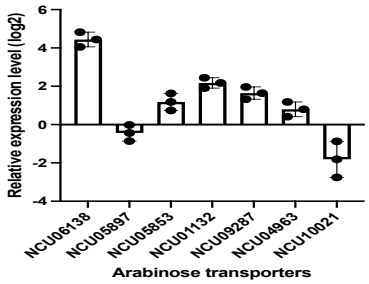
Objectives: The primary goal of the study is to explore membrane transporters, which play an essential role in regulating the secretion of cellulase/hemicellulases using *Neurospora crassa*. There are many inducers known to play an important role in cellulase/hemicellulase secretion. However, their underlying mechanism is not completely elucidated. Further, understanding transporter crosstalk shed light on the new mechanism underlying cellulase/hemicellulase secretion as multiple STs compete for the substrate.

Methods: Multiple knockouts (KOs) strains were analyzed to investigate their role in cellulolytic enzyme secretion, using both biochemical assays and quantitative RT-PCR studies. Further, we are generating ST KOs in *Neurospora* to investigate potential **crosstalk.**

Findings: After curating the STs from Mycocosm and published literature, we identified 40 STs in *Neurospora*. Further, we re-analyzed the RNA sequencing data of complied transporters from the literature and further validated the findings using RT-PCR in complex polysaccharides. Through biochemical analysis of corresponding ST KOs, we found Arabinose-proton symporter (Lat-1) playing an important role in cellulase secretion, with Lat-1 deletion causing a significant reduction in cellulase enzyme secretion. Since Lat-1 is an arabinose-proton symporter, we further investigated arabinose as an inducer for cellulase production and found it to be a potent inducer. Surprisingly, individual Lat-1 KO did not completely abolish cellulase secretion in arabinose. Based on the literature survey, we found RNA-sequencing data for the STs related to arabinose. Recent highlights also indicate the alternative transporters that can

crosstalk with the primary one. Then, we further analyzed the expression levels of arabinose transporters in Lat-1 KO, of which *xy31*(NCU06198) showed the highest upregulation (Fig. 1). Additionally, biochemical analysis of the individual *Xy31* KO (NCU06198) showed a decline in cellulase secretion *(Fig. 2)*. These findings suggest there might be crosstalk between Xy31 and Lat-1.

Conclusion: As we know arabinose to be an inducer of cellulose inducers, we have investigated the effect of two arabinose transporters, Lat-1 and Xy31, and found that their individual KO did not abolish enzyme activity completely. Therefore, it will be interesting to study the combined effect of their double KOs. The insights from this study will enhance our understanding of how these two transporters' crosstalk will influence the cellulase/ hemicellulase secretion.



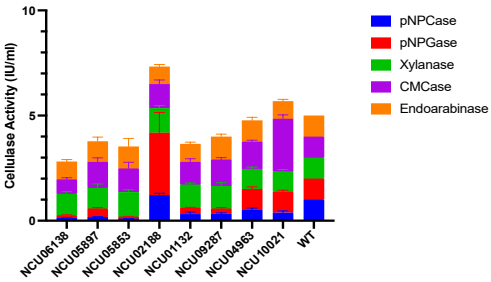


Fig. 1: Quantitative RT-PCR of arabinose transporters in *lat-1* KO.

Fig. 2: Biochemical analysis of individual ST KO.

Abstract ID: 2221 / Poster Number: 127.16P

Identification and Molecular characterization of common uropathogens in catheter-associated urinary tract infections in Limpopo province

Presenting author: Dr. Onele Gcilitshana, University of Limpopo, Polokwane, South Africa

Co-authors: *Joy Maunye*

Introduction: Urinary tract infections (UTIs) have an increasing prevalence in the African sub-Saharan region with South Africa as the second leading country accounting for 67.6% of cases. One of the major risk factors for this prevalence is urinary catheterization. As a result, Catheter-Associated Urinary Tract Infections continue to top the list of the most prevalent nosocomial infections. Due to the use of only conventional methods, these cases remain under reported in most South African hospitals.

Objectives: This study is aimed at identification and characterization of virulence mechanisms of uropathogens isolated from previously catheterized patients in the Limpopo Province of South Africa.

Methods: A cross-sectional, quantitative study analyzed urine samples from catheters of patients admitted in Polokwane Provincial Hospital. Uropathogens were first identified by culturing on Chromogenic agar and Vitek system. Molecular characterization of the virulence genes will be determined using multiplex PCR followed by RNAseq data analysis. Biofilm formation will be determined using crystal violet assay and Scanning electron microscopy.

Findings: Two hundred samples were collected, One hundred and forty samples were confirmed as positive for UTI with identified uropathogens as follows; E. coli (n=50 where n= 14 is polymicrobial occurrences, K pneumoniae (n=29), E. faecalis (n=20) and E. faecium (n=15).

Conclusion: The study is ongoing and molecular studies will identify the virulence genes of these uropathogens and the biofilm assay will also elucidate on their virulence thus assisting in development of relevant treatment strategies.

Abstract ID: 611 / Poster Number: 004.15P

Identification of Endophytic Bacteria Associated with Drought Stress Tolerance in Sugarcane

Presenting author: Mr. Oscar Alejandro Viveros Aguilar, Centro de Investigacion Cientifica de Yucatan, Merida, Mexico

Co-authors: *Luis Carlos Rodríguez Zapata, Miguel Ángel Herrera Alamillo*

Introduction: Sugarcane (Saccharum officinarum L.) is an economically vital crop representing over 76% of global sugar production. However, drought stress significantly impacts its productivity, reducing yields by up to 60%. While chemical solutions and irrigation systems are commonly used to address this challenge, they present economic and environmental concerns, highlighting the need for sustainable alternatives. As a natural strategy, sugarcane cultivation has co-evolved with a great variety of endophytic bacteria, which constitute a diverse community that enhances agricultural yields and helps mitigate stress factors.

Objective: To identify and characterize endophytic bacteria from sugarcane plants that contribute to drought stress tolerance, with the aim of developing sustainable biotechnological solutions for improving crop resilience.

Methods: Endophytic bacteria were isolated from the stems of four varieties of sugarcane collected from drought-affected areas. Isolates were characterized through morphological, biochemical, and molecular methods. Plant growth-promoting traits and drought tolerance mechanisms were evaluated through in vitro assays. Selected isolates were tested for their ability to enhance drought tolerance in sugarcane plants under controlled conditions.

Results: Several endophytic bacterial strains demonstrating significant plant growth-promoting traits and drought tolerance mechanisms were identified. These isolates showed capabilities including nitrogen fixation, phosphate solubilization, and production of osmolytes and plant growth hormones. Inoculation experiments revealed improved drought tolerance in treated sugarcane plants compared to controls.

Conclusion: This study provides valuable insights into the potential of sugarcane endophytic bacteria as sustainable biotechnological tools for enhancing drought tolerance, offering an environmentally friendly alternative to conventional agricultural practices.

Abstract ID: 1653

Identification of genes involved in aggregate associated antimicrobial tolerance of Pseudomonas aeruginosa using Tn-Seq guided discovery

Presenting author: Ms. Lise Goltermann, University of Copenhagen, Copenhagen, Denmark

Co-authors: *Stefano Gualdi, Kasper Nørskov Kragh, Leo Eberl, Tim Tolker-Nielsen*

Introduction: It is estimated that about 60-70% of hospital-acquired infections are caused by biofilms (1). Since current treatment regimens in many cases fail to cure biofilm-based infections, it is critical to identify the mechanisms which underlie biofilm-based antimicrobial tolerance. In biofilms, the bacteria grow in densely packed aggregates concealed in a self-produced matrix of exopolymers and can tolerate high doses of antibiotics (2,3,4).

Objective: The aim of this study was first to develop a transposon sequencing (Tn-Seq) based procedure that enables simultaneous assessment of the relative antibiotic tolerance of hundreds of thousands of individual *P. aeruginosa* aggregates, each established by a single transposon mutant; and second to obtain knowledge about the mechanistic basis of biofilm-associated antibiotic tolerance in the bacterium *Pseudomonas aeruginosa*, which is a model organism for biofilm research.

Method: We use a super-saturated *P. aeruginosa* PA14 transposon (Tn) mutant library (5) consisting of more than 800.000 unique mutants, to identify mutants with increased susceptibility towards a panel of antibiotics. The transposon mutants were grown in agar plates under conditions where each mutant formed an agar-embedded aggregate. The aggregates were treated with the antibiotic of choice or a saline control and surviving bacteria were released from the agar and their DNA extracted. The identity and relative number of the mutants present in the pool of antibiotic-treated and non-treated bacteria were then determined by Tn-Seq analysis.

Findings: Antimicrobial tolerance increases with aggregate age but even relatively young aggregates display tolerance up to 10-fold higher than their planktonic counterparts. Furthermore, the level of c-di-GMP – as measured through a c-di-GMP-monitor (6) - is significantly higher in the aggregates compared to planktonic growth, confirming that the aggregates represent a biofilm-like growth physiology. In our experiment, we cultivated aliquots of the mutant library which in total contained approximately 300.000 aggregates, each formed by a single transposon mutant.

During the ciprofloxacin treatment, Tn-mutants with higher susceptibility were selectively depleted from the antibiotic-treated pool. Subsequently, all identified genes were ordered according to their potential importance for aggregate-associated ciprofloxacin tolerance thus serving as the starting point for exploring the underlying mechanisms.

The top 100 genes were distributed among numerous different functional categories, however, the Cluster of Orthologous Genes (COGs) group “amino acid transport and metabolism” appears to be particularly important for antimicrobial tolerance of the aggregates.

Conclusions: In conclusion, we have developed a Tn-Seq based procedure that enables simultaneous assessment of the relative antibiotic tolerance of hundreds of thousands of individual bacterial aggregates. Using this genome-wide Tn-Seq analysis approach, we found that ciprofloxacin tolerance of *P. aeruginosa* aggregates depends on numerous different genes. Although we could identify genes that appeared to be more important for ciprofloxacin tolerance than others. This emphasizes that aggregate-associated antibiotic tolerance is truly multi-faceted and fundamentally different from resistance mechanisms described for planktonic bacteria.

Using our model, we can continue to expand on these datasets using different selection pressures such as antibiotics or growth conditions. This allows us to identify which genes are important for specific treatments and which are of general importance to the aggregate mode-of-growth.

Abstract ID: 3639 / Poster Number: 093.16P

Identification of gut microbial metabolites affecting insulin sensitivity via high-throughput screening in adipocytes

Presenting author: Dr. Benjamin Diel, Eberhard-Karls University, Tübingen, Germany; Helmholtz Center Munich, Munich, Germany

German Center for Diabetes Research, Neuherberg, Germany

Co-authors: *Amel Makour, Judith Leonie Nono, Andreas Birkenfeld, Reiner Jumpertz von Schwartzenberg, Gencer Sancar*

Type 2 diabetes (T2D) is one of the most prevalent diseases and its incidence is increasing globally. Insulin resistance is a key component in the development and pathophysiology of T2D. Although multiple factors influence whole-body insulin resistance, the dysregulation of insulin signaling in adipose tissue plays a critical role, yet its complex underlying mechanisms remain poorly understood. One of the key contributors in this dysregulation could be the intestinal microbiota and its many derived metabolites. Although it has long been correlated with insulin signaling and adipose tissue dysfunction evidence of causality and possible associated mechanisms remains elusive.

In this study, we investigated the direct effect of microbially associated metabolites on insulin action in a murine adipocyte model. To achieve this, we applied a novel high-throughput screening platform quantifying intracellular lipolysis and evaluating the suppression of lipolysis as a surrogate for insulin action. We analyzed 182 metabolites from Targetmol's Gut Microbial Metabolites library, identifying 36 metabolites that directly affect lipolysis and/or modulate the action of insulin on lipolysis. Five candidates showed a better effect than Rosiglitazone, a clinically effective insulin sensitizer, 29 a similar effect and 2 had negative effect. Many of these metabolites have never been associated with lipolysis or insulin resistance, alongside metabolites previously implicated in the regulation of lipolysis like tauroursodeoxycholate.

Our study is the first establishment of systematic screening for the interaction of microbial metabolites and insulin action. This sets the stage for studying the potential causal relationship between the gut microbiome and insulin signaling in adipocytes.

Abstract ID: 2099 / Poster Number: 157.16P

Identification of human microbial consortia for gut decolonization of multidrug-resistant pathogens

Presenting author: Ms. Cintya González-Torres, Foundation for the Promotion of Health and Biomedical Research (FISABIO), Valencia, Spain

Co-authors: *Alejandro Artacho, Nuria Jimenez, Carles Ubeda*

Multidrug resistant pathogens (MRPs) are a healthcare issue worldwide. Infections caused by MRPs, such as Vancomycin-Resistant Enterococcus (VRE), usually start colonizing the gastrointestinal tract. In homeostatic conditions, the intestinal microbiota inhibits gastrointestinal colonization by MRPs. Alterations caused by antibiotic treatment allow MRPs' colonization which enables subsequent infections. Recent strategies, such as fecal microbiota transplantation (FMT), are being implemented to restore the microbiome and deplete MRPs. However, the efficacy of FMT on MRPs decolonization is suboptimal, cannot be standardized due to intra- and inter- donor fecal material variation, and have inherent risks, including transmission of infective agents. Instead, defined bacterial consortia, that could be propagated *in vitro*, could overcome the potential problems derived from FMT. Here, we have implemented an *in vitro* approach to obtain bacterial consortia from healthy donors' feces. Each generated consortia has a reliable representation of the gut microbiota composition from individual healthy donors, including 60%-70% of bacterial genera present in the original sample, belonging to the most abundant phyla (Actinobacteriota, Bacteroidota, Firmicutes and Proteobacteria). All bacterial consortia can be propagated under anaerobic conditions using commercially available media, and can inhibit VRE growth *in vitro*. Moreover, using a preclinical mouse model that mimics MRPs gut colonization in hospitalized patients, we demonstrated that each individual bacterial consortia abolishes VRE gut colonization *in vivo*. Albeit additional clinical trials should be performed to validate the implemented methodology in patients, this novel and feasible approach could be used as an alternative to FMT to inhibit gut colonization by resistant pathogens.

Abstract ID: 2782 / Poster Number: 417.17P

Identification of keratinolytic microorganisms with promising potential for degrading dog hair during composting

Presenting author: Dr. Saloua Biyada, Vilnius Gediminas Technical University, Vilnius, Lithuania

Co-authors: *Eglė Marčiulaitienė, Rugilė Balbierytė, Daiva Tauraitė, Jaunius Urbonavičius*

Introduction: Worldwide, significant quantities of dog fur from hairdressing salons are discarded yearly and end up in landfill sites. Keratin waste is becoming a major concern throughout the world [1]. Tremendous quantities of keratin waste from dog hair constitute a considerable and scarcely exploited protein resource, from which high value-added products could be derived through efficient keratinolysis [2]. In this respect, and owing to their wealth of organic matter, composting could be an effective waste management and circular economy tool for harnessing this treasure trove of organic matter instead of wasting it and landfilling it.

Objectives: Several studies have been carried out on the management of hair waste, particularly sheep's wool, but barely any studies have been performed on the management of dog hair waste [1,2]. For this reason, the novelty and aim of this study is to explore the feasibility of producing high-quality compost from dog hair, and more specifically to monitor changes in microbial diversity throughout the composting phases, which is considered to be the key factor influencing the quality, maturity and stability of the final compost.

Methods: To achieve this, a mixture of food and dog hair waste was set up for composting. An in-depth characterisation of the microbial communities (in particular keratinophilic microorganisms which are involved in protein degradation) associated with the different composting phases of dog hair waste was achieved using Shotgun metagenomic sequencing.

Findings: Sequencing analysis indicated that the mixture studied (food waste and dog hair) hosted plenty of distinct microbial species. Furthermore, it has been pointed out that microbial communities (bacterial and fungal) change throughout the composting phases. Genera and species belonging to Firmicutes, Actinobacteria, Proteobacteria, Bacteroidota, Ascomycota, Basidiomycota and Apicomplexa were the most abundant in the mesophilic phase, whereas the abundance of bacterial phyla during the thermophilic phase decreased and even species from Bacteroidota phyla were no longer present.

However, for fungal phyla, it is noteworthy that composting dog hair waste has a significant thermophilic profile (above 50°C), which generally prohibits the fungal growth. Nevertheless, an increase in fungal abundance was recorded throughout the thermophilic phase. These new thermophilic fungi might be exploited in the forthcoming future to further boost the degradation of organic matter in general and especially for the specific proteins. Finally, during the maturation phases, an increase in certain genera belonging to the bacterial and fungal communities was reported. Furthermore, and owing to the richness of dog hair with keratin, several microorganisms were identified notably species belong to *Bacillus*, *Streptomyces*, *Microbacterium*, *Pseudomonas*, *Paecilomyces*, *Pichia*, *Geotrichum*, *Candida*, etc. These species are known by their ability to synthesized proteo- and keratinolytic enzymes.

Conclusion: The current study provides valuable data on the highly keratinolytic microorganisms present in dog hair compost, which are suitable for biodegradation of this waste, to address keratin waste mismanagement strategies, and could be used as a database for the onward studies.

Abstract ID: 1946

Identification of metabolic niches and their association to the persistence of antibiotic resistant bacteria in wastewater

Presenting author: Ms. Orestis Kanaris, German Federal Institute for Material Research and testing, Berlin, Germany; Freie Universität Berlin, Berlin, Germany

Co-authors: *Niclas Nordholt, Anne-Christine Schulz, Jan Kreft, Frank Schreiber*

Introduction: Wastewater treatment plants (WWTP) are hotspots for the development and spread of antimicrobial resistance. Antimicrobial resistant bacteria (ARB) can persist in the environment for long periods of time, despite metabolic fitness costs that often arise with resistance. Recent research efforts are striving to uncover the role of bacterial metabolism for the ecology and evolution of antibiotic resistance.

Objectives: The aim of this study is to understand the ecological mechanisms, which allow ARB to persist in the environment. More specifically, we aim to identify metabolic niches that can select for and against resistant bacteria.

Method: 62 *E. coli* strains isolated from different WWTPs with different levels of resistance to 14 antibiotics and 3 disinfectants were assembled, sequenced, and phenotypically characterized. Next, genome scale metabolic models (GEMs) were constructed, and the growth of the strains was simulated in the presence of 298 different carbon sources. Furthermore, the growth rates of the isolates were measured in the presence of 3 carbon sources to verify the model predictions. Competition experiments with synthetic microbial communities consisting of a selection of 10 WWTP isolates, 5 of which were antibiotic resistant and 5 sensitive, were carried out in minimal medium with different carbon sources. Population dynamics modelling was used to simulate the competition of isolates under different conditions.

Findings: The isolates have a wide range of susceptibility to the antibiotics, while disinfectants result in a narrower range of susceptibility. GEMs identified 40 carbon sources that can be utilized for growth only by a portion of all the isolates. The prediction accuracy of the GEMs was 93% in the case of D-malate. A range of WWTP isolates were identified which use D-malate as carbon source and are susceptible to specific antibiotics. In contrast, antibiotic-resistant WWTP isolates were identified that did use sucrose as carbon source but not D-malate. Competition experiments demonstrated that changing the carbon source of the medium from sucrose to D-malate resulted in selection against the resistant isolates. Modelling the competition between isolates

under different conditions suggests that adding a carbon source to a bacterial community under specific conditions could exclude resistant bacteria from a microbial community.

Conclusions: Our data suggest that changing the available carbon source could shift the selection advantage between resistant and susceptible bacterial strains. If this strategy is confirmed experimentally in complex microbial communities, it could be applied to reduce the number of ARB in environments such as wastewater.

Abstract ID: 2187 / Poster Number: 116.17P

Identification of novel molecules supporting the impact of β-lactams against clinically-relevant Gram-negative multidrug resistant organisms

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Antimicrobial resistance spread represents an enormous global health crisis. Particularly worrying are multidrug-resistant Gram-negative (MDR-GN) bacteria, for whom few therapeutic options are available: β-lactams represent frontline antibiotics but resistance towards them is widespread. To limit the impact of this largely unmet medical need, we aim at discovering novel antibiotic adjuvants to be used in combination with β-lactams against MDR-GN bacteria. To achieve this purpose, we first selected five clinically-relevant models of β-lactam resistance in MDR-GN. Then, we proceeded with the high-throughput biological activity-guided screening of a filamentous actinomycetes/fungi-based microbial library (containing 39,000 crude extracts) and of a chemical library (with 9,000 pure compounds) to identify molecules able to restore the activity of β-lactams against the selected resistant isolates. No HITs were identified from the screening of the chemical library, while >200 putative HITs were discovered from the microbial extract library. From them, an activity-guided purification process allowed us to isolate 22 bioactive compounds, which underwent chemical and biological characterization to proceed with dereplication and novelty evaluation. Preliminary data collected attributed the observed bioactivities mostly to metal chelating agents (*e.g.,* gobichelin, 2N-methylcoprogen, coprogen). In the next months, a further characterization of the molecules will be conducted, to better define their mode of action by *in vitro* and *in silico* tests, to evaluate their activity on a wider panel of MDR-GN clinical isolates, and to assess their cytotoxicity on different eukaryotic cells, ultimately paving the way for their possible preclinical development and future medical use.

Abstract ID: 1122 / Poster Number: 011.15P

Identification of phycosphere consortia of dominant Antarctic lake phytoplankton

Presenting author: Mr. Bradley Krzysiak, Miami University, Oxford, United States

Co-authors: *Rachael Morgan-Kiss*

The phycosphere is a critical zone of interaction between algae and bacteria, largely driven by the exchange of metabolites. Algae provide organic carbon in exchange for compounds that support growth, such as phytohormones, vitamins, and micronutrients. However, under nutrient-limiting conditions, this micro-habitat can also host antagonistic interactions, including the production of algicidal or antibacterial compounds. Lakes within the McMurdo Dry Valleys (MDV), Antarctica, represent some of the last perennially ice-covered lakes on the planet. Algae-bacteria interactions within the water column of the MDV lakes are of high interest: the food web is entirely microbial, and fixed carbon is almost entirely autochthonous. The lack of external inputs places a greater importance on photosynthetic algae as major organic carbon producers. In this project, we investigated phycosphere communities associated with key MDV lake phytoplankton from lake water collected at phytoplankton biomass peaks within the permanent chemoclines of East Lake Bonney (ELB) and Lake Fryxell (FRX). We took two approaches to characterize the phycosphere: (i) size fractionation filtration and subsequent 16S and 18S rRNA amplicon sequencing, and (ii) fluorescence-activated cell sorting of single algal cells, followed by whole-genome amplification and metagenomic sequencing. Preliminary findings from the single-cell sorting will be discussed. Overall, samples from both lakes showed enrichment for Gammaproteobacteria and Planctomycetes within the particle-associated fraction, suggesting a potential association with phytoplankton. Size fractionation showed highly distinct bacterial community compositions in planktonic vs. particle-associated communities in ELB. On the other hand, communities were more comparable between the size fractions collected from FRX.

Abstract ID: 3166 / Poster Number: 207.17P

Identification of the metabolic factors driving the successful colonization of bacterial pathogens

Presenting author: Dr. Cristina Herencias, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; Centro de Investigación Biomédica en Red de Enfermedades Infecciosas-CIBERINFEC, Madrid, Spain

Identification of the metabolic factors driving the successful colonization of bacterial pathogens

Antibiotic-resistant bacterial infections have become a major global threat to human health. Decades of antibiotic misuse and overuse have fueled the worldwide dissemination of pandemic antibiotic-resistant clones. These high-risk clones are a critical clinical concern, spreading uncontrollably across both community and hospital settings. Therefore, new approaches are urgently needed to understand and constrain the spread of antibiotic-resistant high-risk clones.

In this study, we propose a multidisciplinary, integrative approach to uncover the mechanistic basis behind the expansion of one of the most successful pathogens of o

ur time: extraintestinal pathogenic *Escherichia coli* (ExPEC). Leveraging a unique collection of strains isolated over 20 years through a longitudinal study, we aim to characterize the molecular mechanisms driving the success of these worrisome strains.

Using genome-scale metabolic modeling, we analyze the growth performance of ExPEC and commensal strains within complex microbial communities. Our findings reveal significant differences in colonization capacity, even within the same microbiome. This approach will enable us to identify key factors underlying the exceptional intestinal colonization ability of ExPEC and, ultimately, its ecological success.

These insights will inform the development of personalized strategies to curb the spread of antibiotic-resistant clones, offering a pathway to more effective interventions against this pressing public health challenge.

Abstract ID: 2285 / Poster Number: 165.15P

Identification of Universal stress proteins (Usps) in multidrug-resistant Klebsiella pneumoniae (MDR-Kp) and their role in the pathogen response to environmental stress

Presenting author: Prof. Shiri Navon-Venezia, Ariel University, Ariel, Israel

Co-authors: *Ziv Lang, Shiri Navon-Venezia*

Introduction: MDR-Kp is an important causative pathogen of various infectious diseases, coping with diverse environmental stressors. Usps, a highly conserved gene family involved in stress response, were studied in MDR-Kp, considering their potential role in facilitating bacterial adaptation to environmental stressors encountered in the host.

Objectives: To investigate the Usps gene family in MDR-Kp and decipher their involvement in response to nutrient limitations and oxidative stress.

Methods: MDR-Kp Usps identification and homology was performed by BLASTN and Clustal Omega. Phylogenetic analysis using MEGA was performed for genetic relatedness with other *Enterobacteriales* genera. Usp genes expression experiments were performed in Kp-B199 BSI clinical strain (GenBank: LJCB00000000.1) using qRT-PCR. Expression levels were quantified in rich and defined media, at different growth stages, and under nutrient limitations and oxidative stress.

Findings: Seven *usp* genes (*usp*A, *usp*C, *usp*E, *usp*F and three alleles of *usp*G) were identified in *K. pneumoniae* genomes, showing high sequence similarity (68.5-99.3%) with Usps of other *Enterobacteriales* pathogens. Comparative transcriptomic analysis demonstrated a significant higher expression during growth in minimal media, and expression of all genes was phase-dependent with pronounced increase during stationary phase (2.3 to 16-fold, $p<0.05$). A significant increased expression under carbon or nitrogen limitation, and in response to H_2O_2 exposure, proved Usps involvement in MDR-Kp response to nutrient limitation and oxidative stress.

Conclusion: Understanding the role of Usps in MDR-Kp contribute to the fundamental knowledge on adaptation systems during infections and could promote the identification of potential stress response biomarkers used as therapeutic targets.

Abstract ID: 3150 / Poster Number: 080.16P

Identifying virulence factors of Streptococcus canis in infective endocarditis using Transposon Directed Insertion Sequencing

Presenting author: Mrs. Miriam Katsburg, Freie Universität Berlin, Berlin, Germany

Co-authors: *Anna Kopenhagen, Etienne Aubry, Simone Bergmann, Marcus Fulde*

Introduction: The opportunistic pathogen *Streptococcus canis* (SC) was found to be involved in almost a quarter of canine infective endocarditis (IE) cases. In humans, cases of IE caused by SC have been described after bite wounds from pets. Biofilm formation is the biggest risk factor for a bad prognosis as it drastically increases the pathogen's antibiotic resistance.

Objective: The goal of this study is to identify virulence factors in SC and find new potential vaccine or drug targets.

Method: A random transposon mutant library (TraDIS) was made by pGh9:ISS1 transposition in a clinical endocarditis SC strain. After infection of human umbilical vein endothelial cells under flow conditions to closely mimic the physiological environment of the endocardium, the non-infectious mutants were sequenced, forming an output pool. Secondly, a biofilm was formed under flow conditions on a fibrin matrix, where the non-adhering mutants created an output pool. By comparing the output pools with the input library, potential virulence factors were identified. After sequence analysis, we confirm these factors by targeted mutagenesis and functional assays.

Findings: Relevant genes in the pathogenesis of SC in IE could be identified. By comparing these genes to known virulence factors in other streptococcal species, we could verify the efficiency of TraDIS in identifying virulence factors and discovering involved genes from different stages of the endocarditis pathophysiology.

Conclusion: The SC genome was efficiently investigated with TraDIS for genes that play a role in different stages of the endocarditis pathophysiology, which provides more insight into IE development.

Abstract ID: 1199

Immobilised BDMDAC particles as an efficient wastewater post treatment strategy with a focus on AMR mitigation

Presenting author: Ms. Marta Redondo, LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Porto, Portugal

Co-authors: *Uli Klümper, Ana Pereira, Thomas Berendonk, Luís Melo, Alan Elena*

Wastewater treatment plants (WWTP) play a key role in antibiotic resistant bacteria (ARB) and ARG removal. Still, available technologies can perform suboptimally making post-treatment alternatives are highly valuable. Benzyl-dimethyldodecyl-ammonium chloride (BDMDAC, a quaternary ammonium compound – QAC) immobilised onto hydroxyapatite to generate functionalised particles (FPs) is here proposed as a novel post-treatment approach that relies on the exposure of treated effluents to FPs in suspension for short periods of time. FPs can be easily removed by decantation following the treatment after which non-detectable BDMDAC residues are left, making this technology also an environmentally safe alternative.

Objective(s): Here we aimed to: i) understand the antibacterial mechanism of FPs and their differential effect on relevant bacterial species, ii) optimise FP concentration and exposure time, iii) evaluate the FP performance on real treated waste water (TWW) samples by molecularly determining total bacterial and ARG removal, iv) determine potential of resistance acquisition, v) analyse the role of FPs in plasmid transfer at suboptimal concentrations, and vi) evaluate particle reusability.

Methods: In order to optimise the FP concentration and exposure time, exponential phase cultures of physiologically different bacteria (Escherichia coli and Pseudomonas putida) were grown in presence of different concentrations of FP, the OD600 was monitored for 20h. Strains harbouring QAC resistance genes were also included to assess the potential resistance to FPs. In order to analyse the potential promotion of plasmid transfer of suboptimal FP exposure, conjugation assays were carried out using P. putida or E. coli donor-recipient systems at across a gradient of FP concentrations. The assessment of treatment efficiency on real life samples was caried out by quantifying 16S rDNA, intI1, blaCTX-M, vanA and qnrS using qPCR. For particle reuse assays, used particles were washed saline and TTSP, experiments were repeated using these particles.

Findings: Photometric assays showed that bacterial growth was stopped for both tested species after 4h of FPs exposure. This effect was achieved using different FP

concentrations for both tested species but remained unchanged for each species disregarding the presence or absence of QAC resistance. FPs had a clear effect on intercelular gene transfer: Culture based assyas revealed that bacteria were killed without lysis,avoiding the release of DNA into the environment that could result in transformation events. Even more, at suboptimal FP concentrations a significant reduction in transconjugant production was observed for both tested species. Applied to a real life scenario, the use of optimal FP concentrations on TWW resulted in a 97% average removal of ARGs and 16S rDNA showing high removal rates of overall bacterial community and an important mitigation of ARG-related risk to the environment. Recycling assays indicate that FPs retained their properties after one reuse but lost efficiency after a second round of reuse.

Conclusion: After FP concentration optimisation, our results highlight the benefits of applying BDMDAC-FPs as post-treatment, including: i) Ease of application and removal with non-detectable QAC release, ii) particle recycling potential allowing to reduce process costs, iii) killing of known resilient bacteria in a short period of time (4h), iv) killing without lysis, avoiding the release of DNA and therefore the risk of transformation events, v) significant decrease of horizontal gene transfer potential even at suboptimal FP concentrations, vi) high ARG and overall bacterial DNA removal, and vii) no selection of qacE, and therefore intI1, carrying isolates.

Abstract ID: 1797 / Poster Number: 179.16P

Immunogenicity and protective efficacy of a novel bacterium-like particle-based vaccine displaying P. multocida PlpE in chickens

Presenting author: Dr. Thu-Dung Doan, National Pingtung University of Science and Technology, Pingtung, Taiwan

Co-authors: *Hsing-Chieh Wu, Thai Ha Nguyen Duc, Chun Yen Chu*

Background: The significance of vaccinations has been underscored by the recent COVID-19 pandemic, which highlighted the urgent need for advancements in vaccine research to better prepare for future outbreaks. The efficacy of vaccines depends on the essential properties of antigens and the contributions of adjuvants. With the exception of live vaccines, most other types require adjuvants to elicit an effective immune response. As vaccinology progresses toward the use of safer and recombinant antigens, adjuvants are becoming increasingly critical in ensuring protective immune responses. Ideal adjuvants enhance cellular immunity, stimulate B cells to produce high levels of neutralizing antibodies, and aid in delivering antigens to immune cells to trigger effective immunity.

Objective: The aim of this study was to establish a novel bacterium-like particle (BLP) platform capable of carrying heterologous recombinant antigens for subunit vaccine development.

Methods: In this study, we displayed the recombinant *P. multocida* Lipoprotein E (PlpE) on the BLP delivery platform using antigen-protein anchor fusions produced by an *E. coli* expression system (rPlpE-PA). Our adjuvant platform was characterized through SDS-PAGE, Western blotting, and transmission electron microscopy (TEM). The vaccine enhancement effects of our adjuvant platform were compared with a chemical adjuvant by evaluating antibody titter and cytokines profiles, followed by a challenge test in chickens.

Results: Our results demonstrated the successful expression of rPlpE-PA at the expected size of 65 kDa. The purified rPlpE-PA protein was effectively displayed on treated *Lactococcus lactis*, serving as a subunit vaccine against *P. multocida*. In a challenge test, our adjuvant platform provided a higher survival rate compared to the antigen-only and chemical adjuvant groups.

In conclusion, our adjuvant platform shows promise as a potential carrier adjuvant, eliminating the need for recombinant protein purification in vaccine development.

Abstract ID: 2133 / Poster Number: 050.15P

Impact of agricultural management, geographic origin, and cultivars of wheat on metabolic profiling and taxonomic composition of soil microbial communities

Presenting author: Manuela Costanzo, PhD, ENEA, Italian National Agency, Rome, Italy

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Introduction: Soil microbiomes (SMs) are involved in all biogeochemical cycles, playing a key role in the turnover of organic matter and in the regulation of nutrient availability. Moreover, SMs play a key role also in climate change being responsible for the production of greenhouse gases (GHGs) which contribute to in global warming. There's therefore the societal need to understand the best agronomic strategies to better preserve healthy soils and reduce GHGs emissions into the atmosphere.

Objective: In the framework of the National Center for Agricultural Technologies (PNRR AGRITECH), soil samples were collected from Northern, Central and Southern Italy to investigate the impact of agricultural management, geographic origin and cultivars of *Triticum durum (Td)* and *aestivum (Ta)* on SMs communities.

Method: Functional diversity was investigated by using the Biolog-ECOPlates™ system (Biolog Inc., USA), with average well color development (AWCD) indicating the level of microbial activity. To assess bacterial diversity and structure, the sequencing of the 16S rRNA gene was performed on the MinION (Oxford Nanopore) platform.

Findings: Preliminary results show that *Ta* had higher metabolic fingerprint than *Td* ($p<0.05$). Further analysis shows that in *Ta*, management and geographic origin affect the metabolic activity of the microbiome ($p<0.05$), the same finding is not obtained in *Td*. Metagenomic analysis will allow us to validate the data obtained.

Conclusion: Our study will allow us to analyze metabolic changes in the microbial community due to different management techniques and geographic origin of the wheat cultivar, identifying the abundance of different species in the central microbiome.

Abstract ID: 2928 / Poster Number: 007.15P

Impact of Agro-Industrial By-Products on the Hemolymph and Gut Microbiota of the Yellow Mealworm Tenebrio molitor

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Introduction: Recently, insect farming has emerged as a sustainable alternative for producing protein-rich food and feed, as well as for bioconverting agro-industrial by-products. The rearing substrate not only influences insect's performance but also plays a crucial role in shaping the insect-associated microbiota, which in turn affects the host's physiology and immune system. Particularly, the gut microbiota of *Tenebrio molitor L.* (Coleoptera: Tenebrionidae), one of the most extensively farmed insect species, has received little attention when raised on different agri-food by-products. As well as the hemolymph, a key mediator of nutritional and immunological homeostasis in insects, remain largely unexplored.

Objective: Aim of this research is to investigate the impact of various agro-industrial by-products on the microbial community associated with the hemolymph and gut of *T. molitor*.

Method: The hemolymph and gut microbiota of larvae reared on different by-products, i.e., 50% wheat bran + 50% brewer's spent grain or 50% wheat bran + 50% tomato peels, and a control diet of 100% wheat bran, were analysed using 16S rRNA high-throughput sequencing. Moreover, the bacterial community abundance was quantified through qPCR on 16S rRNA gene.

Findings: Insects' performance varied depending on the by-products, characterized by different nutrient profiles. DNA extraction protocols were successfully optimized from both hemolymph and gut samples. Microbiological analysis revealed that the larval hemolymph and gut microbiota varied depending on the agro-industrial by-products.

Conclusion: Future studies will be focused to elucidate the dynamics of the hemolymph and gut microbiota, key elements to enhance insect health in mass rearing environment.

Abstract ID: 3512 / Poster Number: 089.16P

Impact of Air Pollutants on Human Gut Microbiome and their probable role in Autism Spectrum Disorders in Children

Presenting author: Dr. Swati M Biswas, ARSD College, university of Delhi, New Delhi, India

Autism spectrum disorder (ASD) is a developmental condition characterized by persistent deficits in social communication and social interaction across multiple contexts, accompanied by restricted, repetitive patterns of behavior, interests, or activities. ASD is a multifactorial condition with unknown etiology, although many genetic and environmental risk factors have been implicated. Gut microbiome homeostasis plays an important role in human health. Gut-brain axis refers to complex interrelationship between central nervous system and gastrointestinal system. Recent studies have highlighted the importance of commensal gut microbes to normal gut function and neurobehavioral health especially the Autism Spectrum disorders (ASD). Air pollutants can cause gut dysbiosis by altering the composition of gut microbiota.

Current study focuses on impact of exposure to potent air pollutants like particulate matter (PM), nitric oxide (NO), ozone (O_3), sulphur dioxide and nitrogen dioxide (NO_2) at prenatal and early childhood stages leading to ASD. Analysis of online data on exposure to pollutants and changes in Gut Microflora could indeed be one of the potent causes of ASD since the gut microbiota plays a role in shaping cognitive networks encompassing emotional and social domains. Decrease in beneficial microbes like *Firmicutes* and an increase in pro-inflammatory species like *E. faecalis, Ruminococcus*.

However further studies are needed to confirm how much exposure time can trigger this condition.

Keywords: *Microbiota, Autism Spectrum Disorder, Neurobehavioural, Dysbiosis*.

Abstract ID: 2668 / Poster Number: 120.16P

Impact of amoxicillin and clavulanic acid on the gut microbiota of calves and on selection of antibiotic resistance genes

Presenting author: Morgane Moustaghfir, PhD, French Agency for Food, Environmental and Occupational Health & Safety, Lyon, France; Ecole Vétérinaire VetAgro Sup, Marcy l'Étoile, France

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Amoxicillin/clavulanic acid (AMC) is an important antibiotic to treat infections in humans and animals. In calves, AMC can be administered to treat omphalitis. AMC can cause dysbiosis in the gut microbiota (GM) and select antibiotic resistance genes (ARGs). Calves' GM is a reservoir of ARGs.

The impact of AMC on calves' GM and ARGs has been poorly investigated. In this study, calves (n = 15) suffering from omphalitis and treated with AMC were enrolled, together with control calves (n=15) not receiving AMC. Stools were collected before AMC treatment (T0), at AMC withdrawal (T1), one month (T2) and two months (T3) after AMC withdrawal. 16S rDNA sequencing (V1-V9) was performed using Oxford Nanopore Technologies. Reads, alpha-diversity and the GM composition were analyzed using epi2me pipeline and R. Quantification of copies' number of the bla_{TEM} gene, relatively to the 16S gene was performed by qPCR.

At T1, a significant decrease of bacterial alpha-diversity was observed in AMC treated calves compared to untreated calves. A restoration of the initial diversity was observed for the treated group at T2 and T3. The bla_{TEM} gene was present in both treated and untreated calves at similar levels at T0. At T1, a 20-fold and a 100-fold increase was observed in the untreated and treated group, respectively, compared to T0, suggesting that the AMC treatment selected bla_{TEM} carriers in the GM of the treated calves. Further analyses are ongoing on fecal samples collected at T2 and T3 and on more ARGs, such as *intI1*, $bla_{CTX\text{-}M}$, *tet(A)*

Abstract ID: 3580 / Poster Number: 122.16P

Impact of anaerobic digestion and composting on antibiotic resistance genes and antibiotic compounds in municipal solid waste treatments

Presenting author: Dr. Alessandra Fontana, Università Cattolica del Sacro Cuore, Piacenza-Cremona, Italy

Co-authors: *Paolo Bellassi, Fabrizio Cappa*

Municipal solid waste (MSW) contains organic fractions, making it suitable for biological treatments such as anaerobic digestion and composting. However, MSW is a notable source of antibiotic resistance genes (ARGs), attributed to extensive antibiotic use in human health, agriculture, and livestock farming. Consequently, a thorough evaluation of how these treatments influence ARGs and antibiotic compounds is crucial.

This study investigates the environmental risks associated with the agronomic use of digestate and compost derived from anaerobic digestion and composting of MSW. Samples from an integrated anaerobic digestion and composting facility treating the organic fraction of MSW were analysed using a multi-omics approach. DNA shotgun sequencing and binning tools identified metagenome-assembled genomes (MAGs) and characterized their ARG profiles. Additionally, untargeted metabolomics provided a comprehensive assessment of antibiotic compounds in the waste treatments.

The two biological processes exhibited distinct impacts on microbial communities and ARG profiles. The composting process significantly increased ARG diversity and abundance. Moreover, the distribution of antibiotic drug classes differed between digestate and compost.

This study offers critical insights into the fate of ARGs and antibiotic compounds in MSW treatments. It highlights how anaerobic digestion and composting differentially shape microbial communities, ARG profiles, and antibiotic content, providing a foundation for assessing the environmental risks of these processes.

Abstract ID: 3985

Impact of cricket protein on gut microbiome

Presenting author: Assoc. Prof. Ilario Ferrocino, Università degli Studi di Torino, Grugliasco, Italy

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The growing demand for sustainable protein sources has increased interest in edible insects, yet their impact on human gut microbiota remains underexplored. This study examines the effects of dietary supplementation with cricket (*Acheta domesticus*) compared to milk and pea proteins on gut microbiome composition and function. Using an in vitro model, we assessed microbial dynamics and metabolomic shifts to explore the potential health implications of alternative proteins. The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) was used to simulate the gastrointestinal environment. A triple M-SHIME® experiment (Figure 1) was conducted to evaluate the effects of cricket, whey, and pea pre-digested proteins (10 g/day for two weeks) on the colonic microbiome. Samples were collected before treatment, during supplementation, and after a post-treatment washout. Microbiome composition, function, and metabolic outputs were analyzed using 16S rRNA sequencing, shotgun metagenomics, and metabolomics. Cricket protein induced distinct microbial shifts compared to pea and milk proteins, promoting *Blautia, Dorea, Lactobacillus, Bifidobacterium,* and *Lachnospira*, along with the highest levels of acetic acid. It modulated gut microbiome by enhancing amino acid metabolism, particularly tryptophan, lysine, and aromatic compounds. Increased shikimate pathway activity suggests a potential impact on neurotransmitter production, benefiting gut and systemic health. While whey protein exhibited a butyrogenic effect, cricket protein influenced acetate production and the generation of potentially beneficial microbial metabolites. These findings highlight the effects of alternative proteins on gut microbiota composition and metabolism and emphasize the need for further research to assess the health implications and safety of insect-based proteins for human consumption.

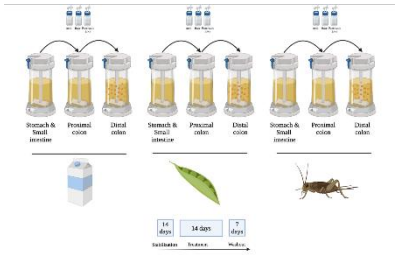


Figure 1: Triple M-SHIME configuration and experiment phases

Abstract ID: 2617 / Poster Number: 021.15P

Impact of Degrading Permafrost and Peatland on Microbial Communities and Dissolved Organic Matter Processing in Small Arctic Catchment

Presenting author: Ms. Nea Tuomela, University of Helsinki, Helsinki, Finland

Co-authors: *Samu Elovaara, Hultman Jenni, Kaartokallio Hermanni, David N. Thomas*

Northern peatlands and permafrost are two of the largest reservoirs of organic carbon on Earth. These environments are facing rapid warming, which is changing their function and releasing organic matter to freshwaters mainly as dissolved organic matter (DOM). Microbial communities and their functionality are one of the key factors in DOM processing. DOM, pH and nutrients are widely recognized as the main drivers of microbes in freshwaters, but their interplay has been less extensively studied in the headwater streams and ponds. Additionally, the recently discovered ultrasmall phylum Patescibacteria can constitute a significant fraction of the free-living microbial communities in freshwater but is poorly captured with standard sampling practices.

Our research integrates metagenomics with DOM quality and quantity metrics to identify the impact of peatland and degrading permafrost on microbial community composition and function in headwaters of a sub-Arctic catchment. Microbial communities affected by high loading of DOM from permafrost were distinct, whereas rest of the catchment had similar microbial communities. Notably, Patescibacteria dominated the aquatic community composition. Our findings offer new insight in microbial community assemblages in small headwater streams and ponds affected by peatland and degrading permafrost. With climate change altering these environments drastically, our results are pertinent to understand the impacts on microbial communities.

Abstract ID: 3969 / Poster Number: 033.15P

Impact of Environmental Changes on the Microbiome of Daphnia magna: The Role of Temperature, Oxygen, and Food Availability

Presenting author: Ms. Ameena Haroon, University of Muenster, Muenster, Germany

Co-authors: *Bettina Zeis*

Daphnia magna, a limnetic zooplankton species is a model system to understand the host-microbiota interaction under changing ecological conditions. The microbiome is composed of diverse bacterial communities that play an important role in shaping the host's physiology, ecological interactions, and growth in a freshwater ecosystem. We assumed that the bacterial communities are expressed differently in temperature shifts, nutrients availability and in lack of oxygen. In order to understand the stability and composition of the microbiome under these varied conditions, we compared animals grown at three temperatures 10°C, 20°C, and 30°C, oxygen conditions; normoxia vs hypoxia and green algae vs cyanobacteria in their diet by using 16S RNA amplicon metagenomics. The comparative statistics analysis builds on the amplicon metagenomics study and adds determination of β-diversity and differential operational taxonomic units' analysis based on replicates amongst the samples on said categories. We found evidence for a large number of bacteria belonging to diverse taxa that are varied in their expression. The dominant of those is *Proteobacteria*, bacterial symbionts in limnohabitats. Other taxa characterized in all datasets include *Bacteroidetes, Verrucomicrobia, Actinobacteria,* and *Plantomycetes*. These investigations are helpful in building a forecast model of specie persistence in mesocosm and natural environmental conditions to give an insight to climate change impact on *Daphnia*-microbe interaction, population of *Daphnia magna*, nutrient recycling and environmental metabolomics.

Abstract ID: 2625 / Poster Number: 108.16P

Impact of heatwaves on Vibrio tapetis-induced brown ring disease and the microbiota of Ruditapes philippinarum clams

Presenting author: Ms. Mélody Lebrun, University of Western Brittany, Brest, France

Co-authors: *Maria Elena Martino, Cynthia Oliveira, Sebastien Petton, Hugo Koechlin, Clement Offret, Massimo Milan, Luca Peruzza, Luca Bargelloni, Charlotte Corporeau, Christine Paillard*

Climate change, including heatwaves, disrupts coastal environments and alters the host-microbiota-pathogen interactions in marine species.

In this context, we focus on the host-pathogen model involving *Ruditapes philippinarum* clams and *Vibrio tapetis*. This bacterium is responsible for Brown Ring Disease (BRD), a condition that can cause mortality. The resurgence of *V. tapetis* has impacted numerous shellfish hatcheries along European coasts. BRD is strongly influenced by environmental factors, since it is exacerbated at a temperature of 14°C and a low salinity. In contrast, a temperature of 21°C and a high salinity are associated with immune system stimulation and disease recovery. Our hypothesis is that summer conditions, with heatwaves, may enhance BRD resistance and recovery by modulating the composition of the microbiota toward anti-*V. tapetis* activities.

In 2024, clams were conditioned to summer conditions, and four heatwaves were simulated in experimental tanks based on the SSP5-8.5 climate scenario. Weekly growth measurements and regular sampling of hemolymph for immune cells, digestive glands, and extrapallial fluids were carried. Summer microbiota collected at the end of experiment will be test *in-vitro* against *V. tapetis* (agar assays) and *in-vivo* by transplantation. After the first and last heatwaves, a bacterial challenge was conducted to assess the susceptibility of clams to *V. tapetis*.

These findings highlight how climate change modifies clam vulnerability to *Vibrio tapetis*, emphasizing the need for management strategies to protect these essential organisms. A potential probiotic strain could be isolated in the near future.

Abstract ID: 3399 / Poster Number: 176.16P

Impact of hygienic conditions on the inactivation efficiency of biocides targeting norovirus

Presenting author: Ms. Swenja Lassen, Technical University of Denmark, Copenhagen, Denmark

Co-authors: *Lisbeth Truelstrup Hansen, Martin Laage Kragh, Ana Herrero Fresno*

Introduction: Human norovirus is a common cause of acute gastroenteritis outbreaks in humans worldwide. The presence of organic matter and other interfering substances can significantly impact the efficacy of preventive disinfection schemes. In this study the influence of such substances was evaluated for ethanol, sodium hypochlorite (NaClO), peracetic acid, and lactic acid combined with sodium dodecyl sulfate (LA-SDS), which have previously shown to reduce viral titers of murine norovirus MNV-1 by >4 log PFU/ml following a contact time of 5 minutes at 22℃.

Methods: Viral suspensions of MNV-1 were supplemented with interfering substances to simulate “clean” (0.3 g/l BSA) and “dirty” (3.0 g/l of interfering substance) conditions. Infective viral particles were quantified by plaque assay after exposure to the four biocide candidates at concentrations with demonstrated virucidal activity for 5 minutes at 22℃.

Results: Preliminary results indicate variability in the virucidal efficacy of the tested biocides under different hygienic conditions.

Conclusion: This study underscores the importance of evaluating biocide performance in the presence of interfering substances to simulate real-world conditions. The findings will provide critical insights into the selection of effective biocidal agents for norovirus control in diverse settings.

Abstract ID: 1687 / Poster Number: 005.15P

Impact of Military Pollution on Soil Microbial Communities in Conflict Zones in Ukraine

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Introduction: Military operations in Ukraine have resulted in significant soil contamination with heavy metals. Development and implementation of biological methods of ecosystem restoration requires the use of metal-resistant microorganisms capable of detoxifying metals in high concentrations in soil.

Objective: The aim of the work was to assess the level of heavy metal contamination of soils affected by military operations in Ukraine and to figure out how military-related pollutants influence soil microbial processes, which are essential for maintaining soil health and ecosystem stability.

Methods: Soil samples from cities polluted and damaged by rocket or artillery fire were analyzed: Kyiv, Hostomel, Kherson, and Kryvyi Rih. Soil elemental composition was assessed using a portable XRF analyzer Niton XL5 Plus. To isolate metal-resistant microorganisms, nutrient agar (NA) containing a concentration gradient of heavy metals from 25 to 200 mg/L was used. Calculations were made of the number of colony forming units (CFU) per gram of dry soil sample.

Findings: The selected samples were significantly contaminated with toxic metals, distributed as follows: Fe(III) > Cr(VI) > Zn(II) > Ni(II) > Cu(II) > Co(II). The presence of tens to hundreds of thousands of CFU/g in the samples indicates that microorganisms have adapted to survive and interact with metals through their metabolic activity.

Conclusion: The findings from this study highlight the potential of metal-resistant microorganisms in soil remediation, offering valuable insights for the development of biological approaches to detoxify contaminated environments.

Abstract ID: 3048 / Poster Number: 024.15P

Impact of Multiple Pollutants on Marine Sediment Microbial Communities: laboratory experiment

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Marine sediments serve as significant reservoirs for pollutants, acting as both sinks and potential contamination sources within marine ecosystems. Consequently, they pose risks to environmental and human health. These sediments host diverse benthic microbial communities that are crucial for ecosystem processes, including elemental cycling, carbon storage, and pollutant degradation. However, these microbial communities are often overlooked in environmental regulations like the Marine Strategy Framework Directive. The effects of pollutants on these communities are influenced by complex interactions among multiple stressors. Yet, research addressing these combined influences remains limited, despite evidence suggesting pollutants may exert cumulative, synergistic, or antagonistic effects when interacting, which cannot be fully understood by examining stressors in isolation. Recognizing these complexities is essential for regulating marine health effectively.

To investigate how multiple pollutants, affect marine sediment microbial communities, a 28-day laboratory experiment was conducted. Fresh marine sediments from two contrasting Croatian sites—polluted Šibenik harbor and pristine Zlarin island—were used to construct a model system. Pollutants tributyltin (TBT), octocrylene (a UV-B filter), and zinc (Zn) were applied in varying combinations: no pollutant, single, double, and triple treatments at two concentrations. The experiment examined bacterial and fungal communities' structural and functional responses using amplicon sequencing, Biology EcoPlate™ assays, and enzyme activity analyses. Findings highlighted microbial dynamics under pollutant stress and the potential resilience of communities from distinct environments. Future studies should explore long-term effects of pollutant mixtures and recovery capacities while fostering holistic regulations that account for complex stressor interactions and sediment ecosystem resilience.

Abstract ID: 2458 / Poster Number: 174.15P

Impact of Polystyrene Microplastics on Daphnia magna: Morphological Influence on Toxicity and Microbiome Dynamics

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Co-authors: *Van Ve Le, Sang Ah Lee*

Microplastic pollution is a global concern that poses a significant threat to aquatic ecosystems. While *Daphnia* spp. have been widely used as model organisms to study the effects of microplastics on fitness, their microbiome remains an understudied aspect of microplastic exposure. This study examines the impact of ground polystyrene microplastics (GPS) with natural morphologies and commercial polystyrene beads (CPS) on the physio-biochemical responses and gut microbiome of *Daphnia magna*, revealing that GPS is more toxic than CPS. LDA analysis identified significant shifts in microbial community composition, with *Flavobacteriaceae* enriched in the CPS group and *Enterobacteriaceae*, *Cupriavidus*, *Lelliottia*, *Blautia*, and *Subdoligranumlum* enriched in the GPS group. Also, amplicon sequence based functional predictions indicated that the altered microbiome may support the growth of *D. magna* by modulating metabolic pathways. Compared to the control group, gut microbiota in the GPS treatment group showed increased enrichment in pathways related to toluene degradation I, toluene degradation II, and aromatic biogenic amine degradation. Overall, these findings underscore the ecological implications of microplastic pollution and emphasize the importance of understanding how varying microplastic shapes influence *D. magna* and its associated microbiome, providing critical insights into the broader implications of microplastic contamination in freshwater ecosystems.

Keywords: Microplastics, *Daphnia magna*, ecological risk, gut microbiome, environmental pollution

Abstract ID: 3403 / Poster Number: 329.16F

Impact of probiotics supplementation on the gut microbial community of Galleria mellonella and Tenebrio molitor exposed to plastics

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Plastic waste accumulation is a growing environmental issue, driving research into biological solutions for its degradation. Insects such as *Galleria mellonella* and *Tenebrio molitor* have been identified as potential candidates for facilitating plastic breakdown, with their gut microbial community playing a key role. This study examines the effects of plastic-feeding diets and probiotics (bioaugmentation) on the microbial community of insect gut. Plastic-degrading microorganisms were isolated from larvae digestive exposed to plastics (polyethylene, polystyrene, and polyethylene terephthalate) using selective media. Colonies unique to test samples (larvae feeding on plastics) were selected for pure culture isolation and molecular identification. Species from *Microbacterium, Bacillus, Rhodotorula, Staphylococcus, Acinetobacter*, and *Moraxella* were obtained. Two species from the genera *Bacillus* and/or *Rhodotorula* were selected for probiotic testing. Bacterial suspensions (10^7 CFU/g) were applied to the diet supplemented with polyethylene. After 14 days for *G. mellonella* and 28 days for *T. molitor*, DNA was extracted from the gut and sequenced. The bacterial community in both inoculated and non-inoculated specimens revealed a dominant presence of *Bacilli*. Probiotic fortification increased biodiversity in *T. molitor*, while no significant change was observed in *G. mellonella*. These findings enhance the understanding of insect microbiomes in plastic biodegradation and highlight the potential for using probiotics to improve plastic waste management through bioaugmentation and insect-based bioremediation.

Abstract ID: 3636 / Poster Number: 187.17P

Impact of recombination functions on the antibiotic heteroresistance phenotype

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Introduction: Heteroresistance (HR) is a transient antibiotic resistance phenotype driven by various molecular mechanisms, including tandem gene amplifications of resistance genes. In Gram-negative bacteria, this amplification usually relies on homologous recombination between identical tandem repeats flanking the amplified. However, how specific recombination factors influence this process remain unclear.

Objective: This ongoing study investigates how different recombination proteins contribute to the HR phenotype caused by tandem gene amplifications in *Escherichia coli*.

Methods: We utilized 20 *E. coli* mutants from the Keio collection, each with a knockout in a non-essential recombination-related gene and three clinical *E. coli* isolates exhibiting HR to gentamicin, tobramycin, and piperacillin-tazobactam due to amplification of resistance genes on plasmids. Plasmids carrying amplified resistance genes were conjugated into Keio mutants and resistance was assessed through MIC measurements.

Findings: Our preliminary findings suggest that loss of recombination proteins affect resistance gene amplification on plasmids. MIC values for gentamicin and piperacillin/tazobactam were lower in a majority of the Keio mutants (*recB*, r*ecX*, *sbcC* and *sbcD)* compared to the Keio wild type, indicating impaired gene amplification on the plasmid. For both antibiotics, deletions of *sbcB* and *recC* had no effect on MIC as compared to Keio wild type, suggesting that these proteins might be less critical for gene amplifications. Further analyses on amplification rates and HR phenotype are ongoing and will be presented at the conference.

Conclusion: These results provide new insights into the role of recombination factors in the emergence, maintenance and reversion of this transient antibiotic resistance phenotype.

Abstract ID: 580 / Poster Number: 069.17P

Impact of Scutellaria baicalensis and Andrographis paniculata Extracts on MRSA Growth, Toxicity, and Extracellular Vesicle Composition

Presenting author: Shih-Chun Yang, PhD, Soochow University, Taipei, Taiwan

Co-authors: *I-Fang Wang,*

The discovery of antibiotics has enabled humans to combat many pathogenic bacteria. However, the misuse of antibiotics has led to the emergence of numerous drug-resistant strains. Despite continuous efforts to develop new antibiotics, bacterial infections remain a significant threat to human health, with the rate of resistance development outpacing the discovery of new antibiotics or antibacterial agents. Many Chinese herbal medicines exhibit antibacterial activity. In this study, we found that extracts of Scutellaria baicalensis (HC) and Andrographis paniculata (SB) demonstrated biological activity against MRSA, influencing extracellular vesicles (EVs) and modulating the microbiota. Specifically, these extracts inhibited MRSA growth and biofilm formation. In bacterial population studies, HC and SB significantly reduced MRSA levels while increasing the proportion of Staphylococcus epidermidis. Furthermore, HC and SB altered the size, surface potential, and quantity of MRSA EVs, thereby mitigating the inflammatory response induced by these EVs. Based on our findings, we conclude that HC and SB can influence bacterial EV secretion and interbacterial communication, effectively reducing MRSA infectivity.

Abstract ID: 1173 / Poster Number: 278.16P

Impact of short-chain fatty acids on the anaerobic growth of Escherichia coli K12 and Enterococcus hirae ATCC9790

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Imbalanced levels of short-chain fatty acids (SCFA) can disrupt the gut microbiome and gut-brain axis, potentially contributing to neurodevelopmental disorders. Therefore, in this study, we examined the growth parameters of *Escherichia coli* K12 and *Enterococcus hirae* ATCC9790 strains under anaerobic conditions in the presence of SCFAs. The growth of strains was assessed by specific growth rate (μ) using a spectrophotometric method.

Results showed that μ of *E. coli* K12 without glucose and SCFAs in anaerobic conditions (control) was 0.5 h^{-1}, but increased by ~2 fold with the addition of 0.4% glucose and 10mM acetic acid. The addition of 3.34mM butyric acid increased μ by ~1.5 fold. Interestingly, the addition of 3.34mM propionic acid and the mixture of SCFAs did not impact μ. The pattern is different in *E. hirae*. The addition of acid increased the μ. In the presence of acids mixture, in *E. hirae* its addition increased growth by ~2.7 fold. The study shows that the response to SCFAs is species-specific under anaerobic conditions. In *E. coli*, limited growth enhancement was detected, likely due to the strain's low ability to utilize SCFAs anaerobically. Conversely, *E. hirae* showed growth increase with all SCFAs, including their mixture. This can be due to the fact that *E. hirae* is a lactic acid bacterium, which probably makes this bacteria have more mechanisms to utilize SCFAs. These findings emphasize the unique metabolic adaptations of Gram-negative and Gram-positive bacteria to SCFAs and highlight their potential impact on the composition of gut microbiota.

Abstract ID: 4137 / Poster Number: 381.17P

Impact of Soil Biostimulant Agents on Vitis vinifera Rhizobiome and Correlation with Plant Physiology

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Co-authors: *Maria Elena Antinori, Filippo Vaccari, Edoardo Puglisi*

Microbial diversity is a key indicator of soil health and is especially relevant in agricultural systems specially in the context of viticulture, where sustainable practices seek benefit from ecosystemic services provided by microorganisms. Thus, effective ways of managing soil diversity are needed.

This study aimed to elucidate the effect of a commercial soil biostimulant on the rhizobiome structure. Six different vineyards where analysed, before and after the treatment with the biostimulant. To understand the rhizobiome variation, DNA extraction and high- throughput sequencing was performed, targeting 16S and ITS genes. Additionally, information of the vine physiological status, vigor, yield, and berry composition were acquired.

Overall, the differences in relative abundance, alpha and beta diversity was not possible to observe a significant difference when the vines were grouped together, except for the beta diversity of fungi. However, when vines were analysed by vineyard, was possible to observed differences in the alpha diversity. Additionally, the treated vines had higher crop production, malic acid, and yeast assimilable nitrogen (YAN), as well as a reduction in the sugar accumulation. These changes correlated with the presence of different genera such as *KD4-96, Bacillus, Streptomyces, Mortierella* and *Solicoccozyma*.

The use of soil conditioners and biocontrol stimulants is a powerful tool to cope with current challenges in viticulture. Yet, the real impact and effect of soil microbiome variations, and the effect on the final grapes composition remain unclear. Nevertheless, the genera found in this study might be promising for future research and applications.

Abstract ID: 3419 / Poster Number: 029.15P

Impact of the microbial community in the mixing zone between Rio Tinto and Jarama river

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Co-authors: *Katherine Thompson, Sergey Abramov, Sara Kleindienst*

Extremophiles are microorganisms that thrive in extreme conditions, such as extreme acidity or alkalinity. The Rio Tinto (RT) in Spain exemplifies an extreme environment with its consistently low pH and high metal content, shaped by natural and mining activities. Furthermore, RT receives input from other water sources, and while RT microbial composition has been studied, it is unknown how the microbial community changes when RT mixes with these new inputs.

This study examines the mixing zone between RT and the Jarama River (JR) to assess how freshwater inputs affect the microbial community dynamics. Water samples were collected in January 2024 from seven sites in the mixing zone between RT and JR (Key sites: 1- upstream RT, 4 and 5 – mixing zone, 7 - post complete mixing) for DNA extraction and 16S rRNA gene amplicon sequencing. The results show that the microbial community shifts across sites due to the Jarama River's influence. Site 1 is dominated by *Acidithiobacillus* (~40%) and *Leptospirillum* (~20%), with *Ferroplasma* and *Pseudomonas* in lower abundance. At Site 4, these acidophiles decrease, while *Flavobacterium* and *Pseudomonas* increase, reflecting a more diverse community. Site 5 shows a further decline in *Acidithiobacillus* (~25%) and *Leptospirillum* (~15%), with a rise in non-extremophiles. However, at Site 7 (Post Complete Mixing), *Acidithiobacillus* (~40%) and *Leptospirillum* (~20%) recover, non-acidophiles decline, and Río Tinto's extreme conditions regain control. Our findings suggest that RT's conditions ultimately determine the final microbial community composition, despite the temporary shifts observed at mixing sites.

Abstract ID: 1939 / Poster Number: 103.17P

Impact of the urban wastewater treatment plant on co-selection and co-localization of antibiotic resistance genes in bacterial genomes in the river ecosystem

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Introduction: Worldwide reported microbial antibiotic-resistance remains one of the main challenges for public health. The co-occurrence of multiple antibiotic resistance genes (ARGs) on mobile genetic elements (MGEs) enhances their co-selection and accelerates their dissemination.

Objective: To investigate the impact of a municipal wastewater treatment plant (WWTP) on a river ecosystem, focusing on co-localization of ARGs in bacterial genomes.

Method: The Poland's largest WWTP, discharges its treated effluent into the Vistula River. During winter and summer of 2023 effluent samples from its discharge site, as well as upstream and downstream sediments from the river were collected. Following DNA isolation and Illumina sequencing, metagenomic analysis comprised taxonomic profiling, plasmid contigs prediction (using PlasME, PlasX, geNOMAD), and ARG identification (using AMRFinderPlus), with statistical analyses performed in R.

Findings: 827 unique ARGs belonging to 25 classes have been identified. Among them, the most concerning is the presence of colistin, cephalosporins, tetracyclines, macrolides and rifamycins resistance genes. Multiple ARGs were found on single contigs, suggesting the presence of multi-drug resistant strains. Moreover, similar clusters of genes were found in various seasons and sampling sites. No significant correlation between the length of the contig and the number of ARGs was found, suggesting that their co-localisation (and therefore possible co-selection) is of an evolutionary importance.

Conclusion: The study highlights the prevalence of ARGs in the river ecosystem and points the role of municipal WWTP in shaping river's resistome. Moreover, it shows the role of co-localization and co-selection in ARGs dissemination and maintenance in aquatic environments.

Abstract ID: 3499 / Poster Number: 148.15P

Impact of tillage practices and white mustard cover cropping on soil microbial diversity

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Co-authors: *Gražina Kadžienė, Danas Baniulis, Inga Tamošiūnė, Skaidrė Supronienė*

Introduction: Conventional tillage and cover cropping are key agricultural practices that have distinct effects on soil microbial communities. Soil microbiota plays a critical role in nutrient cycling, organic matter decomposition and maintaining soil fertility. Cover crops, like white mustard (*Sinapis alba*) due to biofumigation properties can suppress soil-borne pathogens, potentially altering the soil microbial composition.

Objectives: This study aims to evaluate the effect of three different intensities tillage systems and white mustard as cover crop on soil microorganism diversity and relative abundance in wheat field.

Methods: The research experiment consisted of two factors (1) tillage: a) conventional, b) reduced tillage and c) no-till and (2) cover crop (white mustard) use: a) with and b) without. Applied crop rotation: winter oilseed rape - peas - winter wheat - spring wheat. In crop rotation with cover crops, white mustard was sown two years in a row: after harvesting peas and winter wheat. Soil samples were collected from the spring wheat root zone in June. DNA was isolated from freeze-dried soil samples and 16S and ITS metabarcoding analysis was performed using Illumina NovaSeq sequencing at BMKgene (Germany).

Findings: The 16S and ITS metabarcoding analysis revealed greater diversity among bacterial communities, identifying a total of 30 bacterial phyla. The most abundant were Actinobacteria, Proteobacteria, Acidobacteria, and Chloroflexi. In contrast, eight fungal phyla were identified, with Ascomycota, Mortierellomycota, Basidiomycota, Chytridiomycota, and Mucoromycota being the most prevalent. Bacterial alpha-diversity remained consistent regardless of tillage intensity or the presence of cover crops. Similarly, fungal alpha-diversity did not vary significantly across treatments; however, species richness was notably higher in soils managed with no-till practices and cover cropping with white mustard. Several pathogenic fungal genera, including *Cyclospora*, *Plectosphaerella*, *Dactylonectria*, *Fusarium*, *Stachybotrys*, *Didymella*, and *Neocamarosporium*, were detected in the soil. While white mustard cover cropping did

not reduce the relative abundance of these pathogens, it was associated with a decrease in pathogen species diversity.

Abstract ID: 794 / Poster Number: 008.15P

Impact of UV Radiation on Airborne Gram-Negative Bacterial Strain: Adaptation and Resilience in Polluted Environments

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Background: Air pollution is a major health concern causing severe respiratory and cardiovascular disorders. Polluted air consists of different fractions: abiotic part (particulate matter and chemical compounds), and biotic part (microorganisms). These pollutants interact with ultraviolet (UV) radiation. Our hypothesis is that air pollution may increase bacterial virulence, thereby promoting human infections.

Objectives: Assess the effects of UV radiation on microbial fraction.

Methods: The bacterial strain *Pseudomonas fluorescens* MFAF76 was used as a model for airborne microorganisms. A bacterial monolayer was exposed to different types of UV radiation (UV_A, UV_B, UV_C) under controlled conditions.

Bacterial survival was evaluated through cultivability tests and growth kinetics. Membrane integrity and cell viability were assessed using flow cytometry. Expression of specific genes was analyzed using quantitative PCR (RT-qPCR) to investigate the molecular impact of UV exposure. Antibiotic resistance tests were carried out.

Results: Bacterial growth varies depending on the nature and intensity of UV radiation. UV_A exposure had no significant effect on growth or cultivability of *P. fluorescens* MFAF76. In contrast, UV_B and UV_C proved considerably more harmful.

Exposure to UV_B and UV_C also led to a significant increase in membrane fluidity and resistance to Polymyxin B. Flow cytometry analyses revealed that UV exposure did not affect membrane permeability, although UV_B and UV_C significantly reduced bacterial viability. The lethality of UV_B and UV_C appears to be primarily due to DNA damages, confirmed by RT-qPCR, rather than oxidative stress.

These findings enhance our understanding of adaptive mechanisms and resilience of bacterial pathogens to urban pollutants.

Abstract ID: 3224 / Poster Number: 114.15F

Impact of water column hypoxia on microbial community structures and associated sulfur cycles in coastal sediments of the Jinhae Bay, Korea

Presenting author: Haneul Kim, Hanyang University, Ansan, South Korea

Co-authors: *Joo-Han Gwak, Jin-Sook Mok, Sangbeom Baek, Sung-Keun Rhee, Jung-Ho Hyun*

Water column hypoxia (WCH) in shallow coastal environments leads to shifts in benthic biogeochemical processes, particularly towards active anaerobic respiration using alternative electron acceptors. To elucidate the impact of WCH on microbial structures associated with sulfur cycles, we combined biogeochemical analysis with metagenomics in the Jinhae Bay, characterized by a seasonally recurring WCH.

The Dangdong (DD) site exhibited higher sulfate reduction rates and accumulation of reduced sulfur compounds in surface sediment compared to the background site in the center of the bay (CB) during WCH. Microsensor profiles (sulfide, DO, and pH) in surface sediment further corroborated the active sulfur cycles in the DD site. Increased metagenome-assembled genomes (MAGs) affiliated with various sulfate-reducing bacteria of the Desulfobacterota during WCH evidenced the biogeochemical observations. Additionally, MAGs of sulfur-oxidizing bacteria, specifically Chlorobiaceae, photolithotrophic green sulfur bacteria, harboring the rDSR (reverse dissimilatory sulfite reductase) system, were more abundant in the DD site compared to the CB site where Halieaceae harboring the complete-Sox system was detected under relatively less sulfidic condition. Notably, the sigmoid-shaped pH profile observed during post-WCH at the DD site was attributed to sulfur oxidation by nitrate-accumulating Beggiatoaceae, which is supported by increased abundance of their MAG. Consequently, a combination of comprehensive biogeochemical and metagenomic analyses revealed that spatio-temporal changes in the intensity of WCH influence the microbial community structures and associated sulfur cycles in sediments of the Jinhae Bay. This study provides valuable insights into sulfur cycling in hypoxic marine environments, which potentially influences other biogeochemical processes of elements.

Abstract ID: 428 / Poster Number: 184.15P

Impacts of Heavy Metals Stress on the Symbiotic Dynamics and Functional Diversity of Arbuscular Mycorrhizal Fungi and Mycorrhizal Helper Bacteria in Ultramafic Rhizospheric Microenvironments

Presenting author: Prof. Bing-Mu Hsu, National Chung Cheng University, Chiayi, Taiwan

Previous studies have examined the distribution of Arbuscular Mycorrhizal Fungi (AMF) and bacterial communities in serpentine soils, but their co-existence within hyperaccumulator plant rhizospheres remains unexplored. This study hypothesizes a synergistic interaction between AMF and hyperaccumulator plants that immobilizes heavy metals (HMs) and facilitates nutrient cycling, with Mycorrhizal Helper Bacteria (MHB) playing a crucial supporting role. Using high-throughput PacBio sequencing of microbial 18S and 16S rRNA genes, the study aimed to: (1) map the distribution and co-existence of AMF and MHB communities in serpentine rhizospheres, (2) identify their roles in HM immobilization and nutrient cycling, and (3) investigate how abiotic factors, such as HM concentrations, influence their interactions. Key findings revealed that AMF species, including Rhizophagus irregularis, Glomus mosseae, and Claroideoglomus claroideum, and MHB species such as Pseudomonas fluorescens and Bacillus pabuli, formed adaptive associations under stress from HMs like chromium, cadmium, and nickel. These interactions enhanced nutrient cycling, particularly NPK cycling, and HM detoxification, supporting plant resilience in HM-contaminated, nutrient-poor soils. Microbial community composition was significantly influenced by pH, moisture content, and HM levels. Specific associations, such as Pseudomonas chlororaphis with Rhizophagus irregularis, were enriched in the rhizospheres of plants like Panicum maximum and Bidens pilosa. This research underscores the importance of AMF and MHB in mitigating HM stress and improving nutrient cycling. These findings offer valuable insights for designing sustainable phytoremediation strategies and enhancing soil health in HM-stressed ecosystems.

Abstract ID: 3019 / Poster Number: 145.15P

Improved bioleaching of Skutterudite using the As(III) oxidizer Acidiphilium acidophilum CJR1

Presenting author: Ms. Cristian Felipe Jorquera Roman, TU Bergakademie Freiberg, Freiberg, Germany

Co-authors: *Michael Schlömann*

Introduction: The increasing demand for critical metals has intensified the interest in unconventional resources like skutterudite ($CoAs_3$). Even though bioleaching processing alternatives have proven to be effective for $CoAs_3$, arsenic toxicity inhibits the process mainly due to the release of arsenite (As(III)). As(III) is more toxic and mobile than arsenate (As(V)), making it crucial to oxidize As(III) in order to to reduce its toxicity and immobilize arsenic.

Objective: In order to leach $CoAs_3$ more effectively, we tested the use of *Acidiphilium acidophilum* CJR1, an autotrophic acidophilic arsenite oxidizer, in conjunction with the arsenic-adapted Fe^{2+} oxidizing culture BiCoNi4.

Methods: Solid loads of 1% and 2% wt/v $CoAs_3$ were leached by BiCoNi4 in presence of strain CJR1 or without it. Cell growth was followed using an improved Neubauer chamber, and arsenic speciation was determined by ion chromatography. Additionally, pH and ORP (vs. Ag/AgCl) were measured.

Results: For the 1% condition in presence of strain CJR1, after 4 days there was a 4.8-fold increase in cell counts, coinciding with an ORP of 460 mV instead of 351 mV the former peaking at 574 mV on day 7. In the case of 2% solid load in presence of CJR1, after 8 days there was a 7.5-fold increase in cell counts and an ORP of 440 mV (instead of 344 mV) peaking at 566 mV on day 11. Arsenic release increased concomitantly as ORP rose.

Conclusion: The results suggested improved cobalt release when CJR1 was added. The measurements of cobalt concentrations are still in process.

Abstract ID: 2772 / Poster Number: 380.17P

Improved lipogenesis capacity of Yarrowia lipolytica ACA-DC 50109-derived strains obtained through adaptive laboratory evolution

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Industrial production of biodiesel mostly uses vegetable oils as feedstock competing with food production. Harnessing oleaginous microorganisms for single cell oils (SCO) production circumvents this issue, converting low cost industrial by-products into lipids for biodiesel manufacture. However, the SCO fermentation cost is high and very efficient oleaginous microbial strains are required for an economically viable large-scale production of SCO. Without resorting to genetic engineering, adaptive laboratory evolution (ALE) strategies can enhance the oleaginous microorganisms' biotechnological potential, using selective pressure to accelerate the generation of improved phenotypes.

The present study aims to characterize ten evolved clones of *Yarrowia lipolytica* ACA-DC 50109 obtained through an ALE experiment based on the collection of populations with increased lipid content that float upon centrifugation of the culture.

The individual clones obtained by selecting the biggest colonies formed on carbon-free media after different evolutionary rounds (Daskalaki et al., 2019), were cultivated in nitrogen-limited media with 7% (w/v) glucose and monitored daily until glucose depletion. After harvesting biomass, total lipid, protein, and polysaccharide contents were determined, evaluating the performance of the lipogenic machinery of the evolved clones.

The results indicated differences in terms of substrate assimilation and lipid production. The evolved strain P1C2D291024 exhibited the greatest improvement in lipid accumulation (from 24.98±1.06% to 40.81±0.15%), correlated with a reduction in polysaccharide content (from 32.68±1.58% to 23.86±0.91%). ALE strategies produced improved oleaginous clones, indicating a metabolic shift towards lipogenesis, to the detriment of competing pathways. Further testing on low-cost substrates will determine the biotechnological potential of *Y. lipolytica* P1C2D291024.

Abstract ID: 3599 / Poster Number: 299.15P

In *Escherichia coli*, the PatZ N-acetyltransferase modulates the binding of the RutR protein to the promoter driving the synthesis of pyrimidine catabolic enzymes

Presenting author: Mr. Nicholas Athanasatos, University of Georgia, Athens, United States

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Introduction: Reversible lysine acetylation (RLA) regulates the function of proteins involved in many aspects of prokaryotic cell physiology and metabolism. The transcription factor RutR, which regulates aspects of pyrimidine and purine metabolism, has been shown to be a target of RLA in *Escherichia coli*.

Objective: To investigate the effect of acetylation on RutR function.

Method: To identify acetylated residues in RutR we used mass spectrometry after incubation of RutR with PatZ and acetyl-CoA. Electrophoretic mobility shift assays were used to quantify the effect of RutR acetylation on its ability to bind to to the promoter of the *rutABCDEFG* operon (P_{rut}).

Findings: Recent studies by others showed that the PatZ acetyltransferase of this bacterium acetylates RutR nine times. We found an additional RutR lysyl residue, namely Lys101, that is also acetylated. Lys101 hydrogen bonds to uracil potentially stabilizing its binding to the protein. Uracil is the effector molecule that modulates RutR binding to P_{rut}. Results of mass spectrometry studies showed that when RutR was bound to P_{rut} the level of PatZ-dependent acetylation decreased by >10-fold. We also found that acetylation decreased the effectiveness of uracil to disrupt DNA binding. This effect was exerted through the modification of Lys101. When we mimicked the acetylation state of Lys101 by changing Lys101 to Gln101, we found a decrease in uracil binding to RutR. Notably, PatZ-dependent acetylation of RutR resulted in increased affinity of the protein for the target.

Conclusion: We conclude that RLA of Lys101 is how *E. coli* modulates the pyrimidine metabolism.

Abstract ID: 2553 / Poster Number: 421.16F

In silico exploration of non-coding RNA regulation of antiviral defense systems in Shiga toxin-producing Escherichia coli O157:H7

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Shiga toxin-producing *Escherichia coli* (STEC) is a major human pathogen that causes severe gastroenteritis. Among the serotypes, *E. coli* O157:H7 is a frequently reported agent associated with STEC outbreaks. This serotype colonizes the recto-anal junction of cattle and is shed through feces, facilitating its transmission from farms into the food chain. Current interventions target food processing stages, with limited on-farm strategies to directly reduce STEC shedding. Novel strategies, such as microbial manipulation and genomic targeting, are needed to decrease STEC populations in cattle. However, these strategies are complicated by microbial antiviral defense systems (ADS) – innate and adaptive mechanisms that protect prokaryotes against foreign nucleic acids. Studies suggest that non-coding RNAs (ncRNAs) may regulate ADS, raising the possibility of using ncRNAs to overcome STEC ADS and improve *in vitro* targeting. Therefore, this study aimed to (1) identify ncRNA candidates in the *E. coli* O157:H7 reference genome (NC_0002695) and predict their targets and (2) identify ADS in the reference genome and assess potential ncRNA regulation. Of the 116 ncRNAs predicted using an in-house bioinformatic pipeline, 42 were predicted to interact with and regulate host genome sequences, with 12 targeting distinct and non-overlapping loci. DefenseFinder identified 135 ADS candidates, including 26 genes linked to 10 complete systems: Restriction Modification (n=3), toxin-antitoxin (n=3), CRISPR/Cas1-IIE (n=1), Zorya (n=1), Druantia (n=1), and a T4 phage defense system (n=1). Preliminary results showed that the identified ncRNAs do not target complete ADS *in silico* but highlighted a complex mechanistic relationship between STEC and lysogenic phages, warranting further investigations.

Abstract ID: 3223

In vitro and in vivo assays to determine biocontrol potential of Lactiplantibacillus plantarum strains against different phytopathogenic fungi

Presenting author: Alessandro Passera, PhD, Università degli Studi di Milano, Milano, Italy

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Current agriculture is facing the challenge of maintaining the productivity levels granted by the use of intensive practices while becoming more sustainable, reducing its environmental impact.

A substantial help in overcoming this challenge can come from microorganisms: several microbes have evolved features that allow them to live symbiotically with plants, providing enhanced nutrition and protection to plants, usually in exchange for the carbon provided by the plant.

In this study we will present the results regarding the efficacy of four strains of *Lactiplantibacillus plantarum* (S61, VC233, VS166, CE84), isolated from olive, as biocontrol agents against several phytopathogenic fungi (*Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium verticillioides*, *F. graminearum*, *F. fujikuroi*) employing *in vitro* or *in vivo* assays.

All four *L. plantarum* strains were able to completely inhibit the growth of *B. cinerea* in dual-culture in vitro assays; only two of them (S61, VC233) managed to reduce the growth of *F. verticillioides*, while none were effective against *F. graminearum*.

The *in vivo* assay using lettuce-*R. solani* pathosystem showed that strain S61 was able to prevent the damping off symptoms caused by this pathogen with the same efficacy as a commercial Trichoderma-based product, allowing for a successful germination rate of around 80%; in a different *in vivo* assay using the rice-*F. fujikuroi* pathosystem the same strain managed to completely inhibit the development of fungal mycelium on rice seedlings.

Of the four investigated strains, *L. plantarum* S61 produced promising results in these biocontrol assays, making it a candidate for further research and future applications.

Abstract ID: 1742 / Poster Number: 190.16P

In vitro Assays for Exploring the Potential of Artemisia annua Essential Oil in the Management of Infected Wounds

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Introduction/Objective: The purpose of the study was to assess the antimicrobial, antioxidant, and hemocompatibility properties of *A. annua* essential oil (EO) to develop topical formulations for the prevention and treatment of infected chronic wounds.

Method: The EO was isolated by hydrodistillation and characterized by GC-MS. The antimicrobial properties were determined against 10 reference and 7 clinical microbial strains, using qualitative (disk diffusion) and quantitative (serial microdilutions) methods. The antibiofilm activity was tested by the violet crystal microtiter assay of the adhered microbial biomass on the inert substratum. The antioxidant activity was quantified using chemical methods (DPPH, CUPRAC, FRAP), and hemocompatibility was assessed according to the ISO 10993-4:2017 standard.

Findings: The average content of the EO obtained from the dry plant material was 0.89 ± 0.12% and the main compounds were camphor, α-pinene and eucalyptol. The EO minimal inhibitory concentrations ranged from 250 – 7.81 µL/mL, the most susceptible being *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical bacterial isolates, as well as the microfungal *Candida spp*. strains. The EO eradicated the adherence of *Serratia marcescens* and *Klebsiella aerogenes* as well as of the *C. parapsilosis* fungal strain. The EO has also disrupted the 72h mature biofilms. The *A. annua* EO exhibited a significant antioxidant effect (IC50: 12.8± 0.46 mg/mL) and the hemocompatibility was below 31.25 µL/mL (hemolysis <5%).

Conclusion: These results confirm the antimicrobial, antibiofilm, and antioxidant properties of *A. annua* EO. The good hemocompatibility demonstrates the potential of this EO as an active ingredient for infected chronic wounds management.

Abstract ID: 2927 / Poster Number: 074.16P

In vitro assessment of the effect of commercial botanical products on the human fecal bacterial community structure

Presenting author: Fabio Angelini, PhD, University of Milano-Bicocca, Milan, Italy

Co-authors: *Robin Duncan, Giorgio Gargari, Rosario Russo, Giacomo Mantegazza, Simone Guglielmetti*

While most of the studies primarily emphasized prebiotic properties, botanical extracts in dietary supplements can also contain compounds with broad-spectrum antimicrobial activity. This study evaluates the impact of three botanical food supplement ingredients: Bromeyal® (pineapple stem extract), Axtragyl® (astragalus membranaceus root extract), and Kalita® (bergamot secondary juice extract) on the human fecal bacterial community structure and specific bacterial strains using in vitro models. To explore their potential influence on gut microbiota, the INFOGEST static simulation of gastrointestinal digestion was conducted, followed by incubation in fecal suspensions from a healthy donor. 16S rRNA gene profiling revealed no significant alterations in the overall community structure, with alpha and beta diversity metrics remaining stable. However, taxon-specific changes were observed: Axtragyl® and Kalita® increased Akkermansia muciniphila levels, while Bromeyal® reduced Collinsella aerofaciens abundance. In single-strain experiments involving nine probiotic or gut commensal strains, Collinsella aerofaciens was consistently inhibited by Bromeyal® at all tested concentrations, corroborating the trends observed in the fecal model. These findings suggest that the botanical products are gut microbiome-compatible and selectively modulate specific bacterial taxa. Such properties highlight their potential to be considered "gut friendly" products.

Abstract ID: 3367 / Poster Number: 398.16P

In vitro biocontrol potential of indigenous Bacillus velezensis strains in suppressing black rot disease in cabbage cv. Futoški

Presenting author: Dr. Aleksandra Jelušić, University of Belgrade – Institute for Multidisciplinary Research, Belgrade, Serbia

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Serbian autochthonous cabbage cv. Futoški is susceptible to various pathogens, including the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of black rot, resulting in significant economic losses.

This study aimed to provide an environmentally friendly solution for controlling black rot in cabbage cv. Futoški by exploring the potential of indigenous *Bacillus* spp. strains.

Bacillus spp. strains were isolated from the phyllosphere and rhizosphere of cabbage cv. Futoški plants, with and without black rot symptoms. The potential antagonistic activity of their whole cultures and cell-free supernatants was tested in vitro, against *Xcc* strain XccFC231, also isolated from the cv. Futoški. Phylogenetic analysis was performed based on concatenated sequences of the 16S rRNA, *rpoB*, and *gyrA* genes to identify the most potent biocontrol candidate(s). The presence and/or absence of lipopeptide genes in candidates was checked using specific primers, targeting the synthesis of bacillomycin D, fengycin, iturin, kurstakin, and surfactin.

Of the 115 isolated *Bacillus* spp. strains, 52 exhibited antagonistic activity of whole cultures, while nine (BFC3, BFC55, BFC68, BFC73, BFC81, BFC88, BFC98, BFC102, and BFC114) demonstrated activity of supernatants (Ø 12–19 mm) as well. All nine biocontrol candidates were identified as *Bacillus velezensis*. Except for BFC98, the rest of the candidates showed potential to produce bacillomycin D, while six strains (BFC3, BFC55, BFC68, BFC88, BFC98, and BFC102) exhibited potential for iturin production.

The nine isolated *B. velezensis* strains show potential for future use in managing black rot on cabbage cv. Futoški, and could potentially be used on other Brassicaceae.

Abstract ID: 3426

In vitro functional assessment of food texturizers on the human gut microbiota, a focus on chitosan and (hydroxypropyl) methyl-cellulose

Presenting author: Davide Addazii, PhD, University of Bologna, Bologna, Italy

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Food additives, like texturizers, are an important part of the modern food industry. The increasing variety and quantity of processed food in diets lead to increased frequency and dose of food additives exposure, which has become a concern of consumers. Some of these texturizers have glycan structure and can express prebiotic activity, but little is known on the general assessment of these additives on the gut microbiota (TUNCIL, Y. E., et al 2017).

Thus far, this research aims to gain knowledge on the impact of some food-texturizer towards the human colon microbiota, employing an in vitro intestinal model, that simulates human digestion (INFOGEST) and colonic fermentation (MICODE). With the aid of microbiomics (qPCR) and metabolomics (SPME-GC/MS) approaches and multivariate statistical analyses we compared and predicted the effects towards the gut microbiota of: i) artisanal chitosan obtained from seafood waste, ii) commercial chitosan, iii) (hydroxypropyl) methyl-cellulose, iv) a postive control in term of prebiotic activity as frcuctooligosaccharides and v) a blank control with no additive.

The results indicate that artisanal chitosan derived from seafood waste improves some general indicators of colonic well-being in healthy humans and reduces some indicators of dysbiosis. Chitosan derived from seafood waste shows similar abilities than commercial Chitosan and both are able to; i) increase the abundance of some beneficial bacteria (*Bifidobacteriaceae, Bifidobacterium* spp., *Clostridium* group IV and *Faecalibacterium prausnitzii*); ii) contain the growth of opportunistic bacteria (*Clostridium perfringens* and *Prevotella* spp.). In addition, there are positive effects on microbial metabolites content as on the increase of short-chain fatty acids production, in particular propionate and butyrate, and on the modulation on indoles and phenols.

Opposite results were instead generated by (Hydroxypropyl) methyl-cellulose, that is not able to fine-tune the gut microbiota in an in vitro intestinal model. This study suggests that different forms and origin of some common food-texturizers can affect differently the

gut microbiota of consumers, generating an eubiotic or a dysbiotic condition. Further investigations are necessary to outline the interplay among food additives and the gut microbiota, but the present work vows translatability of results to the in vivo conditions that could serve as pre-clinical evidence.

Abstract ID: 1556 / Poster Number: 173.16P

In vitro isolation of contemporary Treponema pallidum strains directly from clinical samples of syphilis patients

Presenting author: Dr. Juraj Bosak, Masaryk University, Brno, Czech Republic

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Introduction: *Treponema pallidum* subsp. *pallidum* (*T. pallidum*) causes syphilis, a sexually transmitted disease of global importance. For over a century, *T. pallidum* strains were isolated and propagated in laboratory rabbits. In 2018, a technique for *in vitro* cultivation of *T. pallidum* was developed.

Objective: Development of an *in vitro* protocol for isolation of contemporary *T. pallidum* strains directly from the patients' clinical samples.

Methods: The oral or genital swabs from nine unrelated patients, collected in two hospitals in Brno (Czech Republic), were placed on the 0.4 µm filter and incubated for 2.5 hours. During this period, spiral *T. pallidum* cells were able to pass through the filter pores to the *in vitro* cultivation well.

Results: Out of nine clinical samples, six *in vitro T. pallidum* cultures (MU1-MU6) were successfully derived during 14-50 days-long *in vitro* cultivation. Among them, five strains (MU1-MU5) belonged to SS14-like cluster and shared identical allelic profile 1.3.1. The last *T. pallidum* strain (MU6) was Nichols-like strain of allelic profile 9.16.3.

Conclusion: In summary, the *in vitro* protocol for isolation of *T. pallidum* from clinical material (even frozen) eliminates the need to propagate treponemes in rabbits and allows an easier isolation of contemporary, clinically relevant, treponemal strains.

Abstract ID: 584 / Poster Number: 070.17P

In vitro properties of WMRK-gH625 a novel hybrid peptide against Multi-Drug-Resistant pathogens

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Introduction: Multi-Drug-Resistant (MDR) bacteria represent a substantial global health concern. Antimicrobial peptides represent a promising strategy for treatment of MDR bacteria, due to their broad-spectrum activity, a low potential for the development of resistance and immunomodulatory properties. The WMR peptide (WMRp), a marine peptide derived from the hagfish, has been demonstrated to exhibit significant antimicrobial activity against bacteria. Furthermore, cell-penetrating peptides, such as gH625 derived from herpes simplex virus type I, have attracted attention due to their capacity to enhance the delivery of macromolecules across cellular membranes. The aim of this study has been to assess the in vitro antimicrobial activity of a novel WMRK-gH625 peptide (WMRK-gHp) against Staphylococcus aureus, Escherichia coli, and Salmonella enterica reference and clinical strains.

Materials and Methods: The antimicrobial properties of WMRK-gHp were evaluated using Broth Dilution Method. Bactericidal or bacteriostatic activities were determined by Time-Kill Assays. Finally, in vitro evaluation of the hemolytic properties of the WMRK-gHp and cytotoxic activities in Hela cells were performed to demonstrate the safe use of peptide.

Results: The WMRK-gHp demonstrated exerting bacteriostatic effects against all bacteria. Additionally, WMRK-gHp exhibited safety profiles, including anti-cytotoxic effects in the HeLa cells, anti-hemolytic activity in human erythrocytes, and a substantial reduction in the release of reactive oxygen species.

Conclusions: The results of this study suggest that the WMRK-gHp exhibits promising antibacterial properties and may represent a valuable step forward in the development of novel therapeutic agents for MDR infection's treatment.

Abstract ID: 583 / Poster Number: 352.17P

In vitro toxicology and antimicrobial efficacy of Q-FIELD™, an innovative photocatalytic additive for paints

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Co-authors: *Markus Karlsen, Janne Elin Reseland, Dirk Linke*

Microorganisms can survive on surfaces for long periods of time, even in dry conditions, contributing to the spread of infections (1–3). Q-FIELD™ is a photocatalytic pigment, active in visible light, that is added to paints, making them capable of inhibiting microbial growth and biofilm formation on the painted surfaces (4–6). Thus, in hospitals and other healthcare settings, these paints could be used to complement routine disinfection procedures (3,6).

Also, in other environments where clinical grade disinfection is not easily applicable, such as elderly homes and schools, they could offer a sustainable solution to reduce the burden of infective agents, giving a long-lasting protection without releasing harmful substances in the environment (6).

Here we examine the toxicological effects of Q-FIELD™ particles comparing them to their precursor, i.e. titanium dioxide particles, on primary human cells from likely exposed tissues. Acute cytotoxicity is evaluated by measuring the release of LDH (lactate dehydrogenase) and caspase-3, while secondary inflammatory effects are studied with a multiplex assay detecting various cytokines and chemokines.

The antimicrobial effects of Q-FIELD™-added paints are quantified, in comparison with normal paint, by inoculating painted surfaces with *Acinetobacter baumannii*, a common hospital pathogen that can survive on dry surfaces (1,2). The bacteria will be allowed to proliferate, monitoring temperature and humidity, for established time periods, before sampling the surfaces with RODAC plates.

Based on the properties of Q-FIELD™, we expect it to be not significantly more toxic than titanium dioxide and we expect the remaining microbial burden on the painted surfaces to be significantly lower where Q-FIELD™ is present.

Abstract ID: 3483 / Poster Number: 004.17S

Increased microbial dominance and CO2 emissions are observed in Arctic soils with elevated graminoid cover near Longyearbyen, Svalbard

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Co-authors: *Jana Rüthers, Annina Maier, Moritz Mainka, Jake Alexander, Simone Fior, Marijn Van de Broek, Kristine Bakke Westergaard, Sebastian Doetterl, Aline Frossard, Dario Barillà, Elias Meier, Cara Magnabosco*

Arctic warming has coincided with dramatic changes in plant cover; however, the drivers and impacts of these above ground biomass changes are still not well understood. This study investigates soil microbial community responses to the greening of Svalbard's high Arctic tundra using a space-for-time experimental approach. Thirty-one topsoil samples from three, ~0.3 km^2 study sites that vary in nutrient input, soil chemistry, and plant cover throughout a 15 km^2 region of Svalbard were collected and characterized during the peak growing season of Summer 2022. One site (Gamlebyen) exhibited a local enrichment of graminoids (>80% of the total plant cover) across a ~40 m^2 area that, within meters, transitioned into a landscape of mixed functional plant types. The graminoid-rich area co-occurs with a local peak in soil fertility and exhibits the highest CO_2 emissions measured in this study. The patterns of soil microbial abundance and diversity throughout the region are more closely linked to the above ground biomass changes than spatial or soil properties, displaying the highest abundance and lowest diversity in the graminoid dominated plots. A comparison of soil CO_2 emissions to plant cover and quantitative PCR data reveals that CO_2 emissions are better explained by internal transcribed spacer (ITS) abundances and graminoid cover than 16S rRNA gene abundances. The increase in ITS and graminoid cover is accompanied by a transition from mixed to Ascomycota-dominated soil fungal communities and suggest that above ground biomass changes are predictive of below ground biomass changes in the region. Additionally, the observed correlations between biodiversity patterns and CO_2 emissions highlight the influence of plant-microbe interactions on the Arctic soil carbon cycle and the co-evolution of above and below ground biomass in a greening Arctic.

Abstract ID: 4033 / Poster Number: 170.15P

Increasing the availability of soluble phosphorus in soil using transmissible plasmids between PGPRs

Presenting author: Ms. Maria B. Monzon Nomdedeu, Polytechnic Valencia University, Valencia, Spain

Co-authors: *Eduardo Bueso Ródenas, Gaetano Bissoli*

Phosphorus is an essential macronutrient for plant development, making it a highly valuable resource now and in the future. Although phosphorus is naturally present in the soil, its availability for plants is reduced since forms insoluble complexes with cations. Currently, the phosphorus added in fertilizers comes from the direct extraction of rock, which is a non-renewable resource whose known global reserves could be finished during this century. Moreover, it is known that there are PGPR with the capacity to solubilize soil phosphorus through the synthesis of various organic acids such as gluconic acid. The aim of the study is to clone the bacterial genes responsible to produce gluconic acid into widely ecotransferable vectors and to stabilize a bacterium that expresses this plasmid in soil. For this purpose, two elements related to gluconic acid production were identified, the enzyme glucose dehydrogenase and the cofactor pyrroloquinoline quinone (PQQ). In addition, two vectors used in conjugation assays were selected. The genes of interest were cloned into both plasmids and agronomic studies were performed with several bacterial strains in orange tree and wheat. Preliminary results confirm that adding bacteria is not detrimental to plant development and that bacteria containing PQQ genes increases photosynthesis rate and induces positive physiological changes in the plant.

Abstract ID: 747 / Poster Number: 116.16P

Industrialisation of lifestyles impacts gut microbiome-mediated metabolism of cholesterol

Presenting author: Mr. Emmanuel K. Quaye, Kiel University & UKSH, Kiel, Germany; Global Microbiome Conservancy (GMbC), Kiel, Germany

Co-authors: *Ana P. Schaan, Malte C. Rühlemann, Lucas Silva, Eike M. Wacker, Hannah M. Jebens, Li Pan, Ainara Sistiaga, Mathieu Groussin, Mathilde Poyet*

Cardiometabolic diseases are the leading cause of death worldwide, with their prevalence expected to rise due to increasing urbanisation and the adoption of industrialised lifestyles. These shifts have been associated with significant changes in gut microbiome composition and diversity, yet their impact on critical microbial functions, particularly those involved in cholesterol metabolism, remains underexplored. These are especially relevant because elevated circulating cholesterol levels are a key driver in the development and progression of cardiometabolic diseases. In this study, we present the first investigation into how host lifestyle influences the gut microbiome's ability to convert cholesterol into coprostanol, a non-absorbable sterol excreted in faeces. Using a dataset and biobank from globally diverse populations, we cultured and isolated a novel human gut *Eubacterium* species and validated its cholesterol-to-coprostanol conversion activity *in vitro*. The abundance of sterol dehydrogenase genes involved in cholesterol conversion, as well as the bacterial species carrying these genes in the human gut metagenome, are associated with faecal coprostanol levels and are elevated in non-industrialised populations. This work provides preliminary evidence linking industrialisation to gut microbiome functions critical for cholesterol metabolism, highlighting a potential mechanism through which lifestyle changes contribute to cardiometabolic disease risk.

Abstract ID: 686 / Poster Number: 071.15P

Industrialization alters IgA-bacteria interaction patterns across worldwide human cohort

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Co-authors: *Ana Paula Schaan, Mathieu Groussin, Mathilde Poyet*

Immunoglobulin A (IgA) is the most prevalent antibody in the gastrointestinal (GI) tract. It coats both pathogenic and commensal microbes, preventing or promoting their colonization and thereby modulating the gut microbial community. However, very little is known about how IgA-bacteria interactions vary among human populations and host lifestyles. This study aimed to elucidate the impact of compositional shifts in the gut microbiome associated with industrialization on IgA secretion and bacteria coating. First, we found that fecal IgA levels were significantly elevated in industrialized and urban populations compared to non-industrialized and rural populations. Then, we performed IgA-Sequencing (IgA-SEQ) on approximately 500 human stool samples from eleven countries to specifically identify microbial taxa coated by IgA. IgA-coating indices (ICI) for individual taxa based on the abundance of coated and uncoated bacteria were calculated and compared between industrialized and non-industrialized populations. We found that IgA coating patterns are generally conserved across populations, particularly among Proteobacteria. However, there was also a greater overall frequency of IgA coating of bacterial species in industrialized samples compared to non-industrialized samples. Furthermore, strong collinearity between ICI, lifestyle, and the abundance of individual taxa was observed. As industrialization increases, it is imperative to understand how lifestyle-driven changes to the gut microbiome impact the gut-immune axis. The results of this study have revealed a complex relationship between lifestyle, gut microbial composition, and IgA, and provoke further questions about the functional impacts of these shifts in the context of human health and evolution.

Abstract ID: 1004 / Poster Number: 079.17P

Influence of Origanum vulgare extract on Escherichia coli metabolism

Presenting author: Anush Babayan, PhD, Yerevan State University, Yerevan, Armenia

Co-authors: *Naira Sahakyan*

Introduction: Plant-based preparations with antioxidant and antimicrobial influence can be effectively applied for both the prevention and adjuvant therapy of various diseases.

Objectives: The presented study investigates the antioxidant and pro-oxidant properties of ethanol extract from *Origanum vulgare* (OVEE) collected in Armenia, focusing on its radical scavenging activity, phenolic and flavonoid content, lipid peroxidation inhibition, nitric oxide (NO) modulation, antioxidant enzyme activity, and gene expression in *Escherichia coli* NM111 cells.

Methods: For evaluation of antioxidant properties, chemical-based tests were applied (total phenolic and flavonoid content, antiradical activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) test). Inhibition of lipid peroxidation, modulation of NO, antioxidant enzyme activity, and gene expression were studied in *E. coli* NM111 cells carrying the fused *katG::lacZ* genes.

Findings: The DPPH assay revealed significant radical scavenging activity (IC_{50}=19.97±0.51 μg/ml), comparable to the positive control catechin (IC_{50}=13.08±0.035 μg/ml). OVEE exhibited a high total phenolic (555.08±5.598 μg GAE/mg) and flavonoid content (31.39±1.16 μg QE/mg), correlating with its antioxidant activity. Lipid peroxidation, assessed via TBARS assay, was significantly inhibited by OVEE (20.715±2.47%). OVEE increased NO levels in *E. coli* cells, suggesting potential pro-oxidant activity influenced by phenolic compound structure, pH, and metal ion interactions. Enzymatic antioxidant defense was enhanced, with SOD and catalase activities increasing by approximately 40% and 35%, respectively, under the OVEE treatment. Additionally, OVEE induced the expression of the *katG::lacZ* gene fusion by 1.41-fold, with further induction observed under oxidative stress.

Conclusion: These findings highlight dual antioxidant-prooxidant nature of OVEE, emphasizing its potential antibacterial mechanisms and broader biological significance for therapeutic applications.

Abstract ID: 2534 / Poster Number: 245.15P

Influence of varying irradiation wavelengths on growth, colony morphology and formation of pigments of the basidiomycete fungus Pycnoporus cinnabarinus

Presenting author: Dr. Christoph Walter Schinagl, University of Innsbruck, Innsbruck, Austria

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The demand for fungal metabolites is constantly increasing in various industries, including food, cosmetic, and pharmaceutical. Besides conventional applications, light-activated natural substances, i.a., fungal photosensitizers, have become a focus of pharmaco-therapeutic research interest over the past decades.

Wood-decomposing fungi, which are generally easy to cultivate in pure culture, present a promising source for these novel natural pigments. Extracts from the fruitbodies of *P. cinnabarinus* have demonstrated a broad range of bioactivities, including notable photochemical activity.

The aim of this study was to investigate the effects of highly standardized irradiation conditions and cultivation techniques on three *P. cinnabarinus* strains in pure culture, focusing on (i) the content of cinnabarin, (ii) the biomass, and (iii) morphology. Newly established methods for the extraction and direct quantification of cinnabarin were applied.

- Red, green, blue, and UV-A irradiation (mean irradiance Ee = 1.5 ± 0.18 W m^{-2}) had – with reference to darkness – considerable effects on growth, biosynthesis of cinnabarin, and colony appearance of all three *P. cinnabarinus* strains tested.
- The determined cinnabarin content depended on the applied irradiation wavelength, allowing strain-specific thresholds to be defined. Irradiation with wavelengths below this strain-specific threshold corresponded to a lower cinnabarin content.
- Light microscopy revealed the orange–red pigment as incrusted extracellular plaque on hyphal walls.

This study demonstrated different light dependencies for the formation of pigment and biomass of *P. cinnabarinus* in pure culture. These findings suggest a careful consideration

of applied wavelength and irradiation levels could be highly rewarding for the biotechnological production of natural pigments.

Abstract ID: 1311 / Poster Number: 089.17P

Inhibiting Collagen-Binding Trimeric Autotransporter Adhesins from Clinically Relevant Pathogens

Presenting author: Ms. Emma Marinelli Johansen, University of Oslo, Oslo, Norway

Co-authors: *Dirk Linke, Athanasios Saragliadis*

Background: Antimicrobial resistance is a global health threat predicted to increase mortality, morbidity and economic loss [1]. The stagnation in development of new antimicrobials and the continued spread of resistance make new strategies critical. The antimicrobials currently used target cell viability and growth, creating strong selection pressure for resistance genes. Targeting virulence factors to inhibit infection rather than killing the pathogens is a proposed strategy [2]. Adhesins are virulence factors that allow for the attachment of bacteria to host cells and thus provide the initial steps in pathogenesis.

Objective: A previous screen has identified small-molecule inhibitors of the trimeric autotransporter adhesin (TAA) *Yersinia enterocolitica* Adhesin A (YadA) from binding to collagen [3]. This project aims to measure the efficacy of these inhibitors in preventing TAAs from clinically relevant pathogens, *Acinetobacter baumannii* and *Enterobacter cloacae*, from binding to the extracellular matrix component collagen.

Methods: The TAA head domains, responsible for the binding properties, were cloned, recombinantly expressed and purified. The head domains were used for plate based binding assays, as well as surface plasmon resonance (SPR) which was used to measure real time binding and inhibitory effect. Additionally, whole cell fluorescence-based assays with the full length TAAs were performed.

Findings: Data showing the effect of the small-molecule inhibitors on the TAAs ability to bind to collagen will be presented. Additionally, we will demonstrate how the inhibitors prevent the binding, as indicated by SPR. These results give important insights into the possibilities of anti-virulence factor drug therapies.

Abstract ID: 2046 / Poster Number: 033.17F

Inhibitory effect of Verapamil in treatment of mixed biofilm of Candida albicans and Staphylococcus aureus

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Co-authors: *Lucia Černáková, Helena Bujdáková*

Verapamil is calcium channel blocker used in treatment of cardiovascular diseases. However, some studies also suggest its antimicrobial potential. Concentration level of intracellular calcium is crucial for virulence of yeasts. Changes in abundance of calcium by some blockers can trigger decrease in expression of transcription factors responsible for expression of genes related to morphology, resistance and biofilm development of the yeasts. Hyphal growth in *C. albicans* is necessary for biofilm, especially in mixed biofilm with *S. aureus*. The aim of this research was to study antibiofilm activity of Verapamil in mixed biofilm of *C. albicans* SC5314 and *S. aureus* CCM3953. At first, minimal inhibitory concentration of Verapamil was set to single biofilms. Further, subinhibitory concentration of Verapamil (1mM) was tested on mixed biofilms. Reduction of biomass in mixed biofilm was in 20% and 30% for *C. albicans* and *S. aureus*, respectively. The results were supported by microcopy. Morphology of *C. albicans* was changed and decrease in *S. aureus* cells was observed as well. Quantitative real-time PCR was used to determine changes in the *HWP1* and *ALS3* gene expression in biofilms formed w/wo Verapamil. Results showed a decrease in expression of both genes (2x for *ALS3* and 3x for *HWP1*). *In vivo* experiments with *Galleria mellonella* confirmed antibiofilm activity of Verapamil to mixed infection of *C. albicans* and *S. aureus*. Survival of larvae increased in 30%. Our results suggest that Verapamil regulates morphology of *C. albicans* resulting with changes in composition of biofilm and adhesion of *S. aureus* in mixed biofilm.

Abstract ID: 781 / Poster Number: 312.15P

Innovations in bacterial solute transport: Discovery of a new class of binding-protein dependent secondary transporter

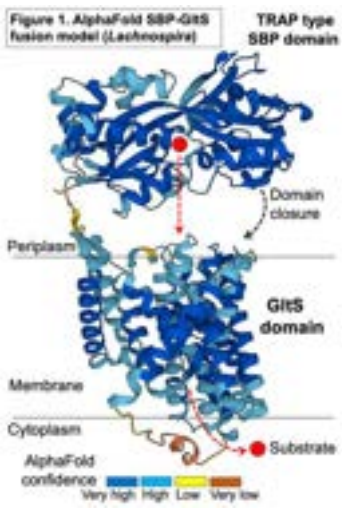
Presenting author: Prof. David Kelly, The University of Sheffield, Sheffield, United Kingdom

Co-authors: *Lily Jaques, Joseph Davies, Vanessa Leone, Christopher Mulligan*

Introduction: How do bacteria scavenge low concentrations of nutrients from their environment? This fundamental question in microbiology was, until quite recently, answered by the existence of the "ABC" (**A**TP-**B**inding **C**assette) transporters that use a high-affinity periplasmic Solute Binding-Protein (SBP) to capture their substrate. The discovery of "TRAP" (**TR**ipartite **A**TP-independent **P**eriplasmic) transporters [1] challenged this prevailing dogma; they also use SBPs for substrate binding, but these are unrelated to the ABC-SBPs. Moreover, solute uptake is driven by a transmembrane electrochemical ion-gradient (usually Na^+) and not by ATP hydrolysis. TRAP transporters are therefore SBP-dependent symporters, and they work by an "elevator" mechanism whereby a larger membrane embedded transport domain moves up and down against a smaller fixed scaffold domain [2]. We have now found that TRAP-type "orphan" SBPs are common in bacterial genomes, and we hypothesised that some of these might be associated with other types of transporters.

Objectives: BP0403 from the human pathogen *Bordetella pertussis* is an unusual SBP with an N-terminal globular domain with a unique fold, a long interdomain helical region and a C terminal domain with homology to a sub-family of TRAP SBPs. Surprisingly, it is encoded next to *gltS* – a known Na^+: glutamate symporter. Our objectives were to (i) characterise the full-length protein and its domains (ii) identify the solute that it binds (iii) clarify the nature of the association with GltS.

Methods and findings: His-tagged BP0403 and its N- and C-terminal sub domains were cloned, over-expressed in *E. coli* and purified by affinity chromatography. Molecular weight analysis using size exclusion chromatography revealed that the N- and C-terminal domains are monomers, but the full-length protein forms a stable dimer; the interdomain helical region is solely responsible for dimerization. Differential scanning fluorimetry and intrinsic tyrosine fluorescence titrations using a diverse ligand library showed that the C-terminal domain of BP0403 binds L-glutamate (but not glutamine) with high affinity (K_d = 180 nM). The genome context of *BP0403* reveals the complete absence of genes for TRAP membrane components but it is co-operonic and translationally coupled with *gltS*, encoding a Na^+: glutamate symporter. GltS is common in many bacteria but normally operates without interaction with an SBP. By extensive homology and gene context analyses across multiple genomes, we identified fused BP0403-GltS homologues, (e.g. from *Lachnospira*, **Fig. 1**). Overall, the data show that a subset of GltS proteins have "captured" a TRAP-type SBP during evolution.



Conclusions:

- Our results indicate the existence of an entirely novel type of binding-protein dependent secondary transporter and show that diverse transporters can employ SBPs beyond the well-known ABC and TRAP systems.
- An SBP-GltS partnership would offer a higher substrate affinity compared with GltS operating alone; an innovation allowing better scavenging in low nutrient environments.

GltS works, like TRAP transporters, as an elevator; perhaps some intrinsic feature(s) of the elevator mechanism is required for the associated SBP to bind/release its ligand during the transport cycle. The identification of further novel classes of SBP dependent secondary transporters will allow this proposal to be tested.

Abstract ID: 3517 / Poster Number: 394.15P

Innovations in Plant-Free Cultivation of Arbuscular Mycorrhizal Fungi (AMF) for Sustainable Agriculture

Presenting author: Ms. Neetu Neetu, Friedrich Schiller University, Jena, Germany; Erfurt University of Applied Sciences, Research Centre for Horticultural Crops, Erfurt, Germany

Co-authors: *Katja Burow, Philipp Franken*

This research focuses on the critical function of Arbuscular Mycorrhizal Fungi (AMF) in boosting plant nutrient uptake, which is essential for sustainable agricultural practices.

Conventional AMF propagation, relying on host plant symbiosis, restricts their accessibility and application in agriculture.Addressing this bottleneck, our research endeavors to pioneer a groundbreaking methodology for the plant-independent cultivation of AMF, facilitating the perennial availability of AMF inoculants. Utilizing Mycorrhizal Helper Bacteria (MHB) from the Plant Growth Promoting Rhizobacteria (PGPR) families Azotobacter and Azospirillum, isolated from grass rhizospheres and roots, we aim to facilitate AMF production without plants. These MHBs can form biofilms on AMF, protecting them from pathogens and improving their vitality. By evaluating biofilm-producing MHB strains for their efficacy in enhancing AMF spore production and introducing phytohormones and sugars as growth additives, this research aims to create conducive conditions for AMF spore development without host plants. Such advancements in plant-free AMF propagation could significantly impact sustainable agriculture, offering a scalable solution for producing AMF inoculants, reducing dependence on synthetic fertilizers, and promoting eco-friendly farming practices, ultimately aiding global food security enhancement.

Abstract ID: 2648 / Poster Number: 135.17P

Innovative Antimicrobial Strategies Targeting the Essential Transcriptional Regulator HP1043 in Helicobacter pylori

Presenting author: Assoc. Prof. Davide Roncarati, University of Bologna, Bologna, Italy

Co-authors: *Federico Antoniciello, Alireza Japoni-Nejad, Andrea Vannini, Elena Chiti, Federica Chiappori, Vincenzo Scarlato, Peter Nielsen,*

Introduction: *Helicobacter pylori* is a globally prevalent pathogen that colonizes the gastric niche, leading to several gastric diseases, including gastric cancer. The rapid emergence of antibiotic resistance in this bacterium has created an urgent need for alternative therapeutic strategies. Transcriptional regulators (TRs) represent promising targets for innovative antimicrobials. Among these, HP1043, an essential TR in *H. pylori*, plays a critical role in cellular processes and cannot be genetically deleted, making it a particularly attractive but challenging target.

Objective: This study aimed to identify compounds capable of inhibiting HP1043's DNA-binding activity and to explore the potential of peptide nucleic acids (PNAs) to knockdown HP1043 expression.

Method: High-throughput in silico screening, molecular dynamics simulations, and drug repositioning were employed to identify small molecules targeting HP1043. Molecular mechanics generalized Born surface area (MMGBSA) analysis further refined candidate selection. The activity of selected compounds was assessed in vitro via electrophoretic mobility shift assays. Additionally, PNAs were designed to target HP1043, utilizing bacterial penetrating peptides (BPPs) to enhance cellular uptake.

Finding: Approximately 14,350 molecules were screened, leading to the identification of seven candidates with promising binding affinity to HP1043. Of these, three compounds significantly reduced HP1043's DNA-binding activity in vitro. Moreover, PNAs effectively modulated HP1043 expression, demonstrating precise gene knockdown capabilities when delivered with BPPs.

Conclusion: This study introduces a dual approach to combating *H. pylori* by targeting the essential transcriptional regulator HP1043. The findings highlight the potential of combining small molecules and PNAs as innovative antimicrobial strategies against this pathogen.

Abstract ID: 165 / Poster Number: 056.17P

Innovative approaches to combat Pseudomonas aeruginosa resistance in cystic fibrosis

Presenting author: Ms. Océane Goncalves, University of Sherbrooke, Sherbrooke, Canada

Co-authors: *Jean-Philippe Côté*

People with cystic fibrosis (CF) are highly susceptible to chronic lung infections caused by *Pseudomonas aeruginosa*, a pathogen with exceptional antibiotic resistance and adaptability. Its ability to survive under hypoxic and nutrient-poor conditions typical of CF lungs complicates treatment, as conventional antimicrobial therapies often fail. Current approaches, such as minimum inhibitory concentration (MIC) testing, are insufficient for predicting *in vivo* drug efficacy due to the unique physiological environment in CF lungs. This underscores the urgent need for novel strategies targeting adaptive resistance mechanisms.

This research aims to identify resistance determinants critical to *P. aeruginosa* survival in CF-like conditions and uncover potential therapeutic targets to combat its antibiotic resistance.

Using high-throughput screening (HTS) of the comprehensive *P. aeruginosa* mutant library, we assessed *P. aeruginosa* grown in artificial sputum media (ASM), which replicates the CF lung environment. Subinhibitory antibiotic concentrations were employed to evaluate the genomic response and identify key resistance determinants.

Our approach revealed genetic determinants and pathways crucial for *P. aeruginosa* resistance under CF-mimicking conditions. The integration of genetic and physiological data provided a comprehensive understanding of how environmental factors influence antibiotic resistance.

This study highlights the potential of HTS combined with ASM to uncover actionable targets for tailored antimicrobial strategies. By focusing on the CF lung's unique environment, these findings pave the way for developing more effective treatments against chronic *P. aeruginosa* infections in CF patients.

Abstract ID: 2362

Innovative rhamnolipid-loaded bacterial cellulose membranes for enhanced anti-biofilm and antimicrobial wound care

Presenting author: Ms. Cátia Gil, 1 i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technol, Caparica, Portugal; UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA Schoo, Caparica, Portugal

Co-authors: *C.A.V Torres, M.A.M. Reis, Filomena Freitas*

Introduction: Chronic wounds remain a critical healthcare challenge, often exacerbated by persistent infections and biofilm formation. Recent estimates reports that chronic wounds affect over 200 million individuals globally, imposing a significant burden on healthcare systems [1]. Hence, there is an urgent need for innovative materials to effectively tackle this escalating health issue. Bacterial cellulose (BC) stands out for wound care due to its excellent biocompatibility, high water-holding capacity, and structural versatility. However, the large-scale industrial production of BC remains constrained by its limited yields and high manufacturing costs. Furthermore, its intrinsic antimicrobial activity is limited [2]. This can be overcome by incorporating microbially derived agents with antimicrobial activity into the matrices, ideally using natural compounds such as rhamnolipids (RL).

Objective: In this sense, this work aims to tackle the global challenge of chronic wounds while addressing the limitations of BC by enhancing its antimicrobial properties through the incorporation of RL. Renowned for their potent antimicrobial and anti-biofilm effects, RLs, when synergistically combined with BC, create a high-value material tailored to effectively prevent chronic wound infections. Furthermore, this study embraces sustainability by utilizing a commonly discarded food waste, such as banana peels, as feedstock for microbial BC production (Figure1).

Methods:



Figure 1: Illustrative process of the methods used for the preparation of RL-loaded BC.

Findings: The valorization of banana peel waste into BC enhanced BC production by an impressive 56% compared to the conventional HS medium, reducing environmental impact and improving cost-effectiveness for industrial applications. Remarkably, after undergoing a soaking process, the RL-loaded BC demonstrated antimicrobial inhibition zones ranging from 0.2 ± 0.12 cm to 0.5 ± 0.1 cm, effectively targeting *Staphylococcus aureus*, which is a prevalent pathogen presents in wound infections. Biofilm inhibition on the membranes surface and surrounding medium reached nearly 100% at a mono-RL concentration of 250 mg/L. Furthermore, mechanical strength surged over 400%, from 0.30 ± 0.14 MPa (native BC) to 1.51 ± 0.14 MPa (RL-loaded BC), ensuring superior durability and suitability for clinical applications.

Conclusions: This study demonstrated the successful development of a sustainable RL-loaded BC membrane. This innovative membrane delivers outstanding antimicrobial, anti-biofilm, and mechanical properties, positioning it as a promising solution for chronic wound care. Furthermore, this research paves the way for sustainable and high-impact medical innovations.

[1] doi: 10.1089/wound.2023.0150 ; [2] doi: 10.5772/intechopen.107021

Abstract ID: 2805 / Poster Number: 324.17P

Innovative Solvent Production Through Genetic Engineering and Microbial consortium approaches

Presenting author: Ms. Cyrielle Debeir, IFP Energies Nouvelles, Rueil-Malmaison, France ; Université Paris-Saclay ; Saclay, France

Co-authors: *François Wasels, Alexandre Delarouzée*

Introduction: With the recent awareness about the consequences of global warming, research for alternatives to fossil fuel resources and petrochemistry has received interest in the last decades. Thanks to their ability to convert lignocellulose-derived sugars into industrially relevant compounds like C2-C4 alcohols, solventogenic microorganisms such as *Clostridium acetobutylicum* are promising candidates for the bioproduction of fuels and platform molecules.

Objectives: While genetic engineering of those bacteria was still complex and time-consuming a few years ago, the development of efficient genetic tools now makes it possible to modify their metabolism. Another strategy to improve fermentation capacities relies on the use of cocultures. Such approaches can contribute to the improvement of carbon yield, the expansion of substrate and product repertoires or the maintenance of a synergistic environment.

Methods: In this work, an ethanol and acetate hyperproducing mutant of *Clostridium acetobutylicum* was constructed using a CRISPR-Cas9 based tool. A bottom-up approach was then implemented to set-up a semi-synthetic consortium comprising the mutant strain and an anaerobic microbial partner.

Results: Effective characterization of the novel *Clostridium acetobutylicum* mutant was undergone. The coculture demonstrated improved fermentation capacities.

Conclusion: A novel ethanol and acetate hyperproducing mutant of *Clostridium acetobutylicum* was constructed. Improved fermentation capacity was achieved thanks to a semi-synthetic consortium approach.

Abstract ID: 2664 / Poster Number: 424.17P

Inoculum of authoctonous biosurfactant-producing bacteria in pesticide contaminated Agri Biobed: evaluation of pesticide abatement and microbial community structure

Presenting author: Dr. Marco Andreolli, University of Verona, Verona, Italy

Co-authors: *Davide Righetti, Alberto Ferrarese, Andrea Zerminiani, Silvia Lampis*

The large use of pesticides in agriculture and their dangerous effects on human health and environment encourages to find new methodologies to control their contamination (Sharma et al., 2019). Biobed system was developed in Sweden in the 1990s as an efficient biological system to limit pesticide spread from a punctual area. Its core consists of a mixture of organic material (biomixture) with the function of either absorb pesticides or support the growth of microbial populations involved in pesticides degradation (Torstensson and Castillo, 1996). On the other hand, the low bioavability of organic compounds can limit their biodegradation. Biosurfactants are amphipathic molecules that can enhance the bioavailability of pesticides improving the bioremediation process (Schalchli et al., 2023).

The aims of this work were: isolate and characterize bacterial strains able to produce biosurfactans from a high polluted Biobed and evaluate the effects in terms of either pesticide abatement or microbial community structure after a bioaugmentation treatment by using these biosurfactant- producing bacteria as inocula.

The isolation was performed through enrichment cultures using three different pesticides (oxadiazon, propiconazol and tebuconazole) as sole source of carbon and energy. The biosurfactant production was tested through the emulsification activity test (%EA) and five strains were selected as inoculum: *Cupriavidus* sp. 1Tox and 1Nox, *Rhodanobacter* sp. 3Tteb and 4Tteb and *Rhodococcus* sp. 3Tox. The treatment was monitored after 38 and 83 days. PCR-DGGE showed the presence of all strains at the beginning of the experimentation, the strains 3Tox, 1Tox and 1Nox after 38 days and only the *Cupriavidus* strains after 83 days.

After 83 days, inoculated soil evidenced a total reduction of 93.1% of pesticides compared to the control, for instance: 97.6, 97.5, 99.5, 86.3, 89.6 and 89.3% abatement of chlorantraniliprole, flutolanil, fluxapyroxad, oxadiazon, propiconazole and tebuconazole respectively. Moreover, DNA metabarcoding analysis showed a shift in

microbial population in biosurfactant-inoculated soil towards Actinobacteria and Basidiomycota, which included many pesticides degraders microbial genera.

Thus, this work evidenced the importance of the biosurfactant-producing bacteria to improve pesticide bioavailability in soil during bioremediation treatments and how their bioaugmentation can influence the microbial community structure increasing the autochthonous microbes able to degrade pesticides. Eventually, the results suggest a new protocol to increase the Biobed efficiency in terms of pesticide abatement.

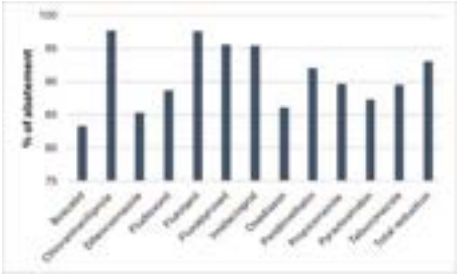


Figure 1: Percentage of pesticides abatement after 83 days of bioaugmentation treatment.

Abstract ID: 4101 / Poster Number: 431.15P

Insecticidal Activity of Methanol Extracts from Culture Filtrates of Azadirachta indica Endophytes

Presenting author: Mr. Rajdip Pavagadhi, Veer narmad south gujarat university, Surat, India

Co-authors: *Dhara Gamit*

In the search for novel potent endophytes derived bioactive compounds for bioinsecticide applications, crude methanol culture filtrate extracts of 19 *Azadirachta indica's* Endophytes were screened for their toxicity. Toxicity tests of all extracts against mealy bugs were performed. The extracts with the highest toxicity were further examined for insecticidal activity against alpha amylase inhibition activity. The results showed that the extracts of five isolates exhibited the highest toxicity to mealy bugs at 50% lethal concentration. These five isolates that obtained from *Azadirachta indica* were identified based on sequence data analysis of 16s rRNA. The extracts used were crude extracts, so their potential as sources of alpha amylase inhibition compounds makes them likely candidates as Gastrointestinal toxic. The GC-MS profiles of the five extracts differed, indicating variations in their chemical constituents. The chemical compounds that were identified were used for docking with the 1JAE alpha amylase of *yellow meal worm* and 6xyu AchE of *drosophila M*. This study highlights the potential of culture filtrate methanol extracts of endophytes as a source of new potential bioactive compounds for bioinsecticide applications.

Abstract ID: 2528 / Poster Number: 126.17P

Insight into Klebsiella species and phylogenomic landscape of K. pneumoniae strains carrying multidrug-resistant plasmid AB595

Presenting author: Assoc. Prof. Kgaugelo Lekota, North West University, Potchefstroom, South Africa

Klebsiella species are of significant public health concern due to their increasing antibiotic resistance. This study assessed the antimicrobial profiles of *Klebsiella* species isolated from water sources and those infecting sheep and goats. Whole genome sequencing was used to determine the antibiotic resistance genes (ARGs) of *Klebsiella* spp. from small stock and water. Comparative genomics was employed on the publicly available 84 genomes. Phenotypic AMR profiles showed that 94% and 91% of the isolates from animals and the environment were resistant to ampicillin. Of the 86 examined *K. pneumoniae* genomes, 70% consisted of the o*qxB* and *oqxA* genes that confer resistance to nalidixic acid. A novel multidrug-resistant plasmid AB595 of *K. pneumoniae* strains was determined that encodes aminoglycoside (*aph(6')-Ia*), trimethoprim (*dfrA-14*), sulphonamide (*sul2*), and tetracycline (*tetA*). The antibiotic resistance genes for beta-lactamases (bla_{LEN}/bla_{OX-1-3}), nalidixic acid (*oqxAB*), and fosfomycin (*fosA*) were found in the *K. varicolla* and *K. michiganensis* genomes. Moreover, the *K. michiganensis* strains additionally contained an aminoglycoside (*aph(3')-Ia*) gene. The sequenced *K. pneumoniae* genomes present novel sequence types with distinct genetic diversity across worldwide strains. Interestingly, the beta-lactamase-resistant gene $bla_{SHV-194}$ was only found in the sequenced K. pneumoniae strains and two strains from Switzerland and Finland *K. pneumoniae*'s multi-drug-resistant plasmid AB595 offer a baseline set of variations for upcoming resistance monitoring and epidemiological research.

Abstract ID: 4091

Insights into hydrogen-driven microbial communities in engineered deep subsurface environments

Presenting author: Mrs. Camille Rolland, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Co-authors: *Olivier Leupin, Rizlan Bernier-Latmani*

Introduction: Hydrogen gas (H_2) transformation by subsurface microbial communities has been investigated for decades, for example in the context of nuclear waste disposal. H_2 consumption enhances the safety of deep geological repositories by alleviating gas pressure buildup caused by H_2 generated from anoxic steel corrosion. Subsurface H_2-fueled communities have primarily been studied in aqueous environments, but quantitative gas-phase characterization under realistic conditions, i.e., unsaturated porous medium, high H_2 concentration, is lacking.

Objectives: Focusing on the context of the Swiss radioactive waste repository, this study aims to:

1. Quantify microbial H_2 consumption rates under realistic repository condition, using partially saturated porous medium, and field-derived porewater.
2. Deconvolute the underlying microbial processes and associated limitations.

Method: The experiment involves two gas-tight reactors filled with a porous medium composed of 80% sand and 20% bentonite clay, which is proposed for gallery backfill in the Swiss radioactive waste repository. The sand-bentonite mixture is partially saturated with porewater (70 or 90% water saturation), extracted from Opalinus Clay, which is the geological formation designated to host the Swiss repository. An overpressure of 4 bars was established with the amendment of H_2 (100%). We investigated the effect of water availability on microbial H_2 consumption by monitoring the gas pressure and composition (mass spectrometry) over 90 days. At the end of the experiment, sacrificial sampling was performed to measure pH and quantify sulfate, sulfide, acetate, and iron concentrations (aqueous and acid extracted). Solid phase iron and sulfur oxidation states were assessed using X-ray absorption spectroscopy and interpreted using linear combination fitting and deconvolution. The microbial community was characterized in a spatially resolved manner with qPCR (Bacterial and Archaeal 16S rRNA gene) and PacBio full-length 16S rRNA gene sequencing (SILVA database, Hellinger transformation and Bray-Curtis distance).

Findings: At 90% saturation, H_2 consumption was rapid (1 bar/day) and complete, and resulted from the simultaneous activity of sulfate-reducers and methanogens. H_2 consumption slowed down after 60 days, either due to an electron acceptor limitation or to alkaline (pH > 10) and high sulfide conditions (250 ppm) resulting from sulfate reduction. At the end of the experiment, the community was predominantly composed of *Geoalkalibacter* (40% rel. ab.) and *Desulfocurvibacter* (20% rel. ab.). At 70% saturation, H_2 consumption was only observed after 70 days (0.2 bar/day), but sacrificial sampling revealed significant sulfate reduction, suggesting that the growth rate may be a limiting factor to observe H_2 consumption in the experiment timescale. Limited water availability resulted in a higher spatial diversity (Wilcox test, $p < 0.001$), with *Desulfatitalea* (20% rel. ab.) and *Vulcanobacillus* (20% rel. ab.) dominating the locations where sulfate reduction was observed (Kruskal-Wallis test, $p < 0.001$).

Conclusion: Under realistic repository conditions, H_2 consumption was rapid but transient in the 90% water saturation condition and delayed but detectable at 70% saturation. By measuring the rate of H_2 consumption and delineating the conditions favorable for this process, this work provides key understanding to inform models of the evolution of the gas pressure in the Swiss deep geological repository.

Abstract ID: 2818 / Poster Number: 023.15P

Insights into the gut microbiome of Atlantic cod (Gadus morhua) as a tool for stock health assessment and ecotype discrimination in Icelandic waters

Presenting author: Elena Radaelli, PhD, University of Bologna, Bologna, Italy

Co-authors: *Giorgia Palladino, Daniel Scicchitano, Daniela Leuzzi, Haseeb Randhawa, Marco Candela*

Introduction: The North Atlantic and Arctic Oceans are characterized by historically intense overfishing and faster temperature changes than average. These stressors can impact on organisms by changing nutrients availability within trophic niches, migration routes, and host-microbiome relationships. Microbiomes can provide key information about health status and behaviour of vertebrates. Their profiling in fish is an emerging goal to monitor anthropogenic impacts on marine species in a changing environment. In this context, Atlantic cod has been a commercially and culturally relevant marine fish for many centuries, due to its role as economic resource in the Northern Europe and North America's eastern coast. For this reasons, Atlantic cod has experienced a consistent population decline during the 1990s.

Objective: This study aims to characterize the gut microbial communities of Atlantic cod to explore the potential gut microbiomes as indicator of health and behaviour in this species, as well as their utility in distinguishing between different cod ecotypes.

Methods: We used 16S rRNA amplicon sequencing to characterize the gut microbial communities of Atlantic cod, considering coastal and frontal individuals collected from a single reference area – Icelandic waters.

Findings and conclusions: Preliminary assessments of the gut microbiome have indicated variations between the coastal and frontal ecotypes of Atlantic cod, whose behavioural and regional variations can vary according to specific genes. However, previous studies relied on comparison across different geographical locations and methodologies. Our research provides a more controlled examination of these ecotypes, contributing valuable data for understanding the health and ecological roles of this species.

Abstract ID: 3208 / Poster Number: 275.15P

Insights into the mycobiome of Wild vs. Cultivated Salicornia ramosissima

Presenting author: Ms. Cristina Torcato, University of Aveiro, Aveiro, Portugal; Centre for Environmental and Marine Studies (CESAM), Aveiro, Portugal

Co-authors: *Rafael Tavares, Cátia Fidalgo, Artur Alves*

Salicornia ramosissima is a halophyte valued for its nutritional content and health benefits and is globally consumed as a cultivated crop and in its wild form. Halophytes harbour rich microbial communities, including underexplored endophytic fungi that may be beneficial for its growth and stress tolerance.

This study aimed to investigate and compare the diversity of endophytic fungal communities in S. ramosissima plants grown in two environments: farmed and wild.

We analysed the endophytic mycobiome associated with belowground (BG) and aboveground (AG) tissues of S. ramosissima (n=10) using Illumina MiSeq sequencing. Shannon's diversity and Richness were consistent across habitats and plant tissues, while Pielou's evenness was higher in BG tissues. PERMANOVA highlighted differences in community composition between plant tissues, and an interaction effect between tissue type and environments.

Using FUNGuild, taxa were assigned to 27 functional groups. Guilds were even across habitats. Despite not being possible to assign any function to most taxa, the higher abundance of taxa associated to animal and plant pathogenesis in AG tissues of cultivated plants was noteworthy.

Further differences between environments were explored using DESeq2. In BG tissues, 7 ASVs were enriched in cultivated S. ramosissima, while 8 were enriched in wild plants. For AG tissues, 13 ASVs were more abundant in cultivated plants, while 14 were more abundant in the wild variant.

This work unveils a complex endophytic mycobiome in S. ramosissima, its relationship with distinct habitats, and highlights the need to further explore their ecological roles.

Abstract ID: 1302 / Poster Number: 426.17S

Integrated biorefinery for the biosynthesis of carotenoids-derived products from the yeast Rhodosporidium toruloides from residual urban biomasses

Presenting author: Stefano Bertacchi, PhD, University of Milano Bicocca, Milano, Italy; National Biodiversity Future Center (NBFC), Palermo, Italy

Co-authors: *Francesca Sabatini, Veronica Termopoli, Heiko Lange, Paola Branduardi*

Microbial diversity is a plentiful repository for the development of biorefineries based on residual biomasses. In this work we focus on the use of the yeast *Rhodosporidium toruloides*, known for the ability to consume pentose and hexose sugars, and to withstand several inhibitory compounds that might arise from lignocellulosic biomasses. At the same time, it naturally produces valuable compounds (*e.g.* carotenoids); nevertheless, its potential is not yet fully exploited.

To explore biodiversity in terms of raw material we used the bark and wood of sessile oak and mulberry trees obtained from the maintenance of the Besozza Park (Pioltello, MI). Their lignocellulosic composition can provide both fermentable sugars (released by enzymatic hydrolysis) and lignin moiety, which can be valorised as biopolymer for the production of nanocapsules. The main goal of the project is to maximise the components of the initial biomasses to obtain microbial carotenoids embedded in lignin-derived nanocapsules.

The first step of the process involves the hydrolysis of the residual biomasses (mulberry and sessile oak wood and bark) to obtain growth media for the yeast, successfully releasing a sufficient amount of sugars. These data, combined with the growth kinetics of *R. toruloides*, demonstrate that they are suitable substrates for growth and for carotenoids production too, which were analysed and quantified by flow-injection analysis coupled with mass spectrometry (FIA-MS). The production of β-carotene and torulene is therefore evaluated during time. To fully utilize the waste biomass, adhering to the principles of bioeconomy and circular economy, the residual lignin from enzymatic hydrolysis is used to produce nanocapsules, which can host yeast-derived carotenoids as cargo for several applications.

This work provides insights on the valorization of lignocellulosic biomass in terms of both microbial based bioprocesses and material science applications, fostering the collaboration between different fields, as core principle of bioeconomy. This project is

funded by the National Biodiversity Future Center (NBFC), a EU funded project aiming to promote sustainable development.

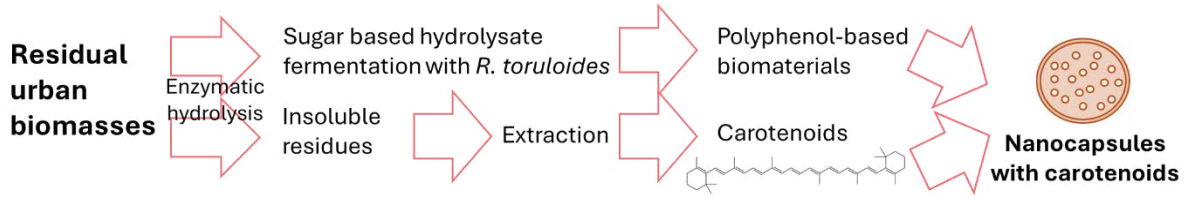


Figure 1: Scheme representing the integrated biorefinery to obtain lignin-derived nanocapsules loaded with carotenoids of microbial origin from residual urban biomasses

Abstract ID: 1893 / Poster Number: 047.15P

Integrated microbiological water quality assessment in recreational water

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Co-authors: *Lúcia Gomes, João R. Mesquita, Eva Amorim, Adriano A. Bordalo*

The number of reported outbreaks of bacterial pathogens linked to recreational bathing is on the rise in the Northern Hemisphere. The World Health Organization guidelines on bathing water quality identify naturally occurring microorganisms of possible concern, and acknowledge the need for research to understand the associated microbial ecology. However, the current European Bathing Water Directive (2006/7/EC) only takes into account fecal pollution. This study aimed to investigate fecal indicator bacteria (FIB), gastrointestinal viruses, and naturally thriving bacteria, combining the information to determine the robustness of the legal criteria to safeguard the health of beachgoers. Monthly water samples were collected from Atlantic beaches. FIB were examined by plate count following ISO guidelines. Gastrointestinal viruses, *Aeromonas* spp., and *Vibrio* spp. abundances were investigated by qPCR. The results showed that during the bathing season, FIB met the European legal criteria, posing acceptable to low risk. However, *Aeromonas* spp. and *Vibrio* spp. were successfully detected throughout the year, with higher abundances during the warmer months. Moreover, gastrointestinal viruses were detected during the bathing season. The results highlighted the public health risk associated with the presence of non-officially monitored indicators in bathing water. Therefore, the development of an integrated microbial risk assessment tool, taking into account new naturally occurring target microorganisms is pivotal to ensure the safety of users.

Abstract ID: 3415 / Poster Number: 004.16S

Integrating multi-omics approaches reveals the responses of Eisenia andrei gut exposed to polymetallic-polluted soils near the industrial zone Gabes, Southern Tunisia

Presenting author: Mr. Filippo Vaccari, Università Cattolica del Sacro Cuore, Piacenza, Italy

Co-authors: *Iteb Boughattas, Edoardo Puglisi, Mohamed Banni*

Phosphate production generates substantial environmental challenges, particularly due to the accumulation of phosphogypsum, a byproduct containing heavy metals and organic pollutants. These contaminants disrupt soil ecosystems, impair microbial activity, and pose risks to biodiversity and human health. Earthworms, such as *Eisenia andrei*, are sensitive bioindicators of soil health due to their ability to reflect environmental stress through changes in gut microbiota and metabolic response. The objectives were to: 1) analyze soil microbiota in contaminated and control sites, 2) evaluate shifts in earthworm gut microbial communities exposed to contaminated soils, 3) investigate metabolic changes in earthworm gut, and 4) integrate DNA sequencing and metabolomic datasets to assess ecological impacts. Soils were collected from sites near an industrial zone in Gabes, Tunisia. This area has been chosen for its varying levels of contamination. Controlled experiments exposed adult *Eisenia andrei* earthworms to these soils for 14 days. DNA from soil and earthworm gut samples was extracted and subjected to 16S rDNA sequencing to characterize bacterial and archaeal communities. Metabolomic profiling was performed using UHPLC-MS, identifying metabolites affected by heavy metal exposure. Data integration employed sPLS-DA via the DIABLO framework to correlate microbial taxa and metabolites across treatments. DNA sequencing analyses revealed shifts in gut microbial diversity, with Proteobacteria increasing and Clostridia decreasing in soils with higher contamination levels. Metabolic profiling identified disrupted pathways related to oxidative stress, amino acid biosynthesis, and energy metabolism. Integration of multi-omics data highlighted specific correlations between microbial taxa (e.g., *Vibrio sp.*, *Myroides phaeus*) and metabolites (e.g., histamine), demonstrating their role in mediating responses to contamination. The findings underscore that heavy metal exposure alters gut microbial and metabolic profiles, reflecting ecological risks and potential soil health deterioration. This study provides novel insights into the ecological impacts of heavy metal-contaminated soils, demonstrating that earthworm gut microbiota and metabolomics offer valuable biomarkers for environmental monitoring. The integration of metagenomic and

metabolomic approaches highlights complex interactions between microbial communities and metabolic responses, advancing our understanding of soil health under industrial pollution.

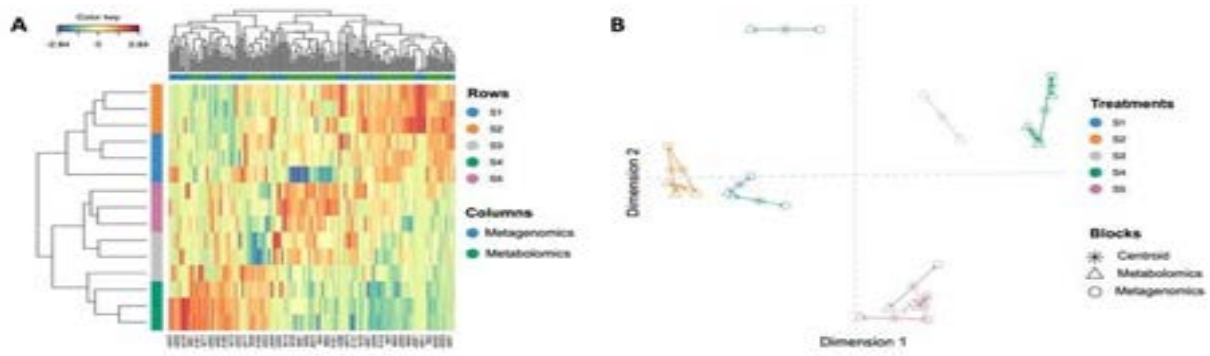


Figure 1: *Data Integration Analysis for Biomarker discovery using Latent variable approaches for Omics studies model of 16S amplicon sequencing and metabolomics block deriving from earthworm's gut samples exposed to 4 polymetallic contaminated soils: S1, S2, S3, and S4. The S5 site was the control soil without contamination. (A) heatmap of the selected features across the two components: samples are in rows, and the datasets are in columns. (B) arrow plot from the integrated model projected into the space. The start of the arrow indicates the centroid between the datasets, and the tip indicates the location of the same sample in each block.*

Abstract ID: 2397 / Poster Number: 357.15F

Integration of DSF quorum sensing with c-di-GMP and fatty acid metabolism in Stenotrophomonas maltophilia

Presenting author: Daniel Yero, PhD, Universitat Autònoma de Barcelona, Barcelona, Spain

Co-authors: *Marc Bravo, Celeste Gómez, Joan-Lluis Pons, Òscar Conchillo-Solé, Xavier Daura, Isidre Gibert,*

Introduction: *Stenotrophomonas maltophilia* is an environmental, multi-drug-resistant bacterium increasingly associated with nosocomial infections. A diffusible signal factor (DSF)-mediated quorum sensing (QS) system, encoded by the *rpf* gene cluster, coordinates bacterial behaviour, and pathogenesis.

Objective(s): Our goal was to elucidate the relationship between components of the DSF QS signalling network and downstream metabolic processes in *S. maltophilia*.

Methods: Wild-type and mutant strains underwent RNA-Seq transcriptomic analysis and phenotypic tests included biofilm formation, motility assays and fatty acid analysis by GC. DSF and c-di-GMP levels were measured via bioassay and immunoassay, respectively. EMSA detected protein–DNA interactions.

Results: We first shown that *S. maltophilia* K279a modulates the intracellular c-di-GMP levels due to the activation of the DSF QS system in response to cell density. The decrease in c-di-GMP triggered a transition from a sessile biofilm state to a motile state. Transcriptomic data revealed significant overlap of differentially expressed genes in mutants of the RpfC/RpfG pathway and the transcriptional regulator Clp. Clp unbound to c-di-GMP acts as a transcriptional activator controlling a wide array of physiological processes that includes genes involved in motility and cell adhesion and a σ54 sigma factor, which in turn controls the master regulator of the flagellar regulon. On the other hand, RpfB and a second σ54 sigma factor controls the DSF QS cascade regulating the fatty acid metabolism.

Conclusions: Our data revealed a complex regulatory cascade involving QS and the second messenger c-di-GMP that controls motility, adhesion, and lipid homeostasis in *S. maltophilia*.

Abstract ID: 3024 / Poster Number: 206.16P

Integrative Analysis of Gut Microbiota and Metabolic Pathways Reveals Key Microbial and Metabolomic Alterations in Diabetes

Presenting author: Ms. Nesma S. Shafie, Misr University for Science and Technology, Giza, Egypt

Co-authors: *2024 GP, Yasser Morsy, Marwa Amer*

Type 2 diabetes mellitus (T2DM) is increasingly recognized as a condition influenced by gut microbiota composition and associated metabolic pathways. This study aimed to investigate the differences in gut microbial diversity, composition, and metabolic profiles between diabetic and healthy individuals. A cohort of 20 individuals, comprising 10 diabetics and 10 healthy controls residing in Egypt, was analyzed. Stool samples were subjected to 16S rRNA gene sequencing using the Illumina MiSeq platform and metabolomic profiling via liquid chromatography-mass spectrometry (LC-MS). Diabetic individuals exhibited significantly greater microbial diversity and evenness, with distinct clustering patterns observed in Principal Coordinate Analysis. Taxonomic profiling revealed an increased relative abundance of *Bacteroidaceae* and *Lachnospiraceae* in the diabetic group, while *Streptococcaceae* was more prevalent in the control group. LEfSe analysis identified key microbial taxa such as *Bacteroides, Blautia*, and *Lachnospiraceae_FCS020_group* enriched in diabetic individuals, potentially linking these taxa to metabolic dysregulation. Metabolomic pathway analysis highlighted disruptions in fatty acid elongation, β-oxidation, glucose homeostasis, bile acid metabolism, and branched-chain amino acid (BCAA) biosynthesis in diabetics. These findings reveal substantial alterations in the gut microbiome's composition and function in individuals with T2DM, leading to disruptions in critical metabolic pathways. This study provides novel insights into microbial biomarkers and potential therapeutic targets for improving metabolic health in diabetic patients.

Abstract ID: 617 / Poster Number: 145.16P

Integrative Metagenomic and Metabolomic Analyses Unveil Host-Microbe Connections for the Gut-Lung Axis in Childhood Asthma

Presenting author: Dr. Shih-Chi Su, Chang Gung Memorial Hospital, Keelung, Taiwan

Introduction: Childhood asthma is associated with microbial dysbiosis in the gut that may impact lung immunity and inflammation, contributing to asthma pathology.

Objective: However, the precise mechanisms of host-microbiome interactions underlying this gut-lung axis in asthma are still elusive.

Method: To fill this gap, shotgun metagenomic analyses of throat swabs and fecal samples, together with metabolomic profiling of sera and feces were perfomed in a cohort of 31 children with asthma and 28 controls.

Findings: Herein, we found a significant positive correlation between asthma-associated airway Prevotella shahii and gut Firmicutes bacterium, as well as a significant negative correlation between airway Leptotrichia wadei and gut Clostridium sp. ($P < 0.05$). Analysis of host-microbe connection revealed that glycan biosynthesis and amino acid metabolisms encoded from both airway and gut microbiome were significantly correlated to fecal metabolomes ($q < 0.2$). Furthermore, levels of fecal methionine were found to have a significantly negative correlation with airway microbiome-derived peptidoglycan biosynthesis ($q < 0.1$), as levels of fecal butyrate and acetic acid were correlated with gut microbiome-derived glycan biosynthesis and with allergen-specific IgE levels ($q < 0.01$).

Conclusion: This study highlights a gut-lung axis in childhood asthma, correlating levels of fecal metabolites with airway and gut microbial functionality, particularly in glycan biosynthesis. Short-chain fatty acids derived from gut microbes, such as butyrate and acetic acid, are crucial in modulating allergic responses, suggesting a therapeutic potential in targeting gut microbiota and their metabolites.

Abstract ID: 2045 / Poster Number: 239.16F

Integron Integrase and Antibiotic Resistance Genes in Onsite Wastewater Treatment Systems

Presenting author: Mr. Gábor Györki, Ludovika University of Public Service, Baja, Hungary; National Laboratory for Water Science and Water Security, Baja, Hungary

Co-authors: *Balázs Kunkli, Gergely Maróti, János Kiss, Judit Knisz*

Onsite wastewater treatment systems provide alternative solutions to centralized wastewater treatment, and are increasing in popularity. However, wastewaters can contain a variety of micropollutants depending on the source, such as disinfectants, antibiotics and other pharmaceuticals in case of domestic wastewater. Continous exposure to these substances can cause microorganisms to develop and spread antibiotic resistance, posing the risk of introducing resistance to pathogenic bacteria. As only little is known about the treatment efficiency of onsite wastewater treatment, these systems can act as a source of antibiotic resistance and resistant bacteria. The importance of micropollutant and antibiotic resistance monitoring is increasing according to recent EU guidelines, and collecting data from onsite systems is also essential. One promising method of assessing antibiotic resistance is through analysing integron integrase genes responsible for spreading resistance. In the current study, we used quantitative PCR to test the presence and abundance of clinical class 1 integron integrase genes, and 9 antibiotic resistance genes commonly detected in wastewater. We also screened the samples for resistance genes using metagenome sequencing. The results were normalized using 16S rRNA gene abundances. We found a number of resistance genes with different abundances depending on the treatment step, and compared the individual resistance gene abundances with the integron integrase gene abundances. Although more analyses are needed to draw reliable conclusions, our results highlight the importance of antibiotic resistance monitoring in onsite wastewater treatment systems.

Abstract ID: 1291 / Poster Number: 012.15P

Interaction between the rhizosphere and endorhizosphere microbiome of ruderal plants and their role in remediation of heavily degraded and long-term oil-contaminated soils

Presenting author: Prof. Anna Galazka, Institute of Soil Science and Plant Cultivation – State Research Institute, Puławy, Poland

Co-authors: *Anna Marzec-Grządziel*

During long-term contamination and self-progressing natural bioremediation, the soil becomes richly populated by spontaneous ruderal vegetation. The rhizosphere of these plants is a unique habitat for various bacteria and fungi with high biotechnological potential. Both microorganisms and plants adapting to growth in contaminated conditions can develop a number of defense mechanisms. Relict vegetation is also characterized by great genetic, physiological and metabolomic diversity. Plants adapted to grow in a polluted area may have (or develop) unique characteristics. The main objective of the study is to explain the role of ruderal vegetation, its rhizosphere, autochthonous microorganisms in the processes of natural, spontaneous bioremediation of soils that have been polluted for many years. The specific objectives of the study include: explaining how relict plants activate and develop their adaptation and defense mechanisms; defining the role and explaining the adaptive mechanisms of microorganisms in the processes of long-term natural bioremediation. Both soils and plants were collected from under crude oil extractors in the historic Oil Mine in Węglówka. These areas have been heavily polluted and degraded (over 100 years of pollution). Nevertheless, from the closure of the mine to the present, crude oil has continued to flow spontaneously from oil wells. The constant flow of crude oil causes permanent pollution of the area while spontaneous, natural remediation is progressing. Soil samples were collected from selected 9 oldest oil wells. 5 species of ruderal plants were selected for research. DNA was extracted directly from the soil and rhizosphere. Isolation and characterization of bacterial and fungal strains isolated from the rhizosphere and endorizosphere of ruderal plants were performed. The strains were assessed on the basis of morphological, biochemical and genetic tests. The following analyses were determined: functional diversity using the Biolog system, next generation sequencing (NGS) of variable regions (16S rRNA for bacteria and ITS for fungi) and viral NGS (Shotgun sequencing). In addition, the chemical parameters of plant and soil samples (Corg, Nmin, Σ16 PAHs and trace elements) were determined. In the plants the

following analyses were assessed: biological activity of selected secondary metabolites, determination of the metabolomic profile.

Abstract ID: 1050 / Poster Number: 192.15P

Interaction of fungi and plant roots drives carbon stabilization in the boreal forests

Presenting author: Anne Tyvijärvi, PhD, Natural Resources Institute Finland, Helsinki, Finland

Co-authors: *Bartosz Adamczyk, Sannakajsa Velmala, Taina Pennanen, Priscila Thiago Dobbler, Pauliina Schiestl-Aalto, Petr Baldrian*

Boreal forests are crucial carbon sinks, storing about one-third of the world's soil carbon stocks [1]. However, climate change and global warming threaten to convert these soils from carbon sinks to sources due to accelerated decomposition of soil organic matter. Despite extensive research on soil carbon dynamics over the past decade, a comprehensive understanding of soil carbon stabilization mechanisms remains elusive [2], [3], [4], [5].

Boreal forest vegetation is often dominated by evergreen and deciduous species, such as Scots pine and Norway spruce, which host diverse fungal communities. These plants also produce secondary metabolites, particularly condensed tannins, that can stabilize soil organic matter by forming complexes with fungal necromass. To investigate how these tannin-fungal necromass complexes influence soil carbon stabilization, we conducted a field experiment in a Finnish pine forest using mesh bags filled with site-collected soil and additions of fungal necromass alone, tannins alone, or both together. We used two mesh sizes: one allowing fungal in-growth but preventing plant root intrusion, and another permitting both plant roots and fungi. After burying the samples in the soil for one growing season, we analyzed fungal community composition through metagenomic sequencing and measured concentrations of ergosterol, chitin, tannins, and soil stable C. We found that root presence correlated with increased fungal biomass and higher concentrations of tannins and stable C. Our results highlight the critical role of plant roots and the importance of tannin-fungal interactions in soil C stability.

Abstract ID: 3316 / Poster Number: 445.15P

Interactions of phages with new microbial lineages in Tunisian tannery wastewater

Presenting author: Mr. Juan Valero Tebar, I2SysBio, Valencia, Spain

Co-authors: *Mária Dzunkova, Vicente Arnau LLombart, Inés Alberola Mora, Rokaia Lejri*

Tannery wastewater pollution is an environmental concern due to high toxicity of chromium used in leather softening. These extreme conditions specifically enrich for microbes resistant to heavy metals, which may aid in bioremediation of polluted surrounding areas. Novel bioremediation pathways might be harboured in genomes of yet uncultured microbes, accessible by culture-independent methods only. Many of these pathways may be transferred across the community as auxiliary metabolic genes, which are generally stimulated by stress, but remain unexplored in industrial extreme conditions. In this study, we sequenced metagenomes and single-cells from four basins of a Tunisian tannery at different stages of the wastewater treatment process. Contigs were assembled using metaSPAdes, and reads were mapped to these contigs with Bbmap. Binning was performed using MetaBAT2, Maxbin2, and MetaCoAG, while DAStool was used for dereplication. The resulting metagenome-assembled genomes (MAGs) and single-amplified genomes (SAGs) were filtered using CheckM2 for quality and contamination, and taxonomic classification was done with GTDB-Tk. The presence of prophages in MAGs and SAGs, free virions in unbinned metagenomic contigs, and active phage infections in SAGs were analyzed using VirSorter2, geNomad, VIBRANT, and DeepVirFinder, with results filtered using CheckV. In total, 140 MAGs and 30 SAGs were obtained, highlighting a diverse array of unidentified taza, including a new bacterial phylum, showing differences in microbial composition across basins. These genomes exhibit metabolic pathways for degrading harmful compounds like chromium, and may highlight the key role in bacterial survival and adaptation in this environment, with future bioremediation implications.

Abstract ID: 119 / Poster Number: 141.16P

Interest of the syndromic approach in the diagnosis of respiratory infection during the period of the COVID-19 pandemic

Presenting author: Prof. Lahcène Benmahdi, Military Hospital, Oran, Algeria

Co-authors: *Seid Lachibi, Mustapha Hanba, Mohamed Anes Lazzaz, Adel Fouathia*

Respiratory infections constitute a major public health problem, characterized by significant morbidity and mortality. Many infectious agents are responsible for these infections, and etiological diagnosis is a significant challenge for optimal patient care.

Objectives: Demonstrate the interest of the syndromic approach by multiplex PCR in the diagnosis of respiratory infections and to describe their epidemiological profile.

Material and Method: This is a prospective recruitment descriptive study, conducted in patients with suspected respiratory infections for 13 months, from March 1, 2021 to March 23, 2022, using Biofire FilmArray RP 2.1 Plus respiratory panel kits.

Results: This study was based on a sample of 91 nasopharyngeal swabs. Among the analyzed samples, 63.7% were positive, with 100% viral etiology. However, no bacteria were detected. The most frequently detected viruses were SARS-CoV-2 (55.17%), followed by Rhinovirus (32.76%). The frequency of co-detection was estimated at 12.07%, most often involving SARS-CoV-2 and Rhinovirus. The people diagnosed positive were from the age group > 80 years (100%), followed by those between 21 and 40 years (72%). The female gender (70% of positives) was more concerned than the male gender (58.8%). The most frequently reported clinical symptoms were cough (45.1%), followed by fever and asthenia with a frequency of 41.8% and 42.5%, respectively. Headache and dyspnea were reported at 20.65% and 14%, respectively.

Findings: The COVID-19 pandemic has not affected the spread of the rhinovirus Confirmation of viral and/or bacterial etiology in a fairly short time could have a positive impact on patient management.

Abstract ID: 1503 / Poster Number: 079.15P

Intracellular Klebsiella pneumoniae Promotes Lung Cancer Cell Survival and Migration

Presenting author: Mr. Tianxiang Yi, The Chinese University of Hong Kong, Hong Kong, Hong Kong

Co-authors: *Sharon Shui Yee Leung*

Introduction: Lung cancer patients are more vulnerable to infectious complications due to their compromised immune system after surgery and/or chemotherapy. Various microorganisms have been identified in multiple tumour tissues, and are closely associated with tumour initiation, progression, and therapy effects. *Klebsiella pneumoniae*, a gram-negative bacterium related to pneumonia, has been found to be one of the most common intratumoral bacterial strains in lung tumour tissues. However, there has been a lack of studies on the interactions between lung cancer cells and intracellular *K. pneumoniae*.

Objectives: To illustrate bacterial invasion into lung cancer cells, and the impacts of intracellular *K. pneumoniae* on lung cancer cell A549-Luc, including the cell viability, survival, and migration.

Method: Firefly luciferase expressing-non-small-cell lung cancer (NSCLC) cells were seeded and infected with indicated bacteria, washed with PBS, treated with 300 μg/mL gentamicin to kill extracellular bacteria, and washed with PBS again, followed by further incubation for 72 h. Cell viability was determined with luciferase assay, and intracellular bacterial amount was determined by plate counting. *K. pneumoniae* invasion into A549-Luc was visualized by fluorescent microscopy. Gene expression changes were assessed with RNA sequencing and qRTPCR. Cell migration was evaluated with a wound healing assay.

Findings: All studied bacteria could enter A549-Luc. While *E. coli* and *S. aureus* decreased the cell viability, other bacteria had no significant impacts. The plate count results indicated *K. pneumoniae* having the largest intracellular amount. This bacterium could also be harboured and coexisted with HCC827-Luc and NCI-H1299-Luc cells (Figure 1A and 1B). *K. pneumoniae* infection did not affect A549-Luc cell colony formation, but the RNA-sequencing results showed that it up-regulated pro-survival genes BCL2A1 and BIRC3, and down-regulated pro-apoptosis genes TNFSF10 and TNFSF15. The corresponding qRTPCR assessment validates the findings, revealing the increased expression of BCL2A1 and BIRC3, and the downregulation of TNFSF10, BAX,

BBC3, CASP9, CASP3, and CASP7. The RNA-sequencing results also displayed that the expressions of pro-migration genes EFNA1 and VEGFA were elevated, while the anti-migration gene RND1 was down-regulated. Wound healing assay complied with gene expression changes, in which A549-Luc cells had stronger migration abilities after being cocultured with *K. pneumoniae*.

Conclusion: Multiple common pathogenic bacteria can be harboured and coexisted within lung cancer cells, and did not affect their viability of cancer cells. *K. pneumoniae* promoted cell survival and migration in NSCLC cells, potentially making the cancer treatment more difficult.

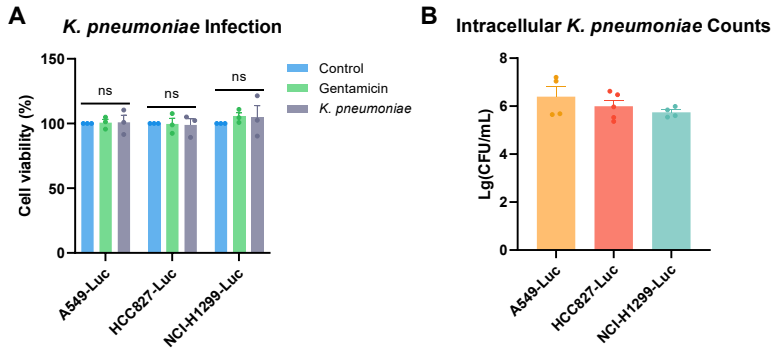


Figure 1: Intracellular *K. pneumoniae* dose not affect NSCLC cell viability.

Abstract ID: 3633

Intra-species genome mining of the bacterium *Burkholderia gladioli* reveals mechanisms of megasynth(et)ase evolution

Presenting author: Dr. Alex Mullins, University of Warwick, Coventry, United Kingdom

Co-authors: *Jinlian Zhao, Ioanna Nakou, Eshwar Mahenthiralingam, Lona Alkhalaf, Matthew Jenner, Greg Challis*

Introduction and Objectives: Microbes are an important source of natural products with antibacterial, antifungal, and anticancer properties in the pharmaceutical and agricultural industries. Two important classes of structurally diverse natural products are synthesised by multimodular megaenzymes called non-ribosomal peptides synthetases (NRPS) and polyketides synthases (PKS) that are encoded by biosynthetic gene clusters (BGCs). The rational design of these BGCs would enable the production of tailored compounds with improved efficacy and the ability to circumvent drug resistance/tolerance. However, previous efforts to manipulate these BGCs often yields dysfunctional systems with low or no yield of the desired product. We hypothesise that successful mechanisms of BGC genetic engineering can be found in Nature. By exploiting genomic data, we aim to find evidence of natural recombination events that occur within NRPS and PKS BGCs. Understanding evolutionary mechanisms will support future genetic engineering of bacterial pathways to enable rational design of bioactive compound derivatives.

Methods: *Burkholderia gladioli* is a well-known producer of multiple of bioactive metabolites. We investigated the evolutionary mechanisms of natural variation in BGCs through intra-species genome mining with Illumina-read mapping. Coverage analyses identified partial gene deletions and their respective splice sites. These strains were subsequently cultured on induction growth media, the natural compound variants confirmed by LC-MS, and their structure elucidated by NMR.

Findings: Recombination events were found in five of the twelve BGCs analysed and included both NRPS and PKS classes. Many of these variant BGCs were functional and resulted in the production of a derivative natural product with titres comparable to the original full-length version of the BGC. A notable example was the anti-cancer polyketide gladiostatin with the loss of two methylene groups from the variant skeletal backbone (Figure 1). This deletion was achieved through three distinct recombination events that evolved in independent lineages. Unexpectedly, one of the these splice sites occurred within a ketoreductase (KR) catalytic domain – a previously unobserved event. To

demonstrate the efficacy of this unprecedented intra-KR domain splice site, we genetically engineered a gladiostatin wild-type producer using the intra-domain recombination to reconstitute the observed variant. This artificially-produced variant retained functionality and synthesised the expected derivative product.

Conclusion: This study demonstrated that large-scale, intra-species genome mining can reveal naturally occurring BGC recombination events that occur via unexpected, and seemingly illogical, splice sites. By characterising and replicating these events *in vivo*, we have illustrated the viability of evolutionary-guided mechanisms as a means to successfully engineer BGCs and produce high yields of natural product derivatives. This research provides a platform for future biosynthesis of novel compound derivatives with improved potency for use in the pharmaceutical and agricultural industries.

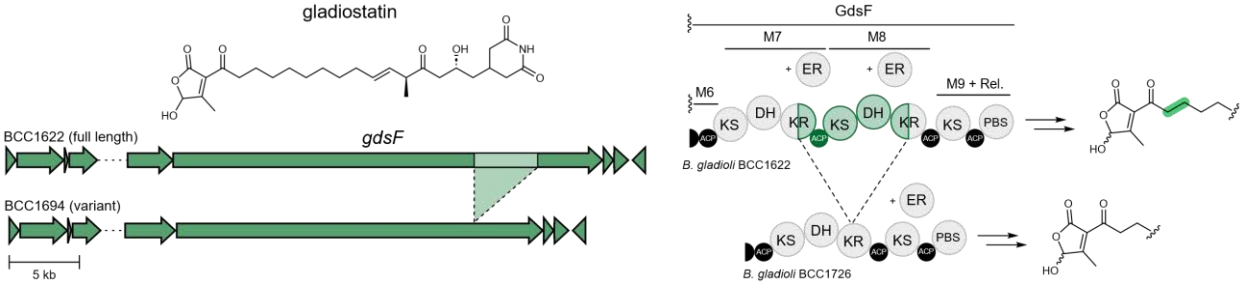


Figure 1: *Structure and BGC of gladiostatin from B. gladioli*. Catalytic domain level architecture of encoded protein showing intra-KR domain splice site and impact on compound structure.

Abstract ID: 2850 / Poster Number: 383.17P

Investigating black soldier fly (Hermetia illucens) larvae gut microbiome shift when reared on high-fiber industrial side streams

Presenting author: Ms. Friscasari Febriani Gurusinga, Fraunhofer Institute for Molecular Biology and Applied Ecology – Bioresources, Giessen, Germany; BMBF Junior Research Group in Bioeconomy (BioKreativ) "SymBioÖkonomie", Giessen, Germany

Co-authors: *Daniel Kreft, Till Röthig, Sabine Hurka, Andreas Vilcinskas, Dorothee Tegtmeier*

Introduction: The gut microbiome of an organism plays a role in facilitating metabolic process such as digestion, while also influencing an organism's overall health and behaviour. Black soldier fly larvae (BSFL) are known as voracious insects capable of breaking down a wide variety of organic materials. Moreover, BSFL have a promising role as aquaculture and livestock feed due to their high protein content. In Germany, agricultural and industrial side streams are available in substantial quantity and often underutilized, representing a valuable opportunity for sustainable waste management within a circular economy framework using BSFL. The effects of different side streams on the BSFL gut microbiome have not been extensively explored and studies on this should contribute to a better understanding of their metabolic adaptability.

Objectives: In our previous research, we demonstrated that the BSFL could successfully be reared on different industrial side streams. From these findings, our current study focuses on analysing the microbial community composition within BSFL gut and predicting microbial functions, particularly their role in nutrient metabolism.

Methods: We investigated the chemical and microbial composition of four different industrial side streams: apple pomace (AP), industrial potato pulp (PPI), lab scale potato pulp (PPL), rapeseed cake (RC), as well as control diet consisting of chicken feed (CF) and the growth performance of BSFL reared on the respective diets.

To analyse the differences in the gut microbial community, we used amplicon sequencing, subsequently microbial function predictions.

Findings: The nutritional composition of the side streams led to varying growth time for the BSFL, 25 days (RC) to 87 days (PPI). The final larval weight in AP was 87% lower in

comparison to the control group (CF). The high crude fiber content and low protein content of AP and PPI might contribute to the poor growth performance of BSFL.

Additionally, amplicon data revealed pronounced differences in the gut microbiota depending on the rearing feed, with slight enrichment of predicted pectin-degrading bacteria in BSFL reared on AP and PPI, whereas predicted nitrogen-fixing bacteria were found in BSFL reared on PPI and PPL.

Conclusion: BSFL have shown adaptability to different side streams, successfully reaching the prepupal stage despite the varied nutritional content of the side streams. Furthermore, the side streams influenced the gut microbial profile, which plays a role in enabling the larvae to complete their life-cycle. This highlights an opportunity to further explore the gut microbial functions and their potential use as probiotics. These findings are expected to contribute to a better feed conversion of various side streams by BSFL.

Abstract ID: 2212 / Poster Number: 118.17P

Investigating degenerate PCR to unlock the potential of uncharacterized biosynthetic gene clusters for antibiotic discovery

Presenting author: Mr. Dries Wéry, KU Leuven, Leuven, Belgium; VIB, Leuven, Belgium

Co-authors: *Sandrien De Smedt, Philip Ruelens, Maarten Fauvart, Jan Michiels*

Antimicrobial resistance poses a major challenge to modern medicine, urging the development of new antibiotics. Metagenomic studies have uncovered a substantial potential in uncharacterized biosynthetic gene clusters (BGCs). Amongst these, ribosomally synthesized and post-translationally modified peptides (RiPPs) are considered underexplored given their structural and functional diversity and malleability. The RiPP microcin B17 (MccB17), for example, has an uncharted mode of action, which is of interest for the design of novel bacterial gyrase poisons. Unfortunately, uncharacterized BGCs are often silent or encoded by unculturable microorganisms and hence not accessible through expression- and culture-based approaches. We propose to investigate degenerate PCR as a sensitive, flexible and scalable alternative to identify and, in a later stage, enrich promising BGCs with known sequence homologs from the metagenome.

As a proof of concept, we designed degenerate primer pairs to amplify sequences conserved amongst eight non-identical, uncharacterized *Pseudomonas* MccB17 BGCs. We tested these primer pairs for their ability to generate amplicons from DNA samples differing in template complexity, and to produce a sufficiently intense signal during qPCR. We found a degenerate primer pair that satisfies both requirements. Ongoing work focuses on primer optimization to maintain a clear signal during qPCR when using more complex DNA samples. This will be key in the development of a platform for high-throughput identification of interesting uncharacterized BGCs from metagenomic libraries. In conclusion, this research is an important first step in exemplifying the value of degenerate PCR to harness uncharacterized BGCs against the intensifying antimicrobial resistance crisis.

Abstract ID: 2062 / Poster Number: 225.15P

Investigating Genetic Expression Changes in Helicobacter pylori: A Novel Approach Using 4f-SAMMY-seq for Chromatin Analysis

Presenting author: Ms. Elena Chiti, Alma Mater Studiorum – Università di Bologna, Bologna, Italy

Co-authors: *Ugo Maria Iannacchero, Valentina Rosti, Andrea Vannini, Eva Pinatel, Chiara Lanzuolo, Davide Roncarati*

Introduction: *Helicobacter pylori* is a primary human pathogen responsible for gastritis, peptic ulcers, and gastric cancer, with infections often persisting lifelong if untreated. Throughout its prolonged lifecycle, this bacterium encounters different growth stages, environmental stresses, and dynamic interactions with the host. To adapt, *H. pylori* modulates metabolism, growth, membrane composition, and the expression of virulence factors, antimicrobial molecules, signalling compounds, and OMVs. These physiological adaptations are intricately linked to modifications in the transcriptional program, and alterations in chromatin structure and compaction are proposed to contribute to orchestrate these regulatory processes**.**

Objective: In prokaryotes, nucleoid organization, genome integrity, and cell cycle regulation are predominantly governed by nucleoid-associated proteins (NAPs) and DNA methylation. This study aims to elucidate the molecular mechanisms driving chromatin remodelling during *H. pylori* growth, pathogenesis and biofilm formation through the innovative application of the 4-fraction Sequential Analysis of MacroMolecules accessibilitY (4f-SAMMY-seq) protocol.

Method: 4f-SAMMY allows the isolation and sequencing of chromatin fractions with varying DNA accessibility, thereby identifying regions with differential NAP complexation and chromatin compaction. These data are then integrated with transcriptomic analyses, to determine correlations between chromosome accessibility and gene expression.

Findings: Application of 4f-SAMMY-seq across various growth phases of *H. pylori* yielded distinct DNA fractions. Preliminary results indicate a notable increase in genome compaction at specific loci during different growth phases.

Conclusion: The novel application of 4f-SAMMY-seq, traditionally employed in eukaryotic systems, offers new insights into bacterial nucleoid architecture and the genomic adaptations that underpin *H. pylori*'s survival and pathogenicity.

Abstract ID: 2634 / Poster Number: 142.15P

Investigating mehcanisms of stress responses and resilience under high ammnonia based piggery wastewater conditions

Presenting author: Ms. Eunbin Joa, Jeju National University, Jeju, South Korea

The growing global livestock industry is causing environmental problems due to wastewater, while securing sustainable energy resources is becoming an important challenge. This study aimed to efficiently treat Piggery wastewater and biosynthesize high value-added biodiesel raw materials using Chlamydomonas sp. Piggery wastewater culture conditions were optimized based on the intracellular N:P ratio of Chlamydomonas. The removal efficiency of ammonia, total phosphorus(TP), and total organic carbon(TOC), neutral lipid content under ammonia stress, and fatty acid composition were analyzed. Gene expression changes in Chlamydomonas were confirmed through transcriptome analysis of the mechanisms of lipid synthesis and high-concentrated ammonia resilience, identifying algal potential to contribute to sustainable alternative energy resource development. This study showed that ammonia removal from piggery wastewater was improved compared to regular medium (BG11-ammonia nitrogen). In addition, it was confirmed that the Chlamydomonas lipid content increased as the ammonia concentration in piggery wastewater increased. Fatty acid composition analysis showed increased ratio of C16:0 and C18:1 suitable for biodiesel. This study demonstrated that Chlamydomonas-based pig wastewater treatment can overcome the limitations of existing treatment technologies and reduce environmental pollution while producing high-quality biodiesel. We present a new understanding of the lipid synthesis mechanism of Chlamydomonas through transcriptome analysis, identifying its potential to contribute to sustainable alternative energy resource development.

Keywords: Piggery wastewater; microalgae; *Chlamydomonas*; intracellular N:P ratio; biodiesel

Abstract ID: 1075 / Poster Number: 046.16P

Investigating the effect of immunosuppression on IgA-targeting of gut microbiota in pediatric solid organ transplant recipients

Presenting author: Ms. Kora Schulze, Hannover Medical School, Hannover, Germany

Co-authors: *Marius Vital, Imeke Goldschmidt, Anette Melk, Martin Böhne, Sabrina Woltemate, Svea Kleiner, Elena Lehmann, Miriam Kramer*

Introduction: Solid organ transplantation (SOT) and subsequent immunosuppression are associated with dysbiosis, however, underlying mechanisms remain understudied. One major factor by which the host immune system interacts with gut microbiota is via secretory Immunglobulin A (sIgA). The objective of this study was to investigate, whether immunosuppression after SOT alters IgA secretion and targeting of gut microbiota.

Methods: Samples were collected from 48 pediatric kidney, liver or heart transplant recipients treated with two main immunosuppressive regimens based on tacrolimus vs. cyclosporin A, and compared to an age-matched healthy cohort (HC; n=16). All fecal samples were subjected to quantitative (based on flow cytometric cell counts) metagenomic analysis and sorted based on IgA-targeting using an optimized protocol; sorted fractions were then analysed by 16S rRNA gene sequencing. Additionally, free and bacteria-bound IgA concentrations were measured using a commercially available ELISA.

Results: Patients showed a reduced diversity and compositional differences compared to HC ($p=0.0438$), especially those receiving heart or kidney transplants that largely clustered separately. Overall a negative correlation between diversity and the percentage of IgA-targeted bacteria was found ($r=-0.4435$, $p=0.0004$). Tacrolimus administration was associated with a lower diversity ($p=0.0241$), lower cell counts and higher IgA-targeting ($p=0.0060$) along with shifts in abundances of specific IgA-targeted taxa.

Conclusion: SOT patients showed long-term dysbiosis of gut microbiota. Immunosuppression was associated with an increased IgA-targeting of gut microbiota, which was accompanied by distinct targeting patterns that might contribute to the observed community alterations.

Abstract ID: 238

Investigating The Efficacy Of A Moving Bed Biofilm Reactor For The Removal Of The Antiretrovirals Tenofovir, Emtricitabine, Nevirapine, Ritonavir And Efavirenz From Synthetic Wastewater

Presenting author: Tara Walmsley, PhD, Vaal University of Technology, Vanderbijlpark, South Africa

Co-authors: *Herman Mokgope, Michael Klink*

Introduction: South Africa utilises more antiretroviral (ARV) compounds per capita than any other nation in the fight against Human Immune Deficiency Virus (HIV) or acquired immunodeficiency syndrome (AIDS). Due to the limited efficiency of conventional biological treatment (activated sludge), ARVs have previously been detected in South African wastewater treatment plant effluents and surface waters. This poses a threat to aquatic environments due to the toxicity of ARVs and can be a potential contributor to ARV resistance due to persistent low level ARV exposure in the general population.

Objectives:

1. To construct, operate and optimise the performance of a laboratory scale moving bed biofilm reactor (MBBR).
2. To investigate the efficacy of the MBBR in the removal of tenofovir, emtricitabine, efavirenz, ritonavir and nevirapine from spiked synthetic wastewater using LC-MS/MS.
3. To assess the shift in the microbial biofilm consortium after the introduction of ARV compounds in the MBBR system.

Method: The capacity of ARV degradation by the MBBR was investigated by spiking synthetic wastewater influent with 10 µg/L of five ARV compounds. Actual removal during treatment was assessed by sampling the inlets and outlets of the reactor. A targeted solid phase extraction method with Ultra High Pressure Liquid Chromatography coupled to quadrupole time of flight mass spectrometry (LC-MS/MS) was used to quantify the five ARV compounds. Microbial diversity (alpha-diversity) of seeded sludge from a full-scale municipal WWTP and biofilm samples from a laboratory scale MBBR system during pre- and post-introduction of ARV compounds was investigated by Illumina sequencing of the 16S rRNA gene.

Findings: After MBBR treatment; Nevirapine, Tenofovir, Efavirenz, Ritonavir and Emtricitabine all showed marked reduction in concentration between the influent and effluent of the MBBR. On average, the percentage removal for Nevirapine, Tenofovir, Efavirenz, Ritonavir and Emtricitabine was 62.31%, 74.18%, 93.62%, 94.18% and 94.87% respectively. Microbial diversity results demonstrated that the introduction of biofilm carriers and antiretroviral drugs affects the bacterial community composition and diversity considerably. The biofilm carriers played a significant role in retaining essential slow-growing organisms, since in the sludge sample Nitrosomonas, Nitrospira and Alicycliphilus showed a lower abundance than in the biofilm samples. Further enrichment of Nitrosomonas, Nitrospira and Alicycliphilus post introduction of ARV compounds in the MBBR suggest the potential of these organisms to degrade the ARV compounds.

Conclusion: The MBBR system investigated in this study showed significant ARV removal capacity, with up to 94.87% removal for Emtricitabine. The shift in microbial diversity to increased abundance of Nitrosomonas, Nitrospira and Alicycliphilus from the activated sludge to the biofilm was noteworthy. Further enrichment of these genera were seen post introduction of ARVs, outlining their potential role in ARV degradation. This study highlights the importance of diverse microbial communities in the degradation of recalcitrant pharmaceuticals and opens the door for future studies on targeted inoculation of WWTPs in combination with moving bed biofilms as a potential method for the improved removal of recalcitrant micropollutants.

Abstract ID: 69 / Poster Number: 459.15P

Investigating the growth dynamics of an extreme obligate alkaliphile Alkalihalophilus marmarensis

Presenting author: Dr. Yağmur Atakav, Marmara University, İstanbul, Turkey; Adana Alparslan Türkeş Science and Technology University, Adana, Turkey

Co-authors: *Eldin Kurpejovic, Soumaya Ben Ali Hassine, Dilek Kazan, Nihat Alpagu Sayar*

Alkalihalophilus marmarensis (formerly Bacillus marmarensis) is an extreme obligate alkaliphilic bacterium that has been reclassified under a new genus as Alkalihalophilus (2021). These reclassifications were prompted by phylogenetic studies revealing the distinctiveness of this group from other Bacillus and Alkalihalobacillus species. Although this group of microorganisms has many biotechnological advantages, the reduction of cellular mass at extreme pH conditions(1) hinders the production of valuable biochemicals. Similar to other extremophiles, A. marmarensis exhibited low cell dry mass (CDM), and notably, CDM was significantly influenced by nitrogen sources such as peptone and meat extract rather than by varying carbon sources. Given the biotechnological importance of these extremophiles, it is crucial to understand the dynamics of these microorganisms to advance future research.

For that reason, in this study, we sought to evaluate how altering the nitrogen source composition, using meat extract and peptone, affects the growth of A. marmarensis by using a response surface methodology inspired model based approach.

Model-based optimization of two input variables (meat extract and peptone) for CDM as dependent variable was performed by the multi-objective optimization using the genetic algorithm-based multi-objective Pareto optimization functionality within MATLAB. The experimental design was conducted via a central composite design. Acquired data was utilized through MATLAB's Gamultiobj function which is part of the Global Optimization Toolbox. Constraints on the independent variables were taken into account during the execution of the optimization process.

The influence of meat extract and peptone concentrations in the presence of 1 % glucose were investigated. Parameter limits varied 0.1-3 g/100 ml and 0.1-5 g/100 ml for meat extract and peptone, respectively. Experiments were carried out at pH 8.8 and 10.5 for 24 and 48 hours. The data for meat extract and peptone was analyzed through mean-effect plots. System behavior was modelled with low order polynomials.

The results indicated that peptone concentration has more impact on the growth compared to meat extract as its lower concentrations halted the growth significantly. A slightly better CDM was obtained at 48 hours and pH 10.5. Interestingly a decrease was observed when peptone concentration was lower than 0.4 % for all samples. It was found that the Quadratic Model described this system the best with a 0.83 determination coefficient (R^2) compared to other models tested. The condition with the highest R^2 value was chosen to construct the three-dimensional figures of the model as seen in Figure 1. Validation experiments showed that the model simulates the growth dynamics of the A. marmarensis accurately.

This study shed a light on how cell growth behavior of this novel bacterium changes depending on the changes in the nitrogen composition, culture duration and the pH of the environment. The model that was created in this study can be used in further research and biotechnological studies helping in decreasing the labor and cost of the studies since Alkalihalophilus marmarensis shows significant potential for biorefining and bioprocess applications.

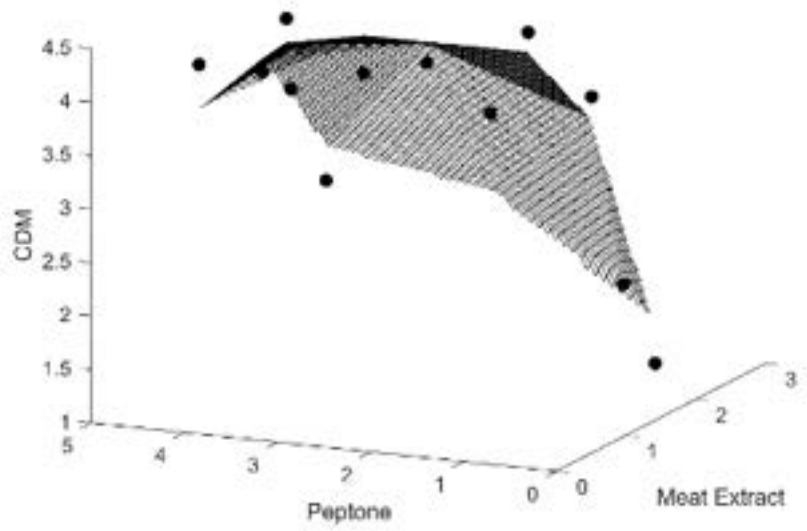


Figure 1: A model-generated representation of response surfaces in three dimensions for the yield of CDM (g/L)

Abstract ID: 3584 / Poster Number: 123.16P

Investigating the Resistome in Cemetery Soil: Combining Traditional, Molecular, and Metagenomic Approaches to Study Antimicrobial Resistance

Presenting author: Ms. Patrycja Tarnawska, Nicolaus Copernicus University, Toruń, Poland

Co-authors: *Maciej Walczak*

This study explores the potential for antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) to be released into the environment through cemetery soils, a site of decomposition as a result of burial customs worldwide. During this process, a high number of bacteria are released, some of which may be antibiotic-resistant.

The research focused on cemeteries near water bodies in northern Poland, with soil samples collected from the surface, under coffins (during exhumation), outside the cemetery (control), and groundwater (piezometers). The number of bacteria resistant to antibiotics of common use: amoxicillin, cefuroxime, doxycycline, and tetracycline, was determined, and the presence of resistance genes, blaSHV, blaOXA, blaCTX-M, blaTEM, tetA, tetB, tetC, tetD, tetE, tetM, Int1, and sul1 in the collected samples. Bacterial identification of selected strains was performed using MALDI-TOF, followed by the disk diffusion method. Also, metagenomic sequencing was performed for selected samples.

The study found substantially more ARBs resistant to beta-lactams than to tetracyclines and the most common resistance genes, tetC, blaTEM, blaSHV, and blaOXA. Of over 120 bacterial isolates, 63 were identified, with 49% of them—such as *Bacillus cereus*, *Rahnella aquatilis*, *Pandoraea pulmonicola*, and *Mycobacterium mucogenicum*—being pathogenic or potentially pathogenic. Although the abundance of ARB and ARGs increased in under-coffin samples, the trend was not statistically significant. Resistome analysis revealed similar ARGs profiles across all samples.

Despite the pollution from cemeteries, the study found limited evidence that AMR poses a major environmental threat. Further research is recommended to examine how the soil microbiome reverts over time after burial.

Abstract ID: 2665 / Poster Number: 375.17P

Investigating the role of microbial communities to hydrogen and VFAs production through dark fermentation of wastes

Presenting author: Dr. Andreas Kaliakatsos, Technical Univerrsity of Crete, Chania, Greece

Co-authors: *Iosifina Gounaki, Angeliki Maragkaki, Ioannis Choinopoulos, Tahereh Jafarpour checkab, Kelly Velonia, Manios Thrassyvoulos, Danae Venieri*

Management of organic waste through anaerobic fermentation enables the production of volatile fatty acids (VFAs), contributing to emission reduction, resource recovery, and economic benefits. The synergistic effects through co-fermentation of multiple substrates have been proven beneficial for production of VFAs. VFAs are common substrates to produce polyhydroxyalkanic acids (PHAs), which are valuable products in industry. Microbial activity drives the conversion of wastes into VFAs and different synthesis thereof have been related to different acid production.

Aim of this study is to investigate the role of the microbial communities derived from different waste streams, such as olive mill wastewater, cheese whey sewage sludge, liquid pig manure, grape residues, dry food waste and mixtures thereof in the production of biohydrogen and VFAs via dark fermentation.

Batch experiments conducted under mesophilic conditions (37°C) revealed that the primary end-products were acetic acid, propionic acid, butyric acid, and ethanol (VFAs concentration 25.1 g/L). Hydrogen in the biogas varied in contents between 25% (cheese whey sewage sludge) and 31% (mixture of cheese whey sewage sludge and liquid pig manure). Metagenomics analysis demonstrate swifts in microbial communities' composition, with the species of *Pseudomonas* dominating in both of the cases after the dark fermentation process.

The optimization of dark fermentation and the understanding of bacteria roles could enhance VFAs yields and thus the production of PHAs from wastes.

Abstract ID: 2432 / Poster Number: 019.15P

Investigating the role of the YrInv and YrIlm inverse autotransporter invasins in Y. ruckeri pathogenesis

Presenting author: Ms. Anna Lislerud, University of Oslo, Oslo, Norway

Co-authors: *Dirk Linke, Athanasios Saragliadis, Hajime Nakatani, Katsutoshi Hori*

Understanding the virulence mechanisms of *Yersinia ruckeri*, the causative agent of enteric red mouth disease (ERM)[1], offers valuable insights into the early stages of host-pathogen interactions and cross-species mechanisms of infection. Adhesins are recognized as key virulence factors in human-pathogenic *Yersinia* species, such as *Y. enterocolitica* and *Y. pseudotuberculosis*, where they facilitate invasion of host cells[2]. This study focuses on two paralogous inverse autotransporter adhesins, *Y. ruckeri invasin* (YrInv) and invasin-like molecule (YrIlm), which are hypothesized to function as virulence factors facilitating adhesion and invasion[3].

To evaluate their roles, single and double-knockout mutants were utilized in *in vitro* adhesion and invasion assays to quantify differences in adhesiveness and invasiveness between wild-type and mutant strains to a CHSE salmonid cell line. Furthermore, a zebrafish infection model[4] was used to assess the relative pathogenicity of the mutants by observing mortality rates and ERM diagnostic markers.

The results indicate that YrIlm plays a prominent role in adhesion and early-stage invasion *in vitro* and in establishing disease *in vivo*, as evidenced by a lower mortality rate when absent. YrInv contributes to later invasion stages *in vitro* but is not essential for *in vivo* disease progression when YrIlm is functional. YrInv and YrIlm synergistically enhance infection severity *in vivo*, though YrIlm is more critical for establishing infection. While compensatory mechanisms may mask the effects of the double knockout *in vitro*, where no differences in adhesion or invasion were observed compared to the wild-type strain, these mechanisms are insufficient *in vivo*.

Abstract ID: 2877 / Poster Number: 260.15P

Investigating the stress response mechanisms of Listeria monocytogenes EGD-e to peracetic acid using a Transposon Insertion Sequencing library

Presenting author: Ms. Hélène Weber, LMU Munich, Oberschleissheim, Germany

Co-authors: *Irmak Şah, Jasna Kovac, Martin Fischer, Sven Halbedel, Claudia Guldimann*

L. monocytogenes is a food-borne pathogen that causes severe disease in susceptible populations, making it one of the most important food-borne pathogens with high hospitalization and case fatality rates. Peracetic acid (PAA) is frequently used as a disinfectant in food processing environments to control this pathogen. *L. monocytogenes* has been shown to develop tolerance to PAA, specifically if exposed to sublethal concentrations. The aim of this study is to investigate the molecular response mechanisms of *L. monocytogenes* to stress conditions caused by sublethal concentrations of PAA to identify genes that contribute to the fitness of *L. monocytogenes*. Identifying these mechanisms will help us understand how *L. monocytogenes* survives when exposed to PAA and to develop novel strategies to combat this pathogen.

A transposon insertion sequencing (Tn-Seq) mutant library of *L. monocytogenes* EGD-e was exposed to 0,01 % PAA for 30 min under optimum growth conditions. Preliminary analysis of results identified several genes associated with phosphotransferase (PTS) systems, which have been shown to contribute to fitness under a range of stress conditions. This suggests their critical importance also for survival under stress caused by PAA. Several hypothetical transcriptional regulators were identified to promote *L. monocytogenes* survival under sublethal PAA stress, suggesting a role in coordinating the expression of genes required to respond to PAA-induced stress. Also, mutations in genes with established roles in fitness under environmental stress, like *sigB and sigL* were identified in this study, confirming the validity of our approach. The full analysis will be presented at the conference.

Abstract ID: 2568 / Poster Number: 214.16P

Investigation of Clinical, Histopathological and Microbiological Changes in Rats as a Result of Fecal Microbiota Transfer from Behçet's Patients and the Healthy Population

Presenting author: Assoc. Prof. Salih Maçin, Selcuk University, Faculty of Medicine, Department of Medical Microbiology, Konya, Turkey

Co-authors: *Rugıyya SAMADZADE, Sümeyye BASER, Özben Özden, Mehmet Burak ATES, Fatma TUNCEZ AKYÜREK, Duygu Fındık*

Behçet's disease is defined by oral and genital aphthous ulcers, uveitis and skin findings. The aim of this study is to show the importance of intestinal microbiota in the pathogenesis of the disease through in vivo changes (immunological and histopathological) that will develop as a result of fecal microbiota transfer from Behçet's patients and healthy donors to rats.

In the study, 26 12-week-old Wistar albino male rats were used and divided into Behçet (n: 10), healthy (n: 10) and control (n: 6) groups. Following a 3-week antibiotic regimen, fecal microbiota transfer was performed by oral gavage for 3 weeks. Control rats were given phosphate-buffered saline. They were euthanized on the 50th day and histopathological analyses (hematoxylin-eosin staining) were performed on duodenal tissues obtained by necropsy technique. IFN-γ and IL-17 serum levels were examined in rat duodenal tissues.

The most important finding in histopathological examinations was the observation of keratitis and uveitis in the eyes of rats that underwent fecal transfer from Behçet's patients. According to the severity of histopathological findings, the eye lesion score was found to be higher in rats that underwent fecal transfer from Behçet's patients (2.20±0.36c). It was determined that the retinal layer of the group that underwent fecal transfer from Behçet's patients was thicker than the group (194.15±7.28b). According to ELISA results, interleukin (IL-17 and IFN-γ) and β-actin) levels of rats in the Behçet group were found to be significantly higher than those in rats that underwent fecal transfer from healthy donors ($p<0.01$).

The study is one of the first original data showing the importance of microbiota in the etiopathogenesis of Behçet's disease in an in vivo experimental animal model via fecal

microbiota transfer. The data may contribute to research on alternative treatments for Behçet's disease.

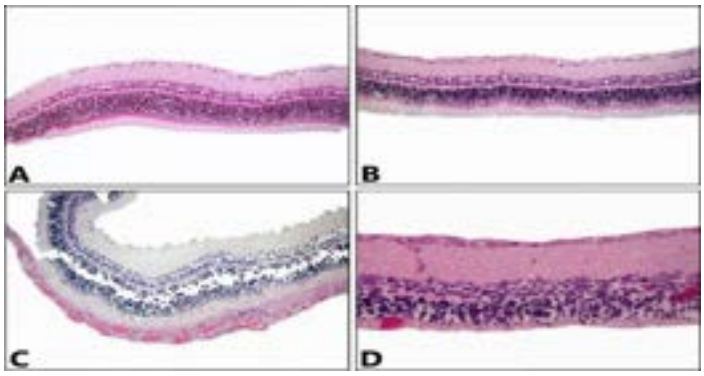


Figure 1. A. Control Group, general appearance of the retina, X20, HXE. **B.** Healthy Donor Group, general appearance of the retina, X20, HXE. **C.** Behçet's disease Group, severe hyperemia in the choroidal layer, perivascular mononuclear cell infiltrations, separation between the layers, thinning of the reticular layer located between the inner and outer reticular layers, local thickenings in the cone cell layer, X20, HXE. **D.** Behçet's disease Group, thinning of the choroidal layer, complete disappearance of the reticular layer located between the inner and outer reticular layers, local necrosis in the cone cell layer, X40, HXE.

Abstract ID: 2955 / Poster Number: 076.16P

Investigation of Salmonella Pathogenicity Island-1 (SPI-1) bistability as a mechanism that shapes epithelial invasion

Presenting author: Dr. Melissa Berni, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Co-authors: *Martina Tambassi, Laura Mazzera, Ilaria Menozzi, Alessandra Dodi, Chiara Bracchi, Marina Morganti, Luca Bolzoni, Erika Scaltriti, Stefano Pongolini*

Introduction: *Salmonella enterica* is a leading cause of foodborne infection and includes over 2,600 serovars with different levels of invasiveness. Salmonella efficiently invades intestinal cells (IECs) by expressing Salmonella Pathogenicity Island-1 (SPI-1) virulence effectors. SPI-1 undergoes bistable expression, with concomitant formation of invasive (SPI-1_{ON} cells) and non-invasive (SPI-1_{OFF} cells) subpopulations that co-exist to divide labour and improve the whole population fitness during infection. SPI-1 bistability was widely investigated in *S*. Typhimurium (STm) laboratory strains, but not across different serovars as a potential mechanism that shapes the invasiveness of Salmonella.

Objective: The aim of this study was to investigate how SPI-1 bistability shapes invasiveness in highly invasive and less invasive serovars.

Method: STm, a virulent and highly invasive serovar and the attenuated S. Derby (SDb), rarely causing illness, were chosen to represent highly invasive and less invasive serovars.

Single cell analysis of SP1-1 bistability and IECs invasion was performed on different SDb and STm strains through fluorescence microscopy. Phylogenetically distant strains were included within each serovar to capture intra-serovar variability.

Findings: STm reference strains showed large proportions of SPI-1_{ON} subpopulation associated with high invasiveness in IECs. Conversely, SDb showed high variability of SPI-1_{ON} subpopulation proportions. SDb strains showing small SPI-1_{ON} subpopulations, corresponding to reduced invasiveness, were observed. Work is ongoing to test additional strains.

Conclusion: The modulation of the SPI-1_{ON} subpopulation size, through SPI-1 bistability, emerged as a significant mechanism leading to attenuation of Salmonella.

Abstract ID: 2034 / Poster Number: 368.15F

Investigation of Temperature Adaptation in Psychrophilic Bacteria Using Single Molecule Tracking

Presenting author: Eveliny Tomas Nery, PhD, Aalto University, Espoo, Finland

Co-authors: *Aakeel Ahmad Wagay, Jarno Mäkelä*

Psychrophilic extremophiles exhibit a series of physiological adaptations which allow them to thrive in extreme cold environments, yet the molecular mechanisms underlying these processes remain largely unexplored. This study focuses on the psychrophilic bacterium Pseudoalteromonas translucida to examine the effects of temperature on intracellular dynamics. To achieve this, I will use cutting-edge super-resolution microscopy to perform single molecule tracking (SMT) to analyse diffusion dynamics of inert probes at different temperatures and bacterial growth phases. A critical step of this research involves developing a platform for the heterologous expression of the selected probes, achieved through plasmid-based expression systems. Here, I will outline the considerations and steps taken in the selection of a novel heterologous expression platform for P. translucida and the methodology utilized in the SMT experiments along with some preliminary results. These data will allow the elucidation of the physical and biochemical adaptations that enable psychrophiles to thrive in low-temperature environments. The findings of this work will contribute to our understanding of microbial temperature adaptation with significant implications for biotechnological applications.

Abstract ID: 3522 / Poster Number: 342.17P

Investigation of the antifungal effect and agricultural applicability of tomato-derived K4CBP6 defensin γ-core peptide derivatives

Presenting author: Dr. Liliána Tóth, University of Szeged, Szeged, Hungary

Co-authors: *Györgyi Váradi, Zoltán Kele, Péter Poór, Zalán Czékus, Gábor Bende, Gábor Tóth K., László Galgóczy, Rebeka Papp*

The control of pesticide-resistant phytopathogenic fungal infection is a huge challenge for agriculture. Therefore, the development of new biopesticides with a different mechanism of action from the available plant protection products is necessary. Antifungal γ-core peptide derivatives of plant defensins may be promising candidates for this purpose. Our research group established that the antifungal activity of γ-core peptide derivatives dissolved in water decreased over time after multiple thaw-freeze cycles, because these peptides cyclize and dimerize under oxidative conditions due to the free sulfhydryl groups of cysteines. The use of the S-tert-butyl group as a cysteine-protecting group may be a solution to this problem.

In the present study, we compared the antifungal activity and agricultural applicability of the γ-core peptide derivative (K4CBP6γ2) of the tomato-derived K4CBP6 defensin and its *S-tert*-butyl modified version (K4CBP6γ2_StBu). Both peptides have antifungal effect against three important phytopathogens of the tomato plant (*Botrytis cinerea, Cladosporium herbarum, Fusarium oxysporum*) at low minimal inhibitory concentration (12.5-50 μg ml^{-1}). Neither of the peptides has a cell membrane-destructive effect or causes the death of *Galleria mellonella* larvae. However, K4CBP6γ2_StBu demonstrated species-specific effects on germinating plants. At MIC values, the peptides have no or only moderate toxic effects on tomato leaves and fruits but inhibit the spread of *B. cinerea* and *C. herbarum* in infected areas.

Our results suggest that peptide derivatives of plant defensins could serve as the basis for new antifungal biopesticides. Furthermore, the synthesis of S-tert-butyl protected peptides may enhance their short-term efficacy.

Abstract ID: 3527 / Poster Number: 090.16P

Investigation of the Role of Oral Microbiota in Colorectal Cancer Pathogenesis and Detection of Potential Diagnostic Biomarkers Using Next Generation Sequencing Technologies

Presenting author: Özben Özden, PhD, Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Istanbul, Turkey

Co-authors: *Salih Maçin, Esra Saylam, Rugıyya Samadzade, Nurullah Çiftçi, Uğur Arslan, Serdar Yormaz*

Colorectal cancer (CRC) has a high mortality rate worldwide. Recent studies have emphasized the presence of various bacterial members of the oral and intestinal microbiota in gastrointestinal tumors, particularly in CRC. This study aims to investigate the association between CRC and oral microbiota and identify potential biomarkers for CRC diagnosis by analyzing saliva samples from CRC patients using next-generation sequencing technologies.

Saliva samples were collected from newly diagnosed CRC patients (n=14) and healthy controls (n=14) between March 2023 and December 2023. Microbiota (16S rRNA) analyses were conducted on these saliva samples using a next generation sequencing technologies. Sequences were aligned using the BLAST algorithm, and operational taxonomic units (OTUs) were generated based on sequences with ≥60% reference coverage and ≥80% pairwise similarity. Phylogenetic analyses, including alpha diversity, principal component analysis (PCA), principal coordinate analysis (PCoA), beta diversity, biomarker, and phenotype analyses, were conducted using the Qiime2 platform.

Analysis of alpha diversity using the Shannon, Chao-1, and Simpson indexes revealed no significant differences in the oral microbial diversity between CRC patients and healthy controls (p=0.78, p=0.28, p=0.81, respectively). However, beta diversity analysis using Bray-Curtis PCoA indicated significant differences in the microbial community between the two groups (*p=0.003*). Examination of OTU distributions revealed that the *Mycoplasmatota* phylum was undetectable in the oral microbiota of healthy controls but was significantly increased in CRC patients (*p=0.038*). *Metamycoplasma salivarium*, *Bacteroides intestinalis*, and *Pseudoprevotella muciniphila* were undetectable in healthy controls but significantly more prevalent in CRC patients (*p=0.038* for all three species). LEfSe analysis identified eight species with an LDA score >2: *Granulicatella adiacens*, *Streptococcus thermophilus*, *Streptococcus gwangjuense*, *Capnocytophaga* sp.

FDAARGOS_737, *Capnocytophaga gingivalis*, *Granulicatella elegans*, *Bacteroides intestinalis*, and *Pseudoprevotella muciniphila* as potential biomarkers.

The results of this study contribute critical evidence into the role of oral microbiota in the pathogenesis of colorectal cancer. Alterations in the microbiota profile suggest as potential biomarkers for understanding the biological mechanisms underlying CRC and for developing diagnostic and therapeutic strategies.

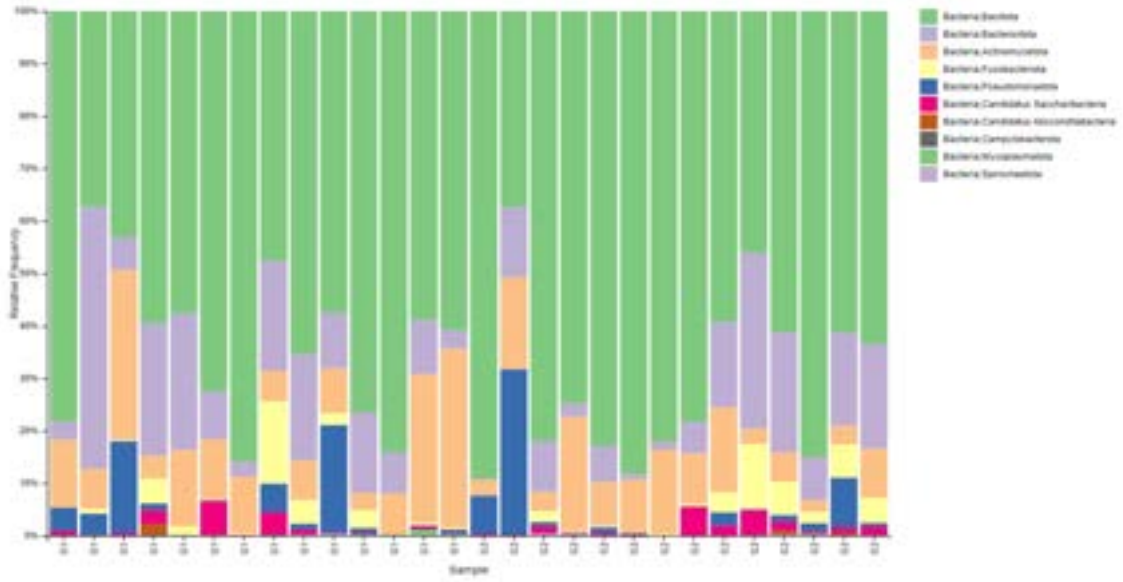


Figure 1. Microbiota Analysis at Phylum Level (GP1: patients, GP2: controls)

Abstract ID: 1945 / Poster Number: 305.17P

Investigation of toxicity and applicability of a heterologously produced Solanum lycopersicum L.-derived defensin in vitro, in vivo in planta experiments

Presenting author: Ms. Papp Rebeka, University of Szeged, Szeged, Hungary

Co-authors: *Zalán Czékus, Péter Poór, László Galgóczy, Liliána Tóth*

The fungicid-resistant phytopathogenic fungi cause enormous crop losses in agriculture. The globally used chemical-based antifungal agents unable to prevent these infections, therefore new antifungal strategies are required. The plant defensins can be a promising candidate due to their wide range of antifungal activity, and high environmental stability. To use these proteins as a biofungicid their industrial production is essential, which can be achieved by the use of heterologous expression systems.

Our research group generated a *Pichia pastoris*-based expression system, for the bulk production (11.5 mg l^{-1}) of a *Solanum lycopersicum* L. defensin (B1N680). We determined the recombinant defensin has antifungal activity against several plant pathogenic fungi (*Fusarium spp*., *Botrytis cinerea*, *Cladosporium herbarum*) at low minimal inhibitory concentration (6.25-25 µg ml^{-1}).

In the present study the toxicity and applicability of the B1N680 was monitored. B1N680 at a concentration of 400 µg ml^{-1} did not harm tomato seed germination, furthermore lateral root development of *Medicago truncatula* and *S. lycopersicum* L. seedlings, although it slightly reduced primary root length. This defensin did not show any toxicity on tomato leaves and fruits at the concentration of 25 and 400 µg ml^{-1}. The defensin inhibited the growth of *B. cinerea* on tomato leaves and *C. herbarum* on tomato fruits. According to these result the B1N680 can be a promising biofungicid due to its antifungal activity and lack of toxicity.

Abstract ID: 1395 / Poster Number: 354.16P

Iontolysis as a bacterial lysis technique suitable for bioassays and environmental application

Presenting author: Ms. Jeongeun Lee, Ewha Womans University, Seoul, South Korea

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Globally, 10% of people are still exposed to unsafe water with high levels of microbial pollution without adequate sanitation. An estimated one million deaths a year are attributed to unsafe drinking water and related diseases, which are largely preventable. Rapid detection of pathogens and water monitoring are critical to contain outbreaks and prevent the spread of waterborne diseases. Whilst nucleic acid-based methods allow early diagnosis by selectively detecting target bacteria in complex samples, these methods have the disadvantage of requiring high levels of expertise and are impractical for field due to multiple pre-treatment processes such as lysis. This study introduces a novel DNA extraction technique, hereafter referred to as iontolysis, suitable for simple, rapid bacterial detection. Iontolysis lyses bacteria using ions released from submerged electrodes with an alternating electric field. Bacterial lysis and nucleic acid extraction can be performed without adding lysis reagents. Using a prototype device manufactured with readily available materials, iontolysis was performed on Escherichia coli K12. Experiments optimized the device by varying frequencies and excitation durations. Once the optimal electrical parameters were identified, fluorescence detection with DNA-intercalating dyes assessed the feasibility of quantification. High linearity (R^2 = 0.97) demonstrated compatibility with fluorescent quantification methods, while minimal interference from byproducts was observed. The device achieved bacterial lysis via electrical excitation (3 Vpp, 5 Hz sinusoidal signal) within 5 minutes and only consumed 25 mW of power. The study demonstrates the feasibility of iontolysis as a pre-treatment for genomic assays and low-power solution for pathogen detection and water monitoring.

Abstract ID: 2825 / Poster Number: 257.15P

Is a Dual-Plant System More Efficient in Nitrogen Cycle Closure? An Italian Study on Clover-Wheat Intercropping

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Diverse agricultural systems, like intercropping, promote ecosystem services, including pest management, climate resistance, soil vitality, and water retention. The functional diversity creates biological synergies that improve nutrient utilization and pest and disease control, increasing system stability and reducing external inputs. Nitrogen is critical for plant growth. Examining its cycle provides insights into soil and vegetation health.

This research investigates how co-cultivating wheat and clover affects soil properties and microbes involved in nitrogen cycling. The study focuses on the first year to evaluate the immediate consequences of intercropping under two ammonium nitrate fertilizer regimens: standard application and a 30% reduced rate. qPCR was used to evaluate the abundance of various phylogenetic groups, the potential microbial functionality of nitrogen cycle genes, and observe differences in plant cover pattern.

Intercropping, under both fertilisation levels, significantly influenced the abundance of ITS fungal genes but not the abundance of total bacterial and archaea genes. In intercropping with reduced nitrogen fertilization, an increase was observed in the abundance of genes associated with denitrification (nirS, norB, nosZ) and nitrification (archaeal amoA). Urease activity was elevated in soil under reduced fertilized intercropping. Both factors and their interaction did not significantly impact SOC, NT, and C:N ratios. Our research suggests that archaea may be the primary factor in activating denitrification processes, and intercropping could influence the nitrogen cycle. Fertilization and intercropping strategies significantly affect the nitrogen cycle, with improved system performance observed in intercropping with reduced fertilization, indicating interspecific complementarity between wheat and clover.

Abstract ID: 3805 / Poster Number: 360.17P

Isolation and Characterization of a Novel Marine Bacterium Capable of Degrading Polyhydroxyalkanoates (PHAs)

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Plastics have been widely used globally for decades. However, their non-degradable nature has led to significant environmental pollution, with millions of tons of plastic entering oceans annually, threatening marine life and contributing to long-term ecological damage through microplastic accumulation. Among the alternatives developed to address this issue, polyhydroxyalkanoates (PHAs) have garnered attention as biodegradable polyesters synthesized by microorganisms. PHAs possess physical properties comparable to conventional plastics and can be produced from renewable resources, offering substantial environmental benefits. Despite these advantages, the biodegradation rate of PHAs is highly dependent on environmental conditions, with slower degradation observed in marine environments. Crystallinity, a key structural property of plastics, poses a major barrier to biodegradation by limiting microbial enzymatic access to the polymer surface.

To address this challenge, a novel microorganism, *Alloalcanivorax* sp. PHAMB_4001, capable of effectively degrading semi-crystalline PHAs in seawater environments. This study aimed to evaluate the biodegradation potential of *Alloalcanivorax* sp. PHAMB_4001 and its ability to overcome the constraints imposed by high crystallinity. Through detailed analysis, the microorganism demonstrated a remarkable capability to degrade PHAs with high crystallinity, thereby addressing a critical limitation in conventional PHA degradation.

These findings present a significant technological advancement in the biodegradation of plastics, particularly in marine environments. The novel strain, *Alloalcanivorax* sp. PHAMB_4001, offers a promising solution to the persistent plastic pollution problem by effectively degrading even semi-crystalline PHAs. This study highlights its potential as an eco-friendly approach to mitigating plastic waste and advancing sustainable environmental practices.

Abstract ID: 1123 / Poster Number: 037.15P

Isolation and enzymatic activity of bacteria associated with the plastisphere in waters of Chengue Bay in the Colombian Caribbean

Presenting author: Dr. Maribeb Castro Gonzalez, Universidad Militar Nueva Granada, Facultad de Ciencias Básicas y Aplicadas, Bogota, Colombia

Co-authors: *Luisa Fernanda Espinosa Diaz, Juan Fernando Saldarriaga*

In Colombian coastal areas it has been estimated that the microplastics (MP) abundance comes from aquaculture, urbanization and poor management of plastic waste. Several research groups in the country have made progress in the quantification and characterization of MP in waters, sediments, fish and zooplankton species in the Caribbean and the Colombian Pacific including estuarine areas where pathogenic bacteria and bacterial diversity have been detected on MP. To complement these findings, and in order to obtain plastisphere associated bacteria with enzymatic potential to degrade organic compounds and reduce nitrate or denitrify, in this research three bacteria separation methods from MPs were compared: vortex (3400 rpm, 10 min), sonication (45kHz for 15min) and stirring (300 rpm 15 min). The MPs were collected in the Chengue bay by trawling with a 300µM Manta net for 10 minutes in sterile containers and then the water samples were filtered through 100 µM to separate MPs of 0.3-5mm in size for subsequent bacterial separation and isolation. Subsequently, bacteria were characterized macro- and microscopically and enzymatic tests were performed on selective media. The results showed that the vortex method allowed us to obtain a greater number of bacteria which were mostly gram+ bacilli with oxidase and catalase activity, a few strains showed proteolytic, amylolytic and cellulolytic activity in addition to the ability to solubilize phosphorus and reduce nitrate. Product derived from project CIAS-4040-2024, funded by the research vice-rectory of Universidad Militar Nueva Granada and INVEMAR under the Special Cooperation Agreement INV-CIAS-4040.

Abstract ID: 2371 / Poster Number: 315.17P

Isolation and Fermentation Optimization of Microbial Strains for Alkaline Protease Production in Sustainable Agriculture

Presenting author: Dr. Beatrice Farda, University of L'Aquila, L'Aquila, Italy

Co-authors: *Amedeo Mignini, Fabrizia Pasquarelli, Daniela Maria Spera, Marika Pellegrini*

Introduction: Proteases are essential in sustainable agriculture because of their various applications that improve plant health and growth. These enzymes can enhance nutrient availability by degrading complex organic debris, thereby making essential nutrients more available to plants, while aiding in disease suppression through destroying the cell walls of pathogens and activating plant defense systems. Besides, proteases can serve in biofertilizer production, decreasing the use of chemical fertilizers and making agriculture ecologically friendlier. Although proteases are widely spread in nature, microbial fermentation is preferred as a source for these enzymes due to their fast growth, limited space needed for cultivation, and easy genetic manipulation to produce new enzymes with modified properties for a wide range of applications.

Objective: This study aims to isolate and identify proteolytic bacteria from various alkaline substrates, optimize fermentative conditions for alkaline extracellular protease (AEP) production, and develop scalable bioprocesses for potential applications in sustainable agriculture.

Material and Methods: Proteolytic bacteria were isolated from various alkaline substrates (e.g., soil, fermented products, and milk processing wastes) using selective media, including BMSM and Alkaline Casein medium with an alkaline pH (from 8 to 10). Clear halos on the agar medium around colonies indicated qualitative proteolytic activity. The strains that showed this ability were morphologically and biochemically characterized and tested for quantitative estimation of AEPs, leading to the final selection of two promising strains that were molecularly identified. The optimization of the fermentative process for AEPs production was conducted using the two selected strains, adjusting key parameters, including media composition, temperature, and pH at laboratory scale. Using the Response Surface Methodology (RSM) analysis, we performed parameter modeling that allowed us to automate the construction of analytical models useful for obtaining ideal bioprocesses.

Main Findings: From a first selection of 21 different strains, the quantitative estimation of AEPs revealed that the most promising strains were BM and SP10, identified as *Bacillus subtilis* BM and *Streptomyces iakyrus* SP10, with 257.1 U mL^{-1} and 338.2 U mL^{-1}, respectively. After optimization, enzyme activities increased significantly, with Bacillus subtilis BM achieving 2509 U mL^{-1} and *Streptomyces iakyrus* SP10 reaching 3127.40 U mL^{-1}. The RSM analysis identified optimal parameters as pH 8.0 and 40°C for BM, predicting a maximum protease activity of 3180 U mL^{-1}, and pH 9.0 and 40°C for SP10, with a predicted activity of 2935.45 U mL^{-1}.

Conclusions: These findings will guide the development of bioprocesses for scaled production in collaboration with a biostimulant enterprise. The ultimate goal is to characterize the proteases produced by selected strains, focusing on their stability and efficiency under varying conditions to support their use in biofertilizers and biostimulants.

Abstract ID: 2413 / Poster Number: 235.15P

Isolation and Functional Analysis of Plant Growth-Promoting Bacteria from Aquatic Plant Biofilms in the Bukhan River, South Korea

Presenting author: Seung Hee Yoo, Korea Agriculture Technology Promotion, Iksan-si, South Korea

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Aquatic plants form unique biofilms on their surfaces, providing a favorable habitat for microbial communities. These microbial populations play essential roles in nutrient cycling, organic matter decomposition, and plant growth promotion by symbiotically interacting with the rhizosphere of aquatic plants and producing extracellular enzymes and bioactive compounds. In this study, the microbial diversity and functional potential of bacteria from biofilms on aquatic plants in the Bukhan River were investigated. Using microbiological and molecular genetic methods to analyze the microbial communities in the biofilms of aquatic plants (*Potamogeton distinctus*, *Nelumbo nucifera*, and *Trapa natans*), it was found that at the phylum level, *Actinomycetota, Bacteroidota*, and *Pseudomonadota* were the most dominant microbial communities.

A total of 215 strains were isolated from the biofilms of aquatic plants, In the course of screening for new bioactive compounds from bacteria, 14 strains with extracellular enzyme production were selected for further studies and were confirmed to possess plant-growth-promoting activities, including siderophore production, nitrogen fixation, IAA production, and phosphorus solubilization. During the study, a novel plant growth-promoting bacterium, *Flavobacterium* sp. IMH1S5-3, was isolated. Whole-genome sequencing revealed approximately 3.62 MB of sequencing data, encompassing 3,027 coding sequences. Gene function annotation identified genes associated with plant growth promotion, including those involved in phosphate metabolism, siderophore production, IAA production, and biofilm formation. These findings suggest the potential of bacteria from aquatic plant biofilms, particularly *Flavobacterium* sp. IMH1S5-3, as valuable resources for plant growth promotion and bioactive compound discovery.

Abstract ID: 2982 / Poster Number: 160.17P

Isolation of amine-resistant variants in Salmonella Typhimurium by evolution assays with lethal treatments

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Salmonella spp. is one of the main pathogens responsible for foodborne outbreaks. The use of antibiotics along the food chain is leading to the emergence of antimicrobial resistance (AMR). This phenomenon is considered a global health problem and is being widely studied. However, it is uncertain whether enhanced resistance might occur against other antimicrobial compounds, such as biocides used in food industry.

The aim of this study was to determine whether exposure to the tertiary amine N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine (APDPD) can lead to the isolation of resistant genetic variants (RV) in Salmonella enterica Typhimurium.

Five evolutionary assay lines of S. Typhimurium parental strain (PS) were conducted (L1-L5). In each of them, PS was subjected to short lethal treatments (13 000 mg/L of APPD for 15 min at 25 ºC). Survivors were incubated at 37 ºC for 24 hours. This cycle was repeated continuously for up to 10 days. Following the evolution assays, the resistance to lethal treatments of the evolved populations was compared with that of PS.

Results indicated that a lethal treatment (13 000 mg/L of APPD for 15 min at 25 ºC) that inactivates 4.6 log cycles of PS was not as effective against the evolved populations. The most resistant populations were L3 and L5, which were inactivated only 1.1 and 1.8 log cycles respectively.

In conclusion, use of APDPD might lead to the emergence of RV of S. Typhimurium. Therefore, appropriate design of cleaning and disinfection protocols are recommended to avoid this phenomenon.

Abstract ID: 2344 / Poster Number: 312.17P

Isolation of ethanologenic yeasts and improvement of their bioethanol production capacities in the presence of lignocellulosic wastes

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Depletion of fossil fuels and environmental concerns cause comprehensive investigations on renewable energy sources. Among them bioethanol has been the most widely used liquid biofuel. Lignocellulosic biomass as a cheap and abundant raw material offers great advantages for second generation bioethanol production. Although *Saccharomyces cerevisiae* has been the most widely used microorganism, investigating alternative microorganisms for fermentation process is important.

The main objective of the current study is to isolate novel ethanologenic microorganisms which can utilize various fermentable sugars present in the hydrolysate obtained after dilute acid pretreatment of pumpkin peel waste. For this purpose 6 yeast strains were isolated from local food company wastes. 50 g/L pumpkin peel was pretreated with 1% H_2SO_4 at 121 °C for 15 min and isolated yeasts were screened for their bioethanol production capacities.

Gas chromatography was used for the determination of bioethanol concentrations. Total reducing sugar concentrations were determined with DNS method. For the measurement of microbial growth, yeast concentration was determined by measuring the turbidity of the sample at 600 nm using a standard curve of absorbance against dry cell mass.

The highest bioethanol was determined as 11.02±0.04 g/L by isolate 1 at 24 hours fermentation time when the initial sugar concentration was 26.46±0.64 g/L. During the fermentation time the highest Qp and Yp/s values were obtained as 0.46 (g/L.h) and 21.28 (g/g), respectively.

It can be concluded that isolate 1 is a suitable microorganism for second generation bioethanol production, and pumpkin peel represents a suitable lignocellulosic substrate in this respect.

Abstract ID: 1406 / Poster Number: 289.17P

Isolation of heterotrophic nitrifying bacteria from activated sludge

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Co-authors: *Yasuraoka Masaki, Shimizu Kazuya, Miura Takeshi*

Currently, wastewater treatment plants use the activated sludge process to remove organic matter and nitrogen. It is known that nitrate-nitrogen is mainly oxidized by autotrophic nitrifying bacteria, but heterotrophic nitrifying bacteria are also known to oxidized from ammonia-nitrogen. However, there are many unknown aspects of the organic matter assimilation ability and nitrification mechanism of heterotrophic nitrifying bacteria. Therefore, the aim of this study was to isolate heterotrophic ammonia-oxidizing bacteria with different polysaccharide utilization capabilities.

Alkaline phosphatase and α-glucosidase were detected in activated sludge collected from wastewater treatment plants located in Gunma and Saitama prefectures. These results indicated the presence of microorganisms that produce starch-degrading enzymes. We then conducted a screening using NM medium and NM starch-modified medium, which are suitable for culturing nitrifying bacteria, and succeeded in isolating 31 strains of nitrifying bacteria that can utilize starch. Within 31 strains, we identified 6 strains using MALDI/TOF-MS. The other strains are now identifying using 16S rRNA gene sequence. Among the identified strains, *Klebsiella spp*. was first determined that the bacteria capable of simultaneously utilizing ammonium ions and nitrate ions.

Abstract ID: 3675 / Poster Number: 234.16P

Isolation of lactic acid bacteria strains with anti-obesity activity from kefir

Presenting author: Prof. Doc. Je-Ruei Liu, National Taiwan University, Taipei, Taiwan

Co-authors: *Yen-Cheng Chen*

The prevalence of obesity poses a significant global health challenge, contributing to various metabolic disorders and cardiovascular diseases. Lactic acid bacteria (LAB) have garnered attention for their potential anti-obesity properties, attributed to their ability to modulate host metabolism and gut microbiota composition. This study aimed to identify LAB strains with anti-obesity effects from kefir. Thirty LAB strains isolated from kefir were screened for their potential anti-obesity activity using the 3T3-L1 cell model. Cell-free extracts (CFEs) of LAB strains were applied to 3T3-L1 adipocytes, and the lipid content and expression levels of adipogenic genes were assessed. LAB strains demonstrating promising anti-obesity effects in vitro were further evaluated in vivo using a high-fat-diet (HFD)-induced obese rat model. Among the tested LAB strains, YPK11 and YPK12 exhibited notable anti-obesity effects. These strains significantly reduced adipocyte lipid content and down-regulated the expression of adipogenic genes, including C/EBPα, PPARγ, SREBP-1c, and FAS, in 3T3-L1 adipocytes. In obese rats, high-dose administration of YPK11 (10^{10} CFU in 0.2 mL PBS/kg BW/day) resulted in reduced body weight gain and adipose tissue accumulation, accompanied by decreased serum and liver cholesterol and total triglyceride levels. YPK11 administration also led to decreased expression levels of SREBP1c, a key lipogenic transcription factor, and increased expression levels of CPT1, a key enzyme in fatty acid β-oxidation, in the liver. Analysis of gut microbiota revealed that HFD increased the Firmicutes proportion and Firmicutes/Bacteroidetes ratio (F/B ratio), which was further elevated with YPK11 administration. The findings of this study highlight the efficacy of YPK11 and YPK12 in inhibiting lipid accumulation in mature adipocytes by down-regulating specific adipogenic-related genes. In an HFD-induced obese rat model, YPK11 administration appeared to mitigate body weight gain and adipose tissue accumulation, along with a trend towards decreased serum and liver triglyceride levels. Analysis of gut microbiota composition following YPK11 administration showed significant alterations. These results demonstrate that YPK11 has the potential to be utilized as a functional food ingredient for the management of obesity.

Abstract ID: 190 / Poster Number: 142.16P

Isolation of *Leclercia adecarboxylata* as a cause of bacteriaemia in a patient with metastatic colon ca (case report)

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Co-authors: *Elias Chytas, Christos Sanos, Chrysi Michailidou, Konstantia Mpani, Prodromos Soukioudoglou, Eleni Vagdatli*

Introduction: Leclercia adecarboxylata is a Gram(-) bacterium belonging to the Enterobacteriaceae family. It is considered a co-infectious agent in polymicrobial infections, while it causes infections in immunocompromised patients.

Objective: Description of a clinical case of bacteremia with *L. Adecarboxylata*.

Methods: The positive blood vial was cultured on Blood/MacConkey agar. Identification and susceptibility testing was done at Vitek-2 (Biomerieux, France) according to EUCAST. Identification was confirmed by mass spectrometry (MALDI-TOF, Bruker, MA).

Results: A patient was admitted to the general on-call day at the hospital's ED, due to daytime fever up to 38 °C. He was placed on intensive intravenous hydration, empiric antibiotic therapy with meropenem, linezolid, and gentamicin (single dose), therapeutic anticoagulation with enoxaparin, and gastroprotection with omeprazole. *Enterococcus faecium* and *L. adecarvoxylata* were isolated from the blood culture. On Blood agar, large, gray, nonhemolytic colonies grew, and on MacConkey, pink, lactose-positive colonies, like those of *Escherichia spp. L. adecarvoxylata* was susceptible to most antimicrobial agents tested, and intravenous antibiotic therapy was continued. The patient developed obstructive jaundice with a parallel decrease in bile drainage from percutaneous transhepatic drainage (PTD), an event that resolved after lavages. The patient remained afebrile, hemodynamically stable, and was discharged with good biliary drainage and no problems with PTD' dysfunction.

Conclusions: The usage of appropriate microbiologic identification diagnostic tools makes it possible to distinguish L. adecarboxylata from other strains of Enterobacteriaceae. However, this pathogen seems to be sensitive to most antimicrobial agents and can result in a favorable outcome with the selection of appropriate treatment.

Abstract ID: 956 / Poster Number: 279.17P

Isolation of Ogataea polymorpha mutants with enhanced ethanol production from xylose and L-arabinose

Presenting author: Dr. Roksolana Vasylyshyn, University of Rzeszow, Rzeszów, Poland; Institute of Cell Biology of the National Academy of Sciences of Ukraine, Lviv, Ukraine

Co-authors: *Justyna Ruchala, Kostyantyn Dmytruk, Andriy Sibirny*

Introduction: Bioethanol production from lignocellulosic biomass requires efficient fermentation of pentose sugars, such as xylose and L-arabinose, at elevated temperatures suitable for simultaneous saccharification and fermentation (SSF). Methylotrophic thermotolerant yeast *Ogataea polymorpha* maximally grows above 50°C and could be suitable for use in the SSF process, especially since it naturally grows and ferments the second most abundant sugar of lignocellulose, xylose. Wild-type strains of *O. polymorpha*, however, produce only negligible amounts of ethanol from this pentose.

Objectives: Development of a simple positive method for isolation of *O. polymorpha* mutants with enhanced ethanol production from xylose and L-arabinose at 45-47°C.

Methods: The A107 strain was obtained through a novel positive selection method that isolated mutants capable of growing on L-arabinose as the sole carbon and energy source.

Findings: The developed strain A107 exhibited a remarkable enhancement in ethanol production, accumulating 20.91 g/L of ethanol from xylose at 45°C, which is over 50 times higher than the ethanol titer produced by the wild-type strain (0.40 g/L). Whole-genome sequencing of A107 identified key mutations in the *API1* and *IRA1* genes. Disruption of the *IRA1* gene increased ethanol production during the fermentation of xylose and L-arabinose in *O. polymorpha* at 45°C. Overexpression of the *API1* gene specifically enhanced L-arabinose fermentation without affecting xylose fermentation.

Conclusion: These results highlight the potential of the developed strains for application in simultaneous saccharification and fermentation (SSF) processes, offering a promising solution for bioethanol production from lignocellulosic biomass.

Abstract ID: 3818

Key players in carbon monoxide consumption in extreme environments

Presenting author: Dr. Marcela Hernández, University of East Anglia, Norwich, United Kingdom

Volcanic eruptions reset ecosystems to barren landscapes, initiating microbial colonisation that often relies on trace gases such as carbon monoxide (CO) for survival in nutrient-poor conditions. This study investigates CO metabolism in volcanic soils from these environments. Metagenomics and enrichment strategies targeting carboxydovores – bacteria capable of oxidising low-to-ambient CO concentrations – were employed to isolate novel strains. From Calbuco Volcano, Chile, two carboxydovores, *Cupriavidus ulmosensis* CV2^{T} and *Paraburkholderia terrae* COX, were identified (Dawson et al., 2025). Whole-genome sequencing and CO consumption assays revealed distinct mechanisms regulating CO metabolism during transitions from heterotrophy to starvation, providing insight into microbial survival in extreme environments. Both strains contain *coxMSL* genes, which encode CO dehydrogenase responsible for CO oxidation, along with uncharacterised accessory genes. This work also explores the active microbes consuming CO in volcanic soils using stable isotope probing (SIP) with ^{13}CO as the substrate (with and without the presence of hydrogen). SIP has shown that CO was primarily incorporated into Pseudomonadota, but only in the presence of H_2. These findings emphasise the significance of CO metabolism in early soil formation and bacterial community establishment, presenting novel model strains to further investigate microbial adaptations in volcanic ecosystems.

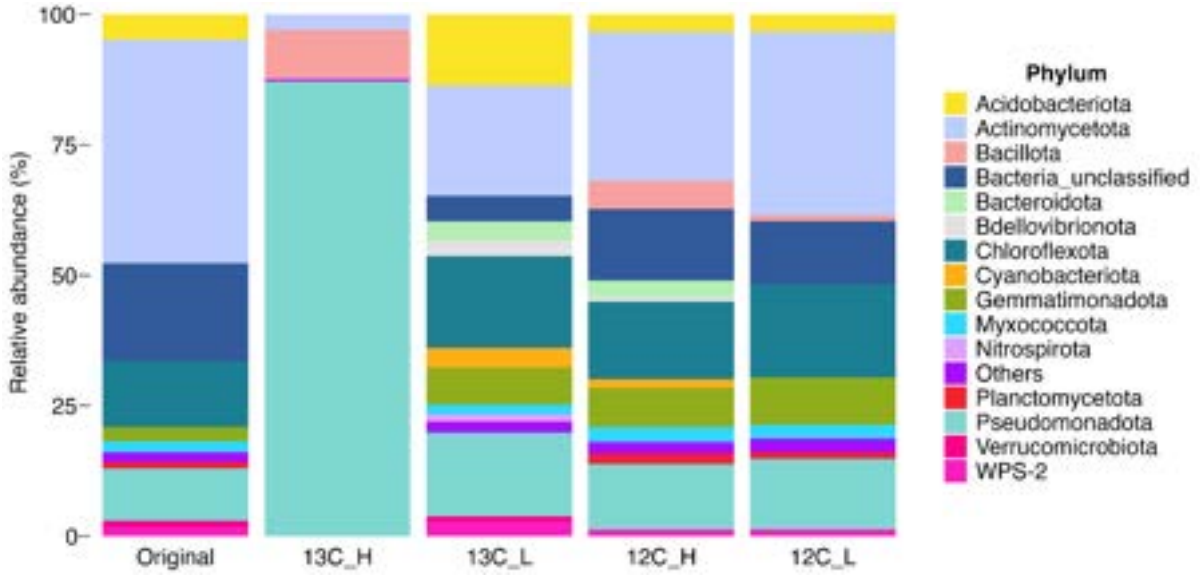


Figure 1: *SIP: total microbial community.* 13C_H: ^{13}C heavy fractions; 13C_L: ^{13}C light fractions; 12C_H: ^{12}C heavy fractions; 12C_L: ^{12}C light fractions.

Abstract ID: 2080 / Poster Number: 205.16P

Key players in the Aspergillus fumigatus secretome shaping Pseudomonas aeruginosa virulence in Cystic Fibrosis

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Co-authors: *Julie Renwick, Julia Nowak, Sean Doyle*

People with Cystic fibrosis (pwCF) are susceptible to co-infections with *Aspergillus fumigatus* and *Pseudomonas aeruginosa*, and co-infections have been linked to reduced lung function, high levels of hospitalisations and respiratory exacerbations. This study aimed to identify metabolites and proteins in *A. fumigatus* supernatants (AFsn) that drive changes in *P. aeruginosa* virulence.

A. fumigatus (AF293) was cultured for 72 hours in five media: Luria-Bertani (LB), Malt Extract Broth (MEB), Sabouraud Dextrose Broth (SDB), Minimal Essential Medium with 10% Fetal Bovine Serum (MEM+FBS), and Artificial Sputum Medium (ASM) and supernatants collected by sterile filtration. Proteins were analysed employing LC-MS and identified using Proteome Discoverer 1.4 and Sequest HT against *the A. fumigatus* Af293 database. For label-free comparative proteomics, MaxQuant with the LFQ algorithm and Andromeda peptide search engine were employed. Metabolites were analysed by RP-HPLC and LC-MS/MS. Peak intensity values were evaluated from the total ion chromatogram (TIC) or extracted ion chromatogram (EIC) using an adduct calculator for m/z values of interest.

A. fumigatus secretes distinct protein profiles depending on the growth medium. A total of 52, 361, 19, 10 and 11 proteins were identified in LB, MEB, SDB, MEM/FBS and ASM, respectively including 16 (LB), 338 (MEB) and 3 (SDB) unique proteins. Metabolomic analysis identified several key *A. fumigatus* secondary metabolites such as Fumitremorgin G (m/z:433) and Fumagillin (m/z:458.5) that are differentially abundant across media types. These findings highlight specific components of the *A. fumigatus* secretome that may alter *P. aeruginosa* behaviour in polymicrobial infections such as those seen in pwCF.

Abstract ID: 1404 / Poster Number: 054.16P

Korean Gut Microbiome Bank (KGMB) for gut microbiome research

Presenting author: Prof. Doc. Jung-Sook Lee, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jeongeup-si, South Korea

Various microbial community studies have reported that human health and disease risks are related to the gut microbiome. To study the relationship between the gut microbiome and human diseases, it is necessary to study the characteristics and functions of various strains that show significant differences between healthy and diseased individuals, and the collection of gut microbiome strains is essential.

The Korea Gut Microbiome Bank (KGMB) project was launched in 2016. We isolated and preserved various anaerobic microorganisms from the feces of healthy Koreans and performed metagenomic analysis on each sample.

To date, more than 13,000 strains have been collected from healthy Koreans (more than 800 individuals), and strains are distributed through the website (https://www.kobic.re.kr/kgmb_dist). To support strains that suit the user's purpose, we provide the results of metagenomic analysis of the gut microbiome and the coding sequences (CDS) of the whole genome sequences of the strains (https://www.kobic.kr/kgmb).

It is expected that this project will help revitalize the microbiome industry in the future.

Abstract ID: 3468 / Poster Number: 341.17P

Lachancea thermotolerans Strains for Enhancing Aroma Profiles in Sour Ale Brewing

Presenting author: Mr. Junhyeok Park, Kangwon National University, Chuncheon 24341, South Korea

Co-authors: *Jangwoo Chun, Heungkuk Lee, Myoungdong Kim*

Lachancea thermotolerans has gained recognition as a promising yeast for sour ale brewing due to its ability to produce organic acids, such as lactic and acetic acids, along with valuable aroma compounds, including phenylethyl alcohol and isoamyl acetate. This study evaluated the aromatic profiles of 10 *L. thermotolerans* strains. A malt wort was prepared by mashing pale ale malt with water at 68°C, then boiling at 100°C for 1 hour. Strains were inoculated into the wort and fermented at 25°C for 96 hours. Isoamyl acetate production was highest in NIYL24154 (34.46±0.53 ppm), followed by NIYL22661 (23.78±0.41 ppm) and NIYL22658 (22.81±0.52 ppm). The control strain, NRRL Y-12663, produced 17.28±0.11 ppm, approximately 70% of the highest-producing strain. Phenylethyl alcohol production peaked in NIYL22658 (16.97±2.69 ppm), with comparable levels observed in NIYL22659, NIYL22661, NIYL22662, and NIYL24154. Furthermore, NIYL22658, NIYL22659, and NIYL24154 demonstrated superior production of other key compounds, including ethyl acetate, acetaldehyde, and isoamyl alcohol, outperforming the control strain. These results highlight the potential of *L. thermotolerans* strains to enhance sour ales' sensory complexity and uniqueness. Their robust production of diverse aroma compounds positions them as valuable candidates for innovative brewing applications.

Abstract ID: 2821 / Poster Number: 256.15P

Lack of *sirB2* gene stimulates virulence, invasion, and small colony variants emergence in *Pseudomonas aeruginosa*

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Co-authors: *Stacy Julisa Carrasco Aliaga, Claudia Antonella Colque, Srikanth Ravishankar, Helle Krogh Johansen, Søren Molin, Moira Paroni, Paolo Landini, Elio Rossi*

Introduction: *Pseudomonas aeruginosa* is the leading cause of death in cystic fibrosis (CF) patients. The production of virulence factors and complex adaptations to the host play a crucial role in its pathogenesis. However, knowledge of the genetic determinants that drive *P. aeruginosa* persistence in the host is limited. Hence, there is a strong need to identify new *P. aeruginosa* persistence mechanisms that can be exploited for new treatment development.

Objectives: This study investigates the role of *sirB2*, a *P. aeruginosa* PA14 gene with unknown function and whose expression is enhanced in the CF lung environment as shown by previous transcriptional data collected from human samples.

Methods: *In silico* promoter analysis, molecular genetics, and biochemical approaches were used to decipher the *sirB2* gene regulation. The generation of PA14 mutants and whole-genome sequencing analysis allowed us to investigate the role of *sirB2* on heterogenous phenotypes essential for *P. aeruginosa* persistence (*i.e.* biofilm formation, *in vitro* and *in vivo* virulence assays on different infection models).

Finding: In *P. aeruginosa*, the *sirB2* gene belongs to the Vfr and AmrZ regulons (Fig. 1A), and its inactivation increases *P. aeruginosa* pathogenic potential in the *Galleria melonella* infection model (Fig. 1B). Similarly, gene deletion stimulates transepithelial migration and biofilm formation in an infection model based on air-liquid interface cultures of the airway epithelium (Fig. 1C). In this context the lack of *sirB2* promotes the production of virulence determinants and the emergence of rugose small colony variants (RSCVs; Figs. 1D and 1E). RSCVs appearance depends on an increased rate of mutations in the *wsp* regulatory circuit, leading to increased c-di-GMP levels (Fig. 1F).

Conclusions: Our data identified the *sirB2* gene as a novel genetic determinant underlying the appearance of heterogenous phenotypes typical of acute and chronic infections through a mechanism involving specific genetic rearrangement in the PA14 genome.

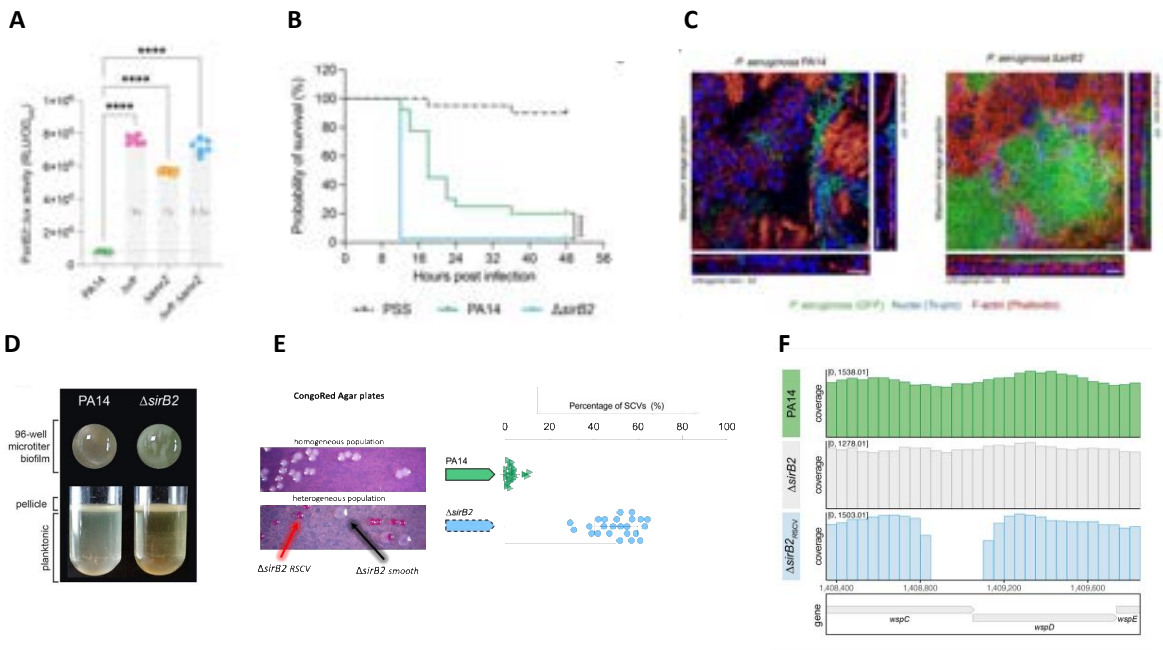


Figure 1: Deciphering the role of the *sirB2* gene through the characterization of its regulation and the effect of its inactivation in *P. aeruginosa* PA14. (A) The activity of the P*sirB2* promoter, reported as RLU/OD_{600}, in *P. aeruginosa* PA14, Δ*vfr*, Δ*amrZ,* and Δ*vfr* Δ*amrZ* knockout mutants. **(B)** Kaplan-Meier plot showing the percentage survival of *G. mellonella* larvae inoculated with *P. aeruginosa* PA14 and Δ*sirB2* strains. **(C)** Confocal microscope imaging of epithelial ALI cell cultures on transwell inserts infected with *P. aeruginosa* PA14 or Δ*sirB2* strains constitutively expressing sGFP. **(D)** Representative pictures of biofilms formed in 96-well microtiter plates and pellicle formed at the air-liquid phase in a tube of *P. aeruginosa* PA14 and Δ*sirB2* strains. **(E)** Evaluation of RSCV appearance in *P. aeruginosa* PA14 and Δ*sirB2* populations. **(F)** Whole genome sequencing analysis on *P. aeruginosa* PA14, Δ*sirB2,* and Δ$sirB2_{RSCV}$ strains.

Abstract ID: 3911 / Poster Number: 129.16P

Lactate stereoisomers are metabolized differently in the gut microbiome

Presenting author: Dr. Maximilienne Allaart, University of Tuebingen, Tübingen, Germany

Co-authors: *Gerben Stouten, Largus Angenent*

Introduction: The gut is home to trillions of anaerobic microbes that are involved in the sequential conversion of our food into short-chain carboxylic acids. One of the intermediate molecules in the gut food web is lactate, which is produced in two enantiomeric forms: D-lactate and L-lactate. L-lactate is native to the human body, whereas D-lactate is not only exclusively produced by the gut microbiome but is also neurotoxic. In healthy individuals, neither of the lactate stereoisomers accumulates, but the fate of the individual stereoisomers in the gut remains to be elucidated.

Objective: This work aims to ecologically and physiologically profile the conversion of D- and L-lactate in the gut microbiome.

Method: To reach this objective, two identical anaerobic bioreactors (V= 1L) that we operated as chemostats were used to enrich lactate-consuming microbial communities at pH 7.0, T = 37°C, and a retention time of 4 days. The bioreactors were inoculated with a human-derived gut microbiome and fed with DL-lactate until steady-state was reached. After steady-state was reached, the bioreactor effluent was used to inoculate chemostats fed with exclusively D- or L-lactate, in duplicate. Finally, when steady-state was maintained for >3 SRTs, the substrate was swapped: D-lactate-fed reactors now got L-lactate and vice-versa. The biochemical conversion profiles of D-, L-, and DL-lactate and the microbial community composition were monitored before and during steady-state operation. We used off-gas mass spectrometry to monitor the dynamic response of the culture to the individual isomers, as well as HPLC measurements of the liquid product spectrum.

Key Findings: D-, L- and DL-lactate were all predominantly converted into acetate, propionate and CO_2. Butyrate, valerate, and hexanoate were measured in smaller amounts. The microbial community of the DL-enrichments predominantly contained the *Anaerostignum* and *Acidipropionibacterium* genera. In the D-enrichments, *Anaerostignum* became the dominant genus, whereas *Acidipropionibacterium* initially dominated the L-enrichments. However, *Propionibacterium* outcompeted *Acidipropionibacterium* in time. Remarkably, the biomass yield on D-lactate was less

than half of the biomass yield on L-lactate, even though the product spectrum was the same. Switching from L-lactate to D-lactate feeding caused no change in the observed metabolic activity but coincided with a steady drop in biomass yield and a community shift to *Anaerostignum*. Switching from D-lactate to L-lactate, however, caused an immediate, significant drop in lactate consumption, leading to the accumulation of L-lactate in the bioreactor. After three days, L-lactate started to be consumed increasingly quickly, concurrent with an increased biomass yield and a community shift to predominantly *Acidipropionibacterium*.

Conclusions: Our results show that the stereoisomers of lactate are consumed by different, specialist microbes in the gut. The significant difference in biomass yield between D- and L-lactate, combined with the highly similar product spectrum comprising acetate, propionate and CO_2, suggests that D-lactate exclusively feeds into the acrylate pathway, whereas L-lactate feeds into the methylmalonyl-CoA pathway. These pathways have the same metabolic output, but a significantly different biochemistry. Overall, our results shed light on the ecological strategies of the underexplored functional group of gut lactate consumers and increase our fundamental understanding of the gut microbiome.

Abstract ID: 2229 / Poster Number: 083.15P

Lag times and spatial structure drive bacterial growth in non-well-mixed environments

Presenting author: Ms. Rita Invernizzi, Humanitas University, Milan, Italy

Objectives: Bacteria create nutrient micro-gradients that shape their local environment, behaviour, and phenotypic responses. This study investigates how nutrients uptake and environmental feedback drive spatiotemporal dynamics, influencing the growth and spatial arrangement of dense colonies under shear forces and fluxes. When exposed to multiple carbon sources, bacteria either co-utilize them or prioritize the most favourable, introducing lag phases before switching. The research aims to understand how these lag phases impact growth in spatially heterogeneous environments.
Methods: This study relies on the implementation of a mathematical model that reproduces the diffusion and hierarchical consumption of multiple nutrients by a bacterial community in a spatially limited setting. The theoretical approach is combined with microfluidics experiments, that allow to perform single cell imaging of bacteria under the influence of multiple nutrient and shear fluxes in a spatially limited setting.
Findings: The mathematical model predicts the behavior of the average growth-rate depending on the diffusion and uptake of the nutrients and the duration of the lag phase (figure 1A). Experimentally (figure 1B) it emerges that the hierarchical consumption of two carbon sources takes place in specific spatial regions of the system, according to the initial concentrations of the sugars

Conclusion: In summary, this research seeks to elucidate how spatiotemporal environmental features affect bacterial growth and to uncover the significance of lag phases in non-well-mixed environments.

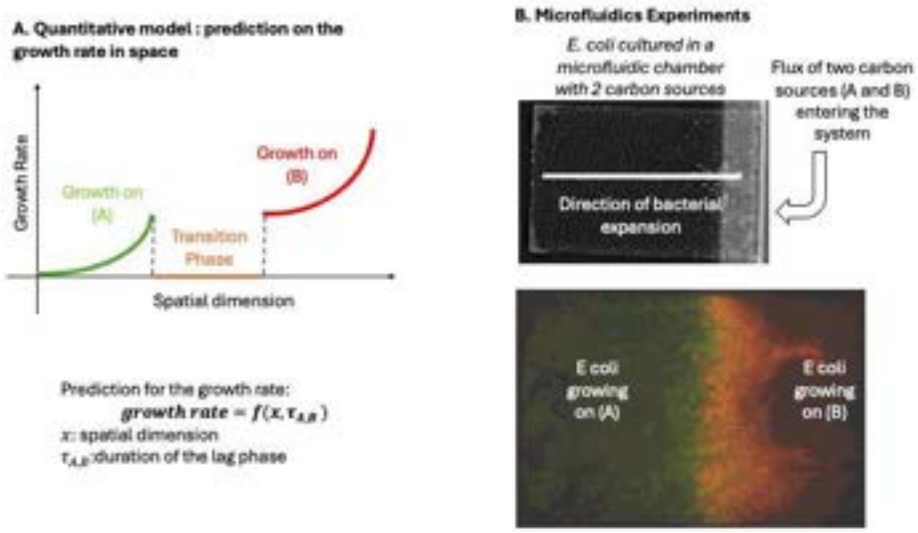


Figure 1: **A** – The mathematical model predicts a growth rate that depends on the concentrations of the nutrients, consequently it is a function of the spatial dimension and of the lag time needed to transition from nutrient (A) to (B). **B**- Microfluidics experiments where E. coli, engineered to express mCherry or GFP according to the carbon source consumed, grows in microfluidics chambers. There, bacteria consume two nutrients. The preferred nutrient (B) is consumed in proximity of the opening of the chamber, until is depleted, while bacteria at the back grow on the second nutrient (A), pushing bacteria in front of them out of the chamber, and entering in regions where the first nutrient is still present, this induces a transition from carbon source (A) to (B).

Abstract ID: 810 / Poster Number: 125.15F

Lake Nizhnee (Enderby Land, East Antarctica) contains DNA from bacteria that may be associated with human diseases

Presenting author: Dr. Katsiaryna Akhremchuk, Institute of Microbiology, NAS of Belarus, Minsk, Republic of Belarus

Co-authors: *Artur Akhremchuk, Yahor Korzun, Leonid Valentovich, Vladislav Miamin, Yuri Hihiniak*

Introduction: Antarctica holds vast water reserves in a liquid and frozen state. Waterborne diseases are recognized as a public health issue, and understanding the presence of potential pathogens in water bodies is crucial for assessing the risks associated with Antarctic ecosystems.

Objective: A comprehensive analysis of the presence of the DNA from bacteria that may be associated with human diseases in the core sample from Lake Nizhnee which serves as a water source for Belarusian Antarctic Station.

Methods: The core sample from Lake Nizhnee was obtained during the 14th Belarusian Antarctic Expedition. Shotgun metagenomic sequencing was carried out using Illumina MiSeq. Detection of operational taxonomic units was done using Kraken 2. Data analysis was performed using R. The database of pathogenic bacteria was created based on the list of pathogens reported by Bartlett et al, 2022.

Findings: The core sample from Lake Nizhnee contained DNA from 691 species of bacteria associated with human diseases. Among the analysed sequences, up to 13.4 % were identified as DNA belonging to pathogenic bacteria according to the revised list of pathogens. The DNA of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Chlamydia psittaci* prevailed among the DNA of bacteria that may be associated with human diseases.

Conclusion: Lake Nizhnee contains DNA from bacteria with an established pathogenic role. It should be noted that the majority of pathogens are commensals, commonly causing infections under specific conditions. Further research is needed to explore the diversity of viable pathogenic bacteria in Lake Nizhnee and other Antarctic lakes.

Abstract ID: 1437 / Poster Number: 274.16F

Large-scale analysis of mechanisms shaping the evolution of T-box riboswitches in Gram-positive bacteria: duplications, horizontal gene transfer, and changes of specificity

Presenting author: Ms. Elizaveta Grigorashvili, Center of Molecular and Cellular Biology, Moscow, Russia

Co-authors: *Anna Shilova, Mikhail Gelfand*

T-box riboswitches, RNA structures that regulate amino acid metabolism in Gram-positive bacteria, play a critical role in bacterial adaptation and gene regulation. Despite their importance, the evolutionary mechanisms driving the T-box diversity remain understudied.

To address this gap, we identified and annotated over 20,000 T-box sequences to investigate duplications, changes in T-box specificity, and horizontal transfer (HGT). We analyzed these sequences by comparing the phylogeny of the studied species with the phylogeny of their T-boxes.

Our analyses revealed several novel evolutionary events. First, we observed a potential HGT event involving the *thrZ* gene and its T-box in *Virgibacillus* species. Second, in contrast to previous studies [1], we observed that ALA-T-boxes in *Bacilli* can regulate genes other than the *alaS* operon. Moreover, in *Leuconostoc mesenteroides*, the *alaS* operon appears to have lost T-box regulation. Third, we identified T-boxes with noncanonical specificities, such as LYS-T-boxes regulating methionine pathway genes and ILE-T-boxes associated with serine metabolism. Estimating the scale of this phenomenon, we suggest that some T-boxes might function as sensors of general amino acid deprivation rather than fine-tuning devices. Additionally, we observed a previously described operon split in *Limosilactobacillus reuteri* [1], where a T-box regulating the *hisS-aspS* operon evolved into distinct HIS- and ASP-T-boxes.

By uncovering novel examples of T-box evolution and filling gaps in existing knowledge, this study lays the foundation for further exploration of their regulatory and adaptive roles in bacterial metabolism.

Abstract ID: 824 / Poster Number: 466.15P

Large-scale insights into the biosynthetic potential of the Bacillus cereus group

Presenting author: Ms. Josefin Blom, Umeå University, Umeå, Sweden

Co-authors: *Hadrien Gourlé, Martin Larralde, Vignesh Ramnath, Johan Henriksson, Laura Carroll*

Introduction and Objective: Bacteria occupying complex, free-living environments often produce a wide array of secondary metabolites (SMs), which are small molecules that confer their producer with a fitness advantage. Due to their rich chemical scaffold diversity, SMs have historically served as a source for natural product-derived drugs. While *Actinomycetes*, in particular *Streptomyces*, have dominated the antimicrobial agent category, other soil-dwelling bacteria remain underrepresented. This includes the *Bacillus cereus* group, which consists of Gram-positive, spore-forming bacteria with high potential as a reservoir for drug discovery. Here, we harness *in silico* methods to mine an unprecedented number of high-quality *B. cereus* group genomes for the gene clusters which govern SM production (biosynthetic gene clusters; BGCs), with the goal of discovering novel BGCs with therapeutic potential.

Methods: *B. cereus* group genomes and associated metadata were downloaded from BTyperDB (www.btyper.app; Fig. 1a). BGCs were detected in each genome using antiSMASH (v6.1.1) and GECCO (v0.9.8; Fig. 1b). The resulting BGCs, together with experimentally validated BGCs in the MIBiG database (v3.1; n = 2502 BGCs), were delineated into gene cluster families (GCFs) using IGUA (v0.0.1; Fig. 1c). Representative BGCs for each GCF (n=2398) were annotated with protein domains to generate a two-dimensional atlas (UMAP; Fig. 1d-e). The atlas and relevant metadata were integrated into an interactive web application (BTyperBGC), constructed using R (v4.3.1) and Shiny (v1.8.1.1; Fig. 1f).

Findings: Overall, we detected 124 097 BGCs among 5976 *B. cereus* group genomes. The BGCs clustered into 2398 GCFs, alongside previously known (MIBiG) BGCs. While the majority of GCFs contained ≥1 MIBiG BGC (*n* = 1597, 66.6%), a significant proportion (*n* = 801, 33.4%) were deprived of previously known BGCs, suggesting novel chemical scaffolds to be explored. For this purpose, the two-dimensional BGC atlas and the full dataset were made available as an interactive web application (BTyperBGC; Fig. 1f). BTyperBGC provides users with options to annotate and subset the atlas based on

features of interest (e.g. species or predicted biosynthetic class of the metabolite), as well as download BGCs for further investigation.

Conclusion: We mined an unprecedented number of genomes to conduct large-scale analysis of the BGCs present among *B. cereus* group members, relying on computational tools and high-quality genomes with standardised metadata. Our research demonstrates the capacity of this group as a candidate reservoir to be leveraged for natural product-based drug discovery and aims to generate novel leads for experimental follow-up.

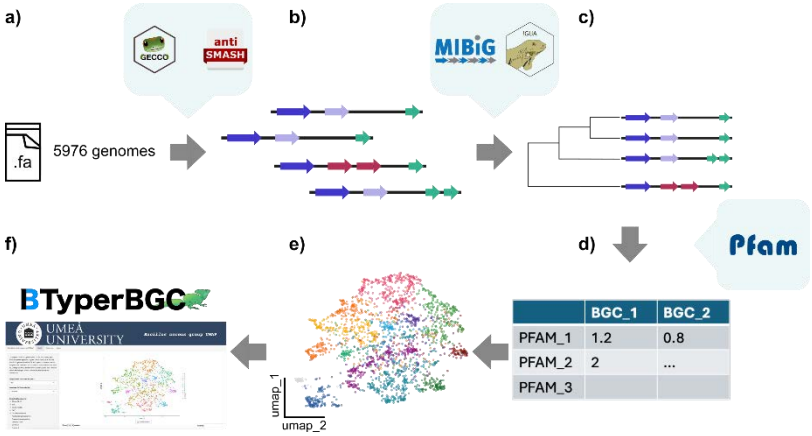


Figure 1: Overview of BTyperBGC atlas construction.

Abstract ID: 2392 / Poster Number: 355.16P

Laser-induced nanostructuring of metal surfaces affects biofilm architecture and reduces biofilm formation

Presenting author: Dr. Olena Moshynets, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukr, Kyiv, Ukraine

Co-authors: *Olga Iungin, Svitlana Rymar, Iaroslav Gnilitskyi, Geert Potters*

Introduction: Biofilms are hard to eradicate due to the matrix in which the bacteria reside, consisting of strong, adhesive and adaptive self-produced polymers such as eDNA and functional amyloids. Targeting a biofilm matrix may be a promising strategy to prevent biofilm formation.

Objective: Investigation of antibiofilm efficiency of nanostructured metal surfaces.

Methods: Femtosecond laser pulses were used to modify stainless steel surfaces to introduce either conical spikes or conical grooves. Two model strains *P. aeruginosa* PA01 and *S. aureus* ATCC29423 were used to develop biofilms which were analysed using CLSM. The adipose-derived stem cell (ADSC) culture were used to investigate the behaviour of eukaryotic cells onto modified surfaces.

Findings: The biofilm structure of both strains, *P. aeruginosa* PA01 and *S. aureus* ATCC29423, formed on such nanotextured metal surfaces, was considerably modified due to a substantial reduction in amyloid production or due to changes in eDNA surface adhesion, leading to significant reduction in biofilm biomass. Altering the topography of the metal surface, therefore, radically diminishes biofilm development, not by reducing of bacteria on the surface but by altering biofilm architecture. At the same time, growth and colonisation of the surface by eukaryotic ADSC culture was apparently enhanced, leading to possible further advantages in controlling eukaryotic growth while suppressing prokaryotic contamination.

Conclusions: Surface nanotexturing can be used to control bacterial fouling in a non-chemical manner using a low-cost and high-speed method of laser irradiation.

Abstract ID: 1603 / Poster Number: 093.17P

Lasso peptides sviceucin and siamycin I have anti-virulence activity and restore vancomycin effectiveness in vancomycin-resistant pathogens

Presenting author: Ms. Solenn Desmadril, Université de Caen Normandie, CBSA UR4312, Caen, France

Co-authors: *Benjamin Baëtz, Abdelhakim Boudrioua, Christophe Goulard, Anne-Claire Groo, Carine Lombard, Sabrina Gueulle, Marie Marugan, Aurélie Malzert-Fréon, Axel Hartke, Yanyan Li, Caroline Giraud*

Antibiotic resistance is a major threat to human health and new drugs urgently need to be developed.

This study aims to investigate on the promising properties of two natural ribosomally-synthesized lasso peptides (LP), sviceucin and siamycin I, on the virulence and the antibiotic resistance of pathogens.

The vancomycin-resistant enterococci (VRE) *Enterococcus faecalis* strain V583 is the model of this study. The LPs effects on bacterial virulence were evaluated using the *Galleria mellonella* insect model. LPs effects on vancomycin susceptibility were assessed by determining the minimal inhibitory concentrations. *G. mellonella* was used to verify the efficacy of LPs and vancomycin as a combined treatment for VRE infection. LPs mode of action (MoA) was analyzed by monitoring gene expression using RT-qPCR analyses and Differential Scanning Fluorimetry was used to study the LPs interactions with their protein targets.

We found that the LPs are able to abolish bacterial virulence of pathogenic enterococci and restore vancomycin clinical susceptibility of VRE *in vitro* and in a surrogate animal model. These activities are linked to their interactions with the histidine kinases (HKs) FsrC and VanS that control the affected phenotypes.

Having multiple clinically-relevant targets further reduces the risk of simultaneous development of resistance to all actions of these molecules. Together with the highly sought-after MoA as HK inhibitors, such properties make these LPs promising candidates for the development of next generation antibiotics. In the future, their targets and effects still need to be further investigated, particularly on different pathogens such as *E. faecium* and *Staphylococcus aureus*.

Abstract ID: 4005 / Poster Number: 430.16P

Lessons from Diversity Promotion in Korean Universities

Presenting author: Prof. Jung-Hye Roe, Seoul National University, Gwanak-gu, South Korea

As of 2023, the foreign population residing in South Korea accounts for approximately 4.8% of the total population, bringing the country closer to becoming a multicultural society. However, the concept of diversity remains unfamiliar in Korean society, either still intangible or even met with resistance. Institutions such as corporations and universities in Korea still lack dedicated diversity offices, with only a few exceptions.

The first organization to promote diversity within a Korean university was established at Seoul National University (SNU) in 2016. Proposed by the SNU Women Faculty Council, the Diversity Council was launched as an advisory body to the university president. Since its establishment, the council has published annual reports addressing gender equality, the status of underrepresented members of the university community, work-life balance, and international exchange efforts. Based on these reports, it has made policy recommendations to enhance diversity. Following SNU's lead, other universities, such as Korea University, KAIST, Kyungpook National University, and Pusan National University, have also established diversity offices.

One of the most pressing gender equality agendas has been the stark underrepresentation of female faculty members compared to the number of female students. In 2017, female professors accounted for only about 15% of the faculty at national and public universities. That year, the SNU Diversity Council supported the launch of the Federation of Women Faculty Councils of National and Public University, and in collaboration with this organization, petitioned the government and the National Assembly to increase the hiring of female professors. As a result, the law governing faculty appointments at national and public universities was revised to encourage universities to make proactive efforts until female professors make up at least one-fourth of the total faculty.

While collective efforts in various aspects of diversity promotion have yielded some tangible results, ensuring the stable operation of diversity offices remains a significant challenge. To encourage each other's efforts and create a broader impact, diversity offices from universities, research institutes, and corporations have recently begun forming alliances to collaborate and strengthen their collective influence. I would like to exchange some challenges and strategies for advancing gender equality and diversity, hoping to shape a more inclusive future with collaborative efforts.

Abstract ID: 2147 / Poster Number: 422.15P

Leveraging 4f-SAMMY-seq protocol in Prokaryotes as a high-resolution technology to study Nucleoid accessibility

Presenting author: Mr. Ugo Maria Iannacchero, National Research Council, Segrate, Italy

Co-authors: *Elena Chiti, Valentina Rosti, Andrea Vannini, Chiara Lanzuolo, Davide Roncarati, Eva Maria Pinatel*

Introduction: Bacterial chromosome self-organizes into the nucleoid, a membrane-less region where Nucleoid Associated Proteins (NAPs) and RNAs regulate its accessibility. Bacterial HiC-based protocols provided insights into nucleoid organization; however, they fail to capture interactions below one kilobase, even pushing sequencing depth. We describe the first application on prokaryotes of 4f-SAMMY-seq (4-fraction Sequential Analysis of MacroMolecules Accessibility sequencing), a crosslink and restrictions enzymes-free technology conceived to study chromatin organization, which achieved HiC-level resolution at lower sequencing depth in eukaryotes.

Objective: We aim to adapt 4f-SAMMY-seq protocol and analysis tools to *Helicobacter pylori* in exponential growth phase, to investigate nucleoid organization at high-resolution.

Method: 4f-SAMMY-seq biochemically separates DNA and associated proteins or RNAs into four fractions according to their physico-chemical properties. Fractions are sequenced, and DNA accessibility is assessed through fraction-pair relative ratios. Generally, accessible fractions correlate with ATAC-seq signals and open chromatin marks, while less accessible fractions with closed chromatin marks. We adopted the nf-core/sammyseq pipeline implemented in Nextflow to generate genome-wide signal tracks and paired comparisons. We analyzed a contact-based matrix derived from individual fractions to define nucleoid domains.

Findings: We adapted 4f-SAMMY-seq to *Helicobacter pylori,* confirming its ability to capture DNA domains according to their different accessibility also in bacteria. We achieved a kilobase domain resolution with 500000 reads per fraction and observed specific signals for some promoters opening the possibility to further decrease domains resolution.

Conclusion: 4f-SAMMY-seq promises an advantageous alternative for studying nucleoid accessibility, and to further assess its potential, we will integrate transcriptomic and ChIP-seq data.

Abstract ID: 3621 / Poster Number: 441.16P

Leveraging AlphaFold for Teaching Microbial Biotechnology: Enhancing Understanding of Protein Structure-Function Relationships

Presenting author: Dr. Elias R. Olivera, Universidad de León, León, Spain

Co-authors: *Ana Ibañez, Carlos Barreiro*

The fast development of computational tools such as AlphaFold has transformed the understanding of protein structures and provided unmatched insights into the intricacies of microbial systems. AlphaFold can be included in the teaching of microbial biotechnology, which will improve students' comprehension of microbial physiology, enzyme activity, and metabolic pathways using structural bioinformatics. Students can use AlphaFold's predictions of protein structures to investigate real-world applications in the fields of microbial engineering, drug discovery, and synthetic biology. By incorporating AlphaFold into biotechnology courses, students can learn through hands-on, interactive experiences. This approach helps them build the skills they need for modern biotechnological research and industry applications.

The incorporation of AlphaFold into the study of microbial biotechnology marks a significant change in the way that students can interact with the molecular components of microbial systems. AlphaFold provides students with a strong computational tool that allows them to anticipate protein structures. This enables students to establish important linkages between protein structure, function, and biotechnological applications. Students who are able to visualize and alter protein structures in a classroom context are more prepared for the changing needs of the biotechnology sector since this ability improves their critical thinking and problem-solving skills. In the future, advancements in artificial intelligence-powered tools such as AlphaFold will probably continue to influence the future of biotechnology education. These breakthroughs will provide students with new chances to learn about the molecular world of microbes.

Abstract ID: 2581 / Poster Number: 133.16S

Leveraging pet dogs as a model to characterise dietary influence on gut microbiome and physiological responses

Presenting author: Ms. Louise Campbell, The University of Sydney, Sydney, Australia; Charles Perkins Centre, Sydney, Australia

Introduction: Diet and the gut microbiome are intrinsically linked and strongly influence health in all animals. The gut microbiome acts as an interface between the host and ingested dietary components, impacting energy balance, absorption of nutrients, and overall gut and immune health. Nutrition-related chronic diseases are a growing concern in both humans and companion animals, particularly pet dogs. Dietary interventions offer a promising, non-invasive strategy for treating these diseases by altering the gut microbiome and emergent outcomes. However, achieving consistent gut microbiome responses remains a challenge. A major factor likely contributing to this is the individuality of each host's gut microbiome composition, with identical dietary changes often leading to different responses across individuals. Improving our understanding of how the microbiome contributes towards variability will help increase the effectiveness of such interventions. Pet dogs are a relevant model for human health due to their shared environment, omnivorous diet, similar disease profiles, and controlled lifestyle. In addition, the canine gut microbiome shares significant functional similarities to that of humans.

Objectives: We leveraged a pet dog model to compare the effects of two commercially available dog foods that differed by processing level and macronutrient distribution, aiming to identify differences in short-term effects between the two diets. Comprehensive measuring of gut microbiome, individual traits and physiological outcomes at baseline and during treatment periods allowed us to determine the extent to which diet drove microbiome response and the predictability of these responses.

Methods: Using a cross-over design, we randomly assigned 24 healthy dogs to one of two groups: all dogs remained on their at-home diet for 1 week, before being transitioned to (A) a minimally processed cooked diet or (B) an extruded kibble diet for 2 weeks each. Dogs were then swapped onto their second experimental diet following an additional transition period. We measured broad physiological outcomes as well as glycaemic, hormone, immune and gut microbiome responses. Serum samples were obtained on three separate days – each at the conclusion of a treatment period – while owners provided 12 faecal samples from their dogs throughout the study.

Findings: 16S amplicon sequencing revealed significant shifts in alpha and beta diversity of the gut microbiome between the two diets and baseline (Fig 1A, B). The two diets drove significantly different gut and appetite-related hormone responses, and post-prandial glycaemic response trended lower on the minimally processed diet compared to the kibble (p = 0.08) (Fig 1C). Although diet was a major determinant of host response, individuality also contributed strongly to observed outcomes, as evidenced by PCoA clustering and differential abundance analyses (Fig 1D). **Conclusion:** Our study reinforces the value of pet dogs as a relevant model for understanding how diet, individual factors and the gut microbiome interact to drive host health outcomes.

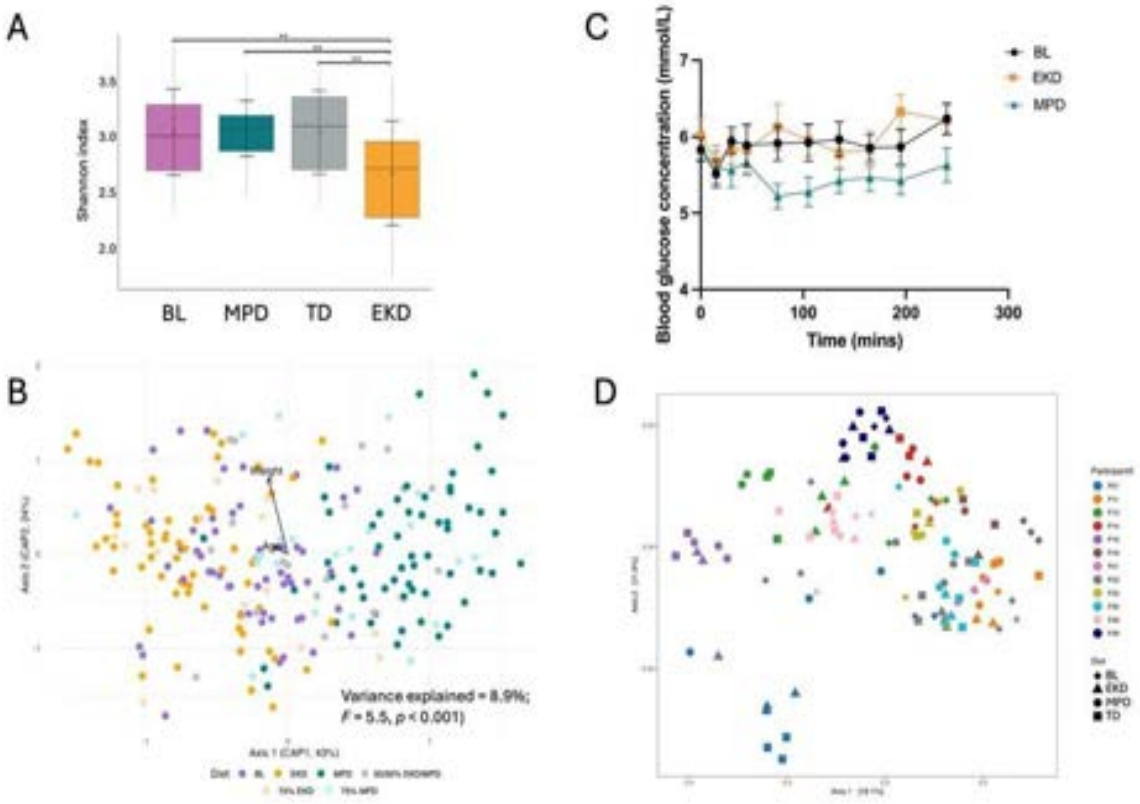


Figure 1: *(A) ASV-level Shannon index by diet period (n = 12, MPD-first participants only)(**p < 0.01); (B) Constrained ordination by dbRDA on Bray-Curtis dissimilarity (n = 24); (C) Average post-prandial blood glucose response over 4 hours per diet treatment (n = 21); and (D) PCoA of Bray-Curtis dissimilarity, coloured by individual (n = 12, MPD-first participants only). BL = baseline diet treatment, MPD = minimally processed diet, TD = transition, EKD = extruded kibble diet.*

Abstract ID: 1078 / Poster Number: 333.16F

Leveraging Phages for Sustainable Dairy Goat Farming in Kenya

Presenting author: Audrey Addablah, PhD, Université Laval, Québec, Canada

Co-authors: *Xavier Minne, Elodie Fortin-Huburtise, Nicholas Svitek, Sylvain Moineau*

With approximately 28 million goats, these animals represent the most abundant livestock resource in Kenya. In addition, goat milk production also plays an essential role in the nutrition and economy of smallholder farmers. However, mastitis, primarily caused by Staphylococcus species, threatens both milk quality and yield, enhancing financial pressures. With the emergence of antibiotic-resistant bacterial pathogens, alternatives are sought after. Bacteriophages (phages) offer a promising, eco-friendly and sustainable alternative for biocontrol by providing targeted action against these pathogens while reducing the reliance on antibiotics. For application purposes, promising phages will need to be produced at high titers.

This study aims to evaluate the production efficiency of phages on non-pathogenic Staphylococcus strains, with the goal of identifying potential phage-host pairs for scalable phage production.

Fourteen staphylococcal phages from the Université Laval collection (phage.ulaval.ca) were tested for their ability to infect strains belonging to non-pathogenic Staphylococcus species, namely S. xylosus and S. carnosus. Killing assays were performed on plates and in liquid media to assess the activity of the candidate phages. Adsorption tests were also conducted to confirm the ability of the phages to bind effectively to these amplification hosts. Based on these evaluations, four phages demonstrated successful production on S. xylosus and S. carnosus and they were further analyzed for production yield. Phage lysates produced in LB with S. xylosus showed superior titers, making it a robust host candidate for scalable phage production.

These results support the potential of integrating phage-based solutions into sustainable dairy goat farming practices.

Abstract ID: 2193 / Poster Number: 229.15P

Leveraging vineyard microbiomes for sustainable agriculture in Vino Nobile di Montepulciano DOCG production area

Presenting author: Simone Rampelli, PhD, University of Bologna, Bologna, Italy

Co-authors: *Nicolò Cinti, Lucia Foresto, Giorgia Palladino, Federica D'Amico, Daniel Scicchitano, Silvia Turroni, Marco Candela*

Viticulture relies on complex microbial ecosystems that shape grapevine health, soil fertility, and, ultimately, wine quality. In the Vino Nobile di Montepulciano area, recent metagenomic and functional studies have highlighted the presence of plant growth-promoting bacteria (PGPB) with key functional traits that can be harnessed to enhance both vineyard sustainability and wine production[1].

This study presents the biodiscovery and characterization of PGPB strains isolated from vineyard soils and plant rhizospheres in the Vino Nobile di Montepulciano terroir, focusing on their potential applications as microbial probiotics in winemaking.

We applied shotgun metagenomics and culturomics to 320 bulk soil and rhizosphere samples collected during the 2022-2024 seasons.

We identified microbial consortia enriched in biofertilization, biocontrol, and stress tolerance functions, with dominant genera including Pseudomonas and Bacillus. These strains exhibited enhanced functions associated with phosphorus solubilization, nitrogen fixation, and the production of bioactive compounds such as indole-3-acetic acid (IAA) and siderophores, demonstrating their potential to support vine growth while reducing the need for chemical inputs. Laboratory-scale experiments confirmed that selected PGPB strains exert the functions detected by a genome-mining approach.

This study provides the first evaluation of native PGPB from Montepulciano vineyards and explores their possible role in sustainable vineyard management. By integrating these beneficial bacteria as biofertilizers and enological probiotics, this research lays the foundation for a microbiome-based circular bioeconomy, reducing agrochemical dependency while enhancing wine quality through natural microbial interactions. Our findings offer a scalable approach to regenerative viticulture, with implications for terroir expression, climate resilience, and sustainable wine production.

Abstract ID: 518 / Poster Number: 402.15P

L-forms of Bacillus subtilis are more susceptible to the human cathelicidin LL-37 than their walled counterparts

Presenting author: Ms. Jessica Wheatley, Newcastle University, Newcastle upon tyne, United Kingdom

Co-authors: *Katarzyna Mickiewicz, Henrik Strahl*

L-forms are bacterial cells that temporarily lose their cell wall, a feature found in most Gram-positive and Gram-negative bacteria. Under stress, particularly from cell wall-damaging agents, certain bacteria can undergo L-form switching, shedding their cell wall to survive in a cell wall-less state. Once stress is alleviated, they can reassemble their cell wall and resume regular division, perpetuating disease. L-forms are implicated in chronic and recurrent infections, as they evade both the host immune response and commonly used cell-wall-targeting antibiotics. Thus, alternative antimicrobial strategies targeting the cell membrane are needed.

Antimicrobial peptides like LL-37 are cationic, amphipathic molecules that disrupt microbial cell membranes. However, the susceptibility of L-forms to antimicrobial peptides remains unclear. In this study, via a variation of genetic mutations and cell-wall targeting agents, I use the model organism *Bacillus subtilis* to generate L-form bacteria and compare their susceptibility to LL-37 with that of their cell-walled counterparts. Growth and viability assays reveal that L-forms are susceptible to LL-37, and in fact display lysis at lower concentrations than walled bacteria. Fluorescence microscopy shows that at lower concentrations, LL-37-treated L-forms exhibit disrupted morphology and significant membrane damage, while walled bacteria only show membrane depolarization, retaining their characteristic shape.

These results suggest that L-forms have an increased susceptibility to LL-37, likely due to the direct accessibility of their membrane, and supports the hypothesis that antimicrobial peptides could serve as a potential therapeutic approach for infections associated with L-form bacteria.

Abstract ID: 2745 / Poster Number: 022.15P

Linkage between Arctic Tundra methane fluxes and microbial communities' structure and function

Presenting author: Lorenzo Tonin, PhD, University of Insubria, Varese, Italy

Co-authors: *Francesca Berini, Mario Muscarella, Nicoletta Cannone, Mauro Guglielmin, Benedetta Turchetti, Pietro Buzzini, Flavia Marinelli*

Permafrost is the largest global pool of soil organic carbon (SOC) and it is almost ubiquitous in the Arctic regions. Among Arctic regions, Alaska suffered the highest warming in the last 40 years, causing permafrost degradation and release of greenhouse gasses (GHGs). Microbial communities are crucial in this process as their metabolic activities control the rates at which SOC is decomposed and GHGs are emitted.

This study explores the microbial communities' structure and function across three distinct types of arctic tundra (dry, tussock, and wet) in Arctic Alaska to better understand their role in the different CH_4 fluxes observed. Soil cores were collected from each site over two consecutive years, 2023 and 2024. Amplicon sequencing for 16S rDNA and ITS was used to characterize the bacteria, archaea, and fungi communities in these samples.

Results relative to CH_4 fluxes showed that tussock tundra and wet tundra acted as CH_4 sources, while dry heath tundra functioned as CH_4 sink. Preliminary analysis using PCoA with Bray Curtis dissimilarity showed that in the first year of sampling, microbial and fungal communities differed significantly across tundra types and varied with sampling depth ($p<0.001$). Metabolic predictions of bacteria and archaea communities conducted with PICRUST 2.0 confirmed that methanogenic pathways were present only in the wet and tussock tundra, whereas methanotrophic pathways were common in the three different tundra types. Ecological prediction of fungal communities with FUNGuild revealed notable differences in the metabolism of SOC among the dry, tussock and wet tundra ecosystems.

Abstract ID: 1569 / Poster Number: 005.16S

Linking Microbial Electron Transport to Arsenic Dynamics in Wetlands

Presenting author: Sarah Zecchin, PhD, University of Milano, Milano, Italy

Co-authors: *Stefania Marzorati, Lucia Cavalca*

Microbial Extracellular Electron Transfer (MEET) is likely widespread in natural environments, yet the identity, metabolic pathways, distribution and ecological role of electroactive microorganisms remain poorly understood. MEET likely plays a crucial role in cycling redox-active elements such as arsenic, which poses a remarkable issue in flooded rice paddies. This study aims at filling the gap between natural MEET processes and engineered bioelectrochemical systems by investigating MEET-driven arsenic cycling in rice paddies.

A microcosm experiment was conducted, where electrical conductivity of a flooded arsenic-contaminated rice paddy soil was modulated by adding electroactive biochar and anodic polarization. The hypothesis is that increased soil conductivity fosters electroactive microorganisms, allowing analysis of their metabolic pathways through metatranscriptomic profiling.

Preliminary results revealed that biochar-soil mix exhibited significantly lower chemical oxygen demand and bacterial 16S rRNA gene copies compared to controls (250 *vs* 40 mg L^{-1} and 10^8 *vs* 10^3 gene copies g^{-1} soil). However, microbial communities enriched in biochar microcosms displayed significantly higher current transfer efficiency compared to controls ($1 \cdot 10^{-4}$ *vs* $3 \cdot 10^{-5}$ A gene copies^{-1}).

These results confirm the hypothesis and support further investigation into MEET-driven arsenic transformation by enriched electroactive microorganisms while addressing arsenic contamination in rice.

This project is funded by the University of Milano (Piano di Sostegno alla Ricerca PSR 2023 Linea 2–Azione A and PSR 2021 Linea 6 1H_HUB project).

Abstract ID: 3271 / Poster Number: 008.16S

Linking microbial taxa to high enzyme activity in soil aggregates

Presenting author: Mr. Abdelhady Elshal, Johann Heinrich von Thünen-Institut, Braunschweig, Germany

Co-authors: *Fatemeh Dehghani, Damien Finn, Thomas Reitz, Evgenia Blagodatskay Blagodatskay, Christoph Tebbe*

Introduction: Soil aggregates are the biologically relevant scale where nutrient cycling and carbon turnover take place. Within these aggregates, microbial communities synthesize enzymes to carry out their functions such as organic matter mineralization. The microbial taxa that comprise each individual aggregate (ca. 6 mg) are simpler than what is typically observed in most studies that extract DNA from 250 mg of soil. Each community is also unique, potentially with taxa associated with distinct enzyme activities, e.g., hyper-producers of cellobiohydrolase. To address whether individual taxa are responsible for unexpectedly high rates of organic matter mineralization, we investigated enzyme activities and microbial communities in soil aggregates incubated with and without cellulose.

Objectives: This study aimed to: (1) characterize the enzymatic activity of β-glucosidase, cellobiohydrolase, and phosphatase to understand if the enzymes follow classical Michaelis-Menten dynamics at the aggregate scale (2) determine how cellulose amendment, incubation time and aggregate size influence microbial abundance and community composition, and (3) identify specific microbial taxa linked to enzyme activity, particularly in aggregates exhibiting unusually high enzymatic activity.

Methods: Soil was incubated over 42 days with and without cellulose. Enzyme activity was measured after 7, 17 and 42 days across a range of 4-methylumbelliferyl (MUB)-substrate concentrations, and microbial abundance and prokaryotic community composition were assessed through qPCR and amplicon sequencing of the V4 region of the 16S rRNA gene, respectively. qPCR and amplicon sequencing data were merged to calculate absolute abundances of individual taxa. Redundancy analysis (RDA) was used to identify relationships between community composition and enzymatic activity, and what specific taxa were associated with high-activity aggregates.

Results: Enzyme activity followed Michaelis-Menten kinetics across time and MUB-substrate concentrations. β-glucosidase and cellobiohydrolase activities were initially higher after cellulose addition but exhibited two distinct patterns by days 17 and 42, with

some aggregates showing activity similar to controls. Phosphatase activity was unaffected by cellulose addition. Certain aggregates displayed unusually high β-glucosidase activity. Prokaryote populations declined over 42 days, while fungi sustained their abundances by utilizing cellulose. Cellulolytic genera such as *Cellulomonas spp.* were enriched in cellulose-amended aggregates. Smaller aggregates showed higher enzymatic activity. Time and aggregate size were the primary drivers of prokaryotic community composition. RDA indicated a linear relationship between community composition and enzymatic activity, whereby specific genera such as *Sphingomonas* and *Comamonas* were consistently linked to high enzymatic activity.

Conclusion: Enzyme kinetics at the aggregate scale generally follow classical Michaelis-Menten dynamics. Fungi utilize cellulose to sustain their abundances, possibly due to their larger cell size. Smaller aggregates with specific genera, despite their smaller population sizes, are more enzymatically active under stressful conditions. This is possibly due to their higher surface area: volume ratios that could facilitate a relatively greater concentration of added substrate for microbial growth and activity.

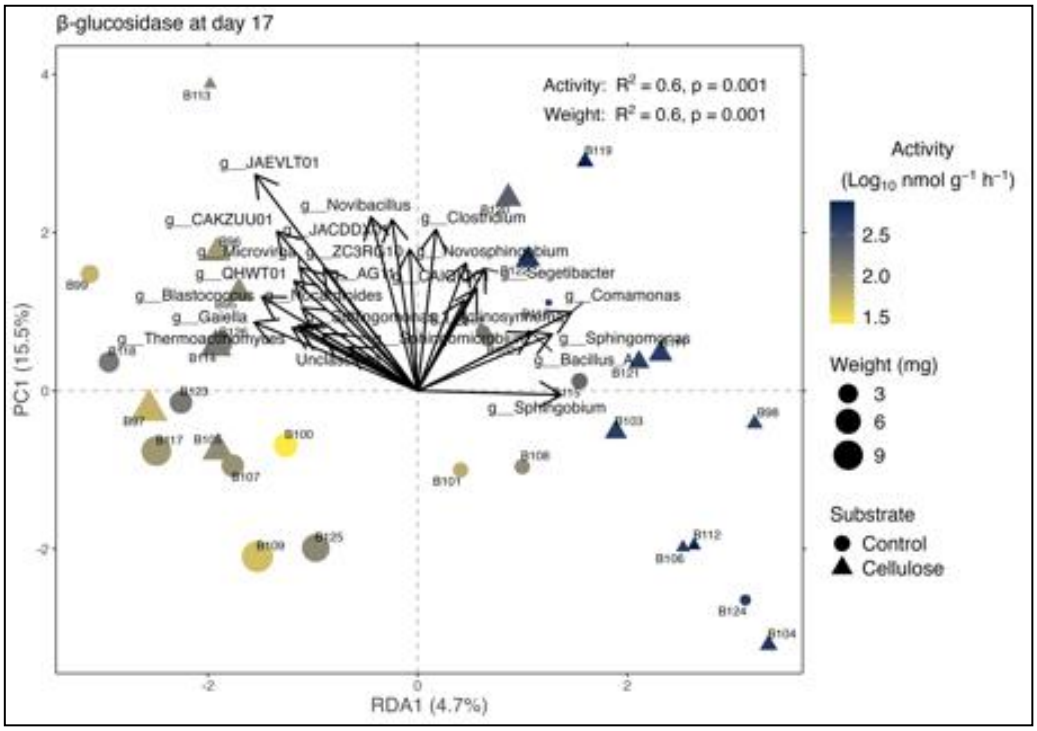


Figure 1: *Redundancy analysis of prokaryotic genera compositions and enzyme activity in aggregates.*

Abstract ID: 3486 / Poster Number: 088.16P

Linking Oral Microbiota Composition to Psychopathy Traits: Insights into the Oral-Brain Axis

Presenting author: Assoc. Prof. Benedita Sampaio-Maia, Faculdade de Medicina Dentária da Universidade do Porto, Porto, Portugal; I3S Instituto de Investigação e Inovação em Saúde, Porto, Portugal

Co-authors: *Carolina Costa, Alexandre Figueiredo, Daniel Radford-Smith, Daniel Anthony, Fernando Barbosa, Philip Burnet*

Introduction: While the Gut-Brain Axis has garnered significant attention for its role in modulating behaviour and neuropsychiatric conditions, the oral microbiota—despite its unique characteristics and proximity to the brain—remains relatively underexplored in this context. The oral microbiota has been implicated in systemic inflammation and neuroinflammatory processes, potentially contributing to cognitive and behavioural outcomes. However, its specific influence on psychopathy, a complex personality construct characterized by traits such as manipulation and antisocial behaviour, remains poorly understood.

Objective: To investigate the relationship between oral microbiota composition and psychopathy traits in the general population.

Methods: A total of 200 participants completed the Self-Report Psychopathy Scale Short Form to whom saliva samples were collected. Their microbiota composition regarding amplicon sequence variant (ASV) was analysed using 16S rRNA sequencing (V4-region). K-means clustering identified psychopathy-related microbial profiles. Differential abundance analyses were performed, followed by correlation and linear regression analyses.

Findings: *Treponema vincentii* was the only species with a significant differential abundance between clusters. Negative correlations were observed between *T. vincentii* and all psychopathy metrics (Interpersonal Manipulation, Callous Affect, Erratic Lifestyle, and Antisocial Behaviour) and Regression analyses indicated that Antisocial Behaviour, Erratic Lifestyle, and the Total score were significant independent predictors of *T. vincentii* abundance.

Conclusion: These findings suggest a potential link between *T. vincentii* and psychopathy traits, highlighting the potential contribution of the oral microbiota to psychopathy traits

within the oral-brain axis. Further research could uncover mechanisms underpinning this relationship, contributing to novel insights into psychopathy and microbiota interactions.

Abstract ID: 2008 / Poster Number: 161.16P

Lipid and carotenoid production by Cystofilobasidium ferigula CCMA 1623 using crude glycerol

Presenting author: Disney Dias, PhD, UFLA – Federal University of Lavras, Lavras, Brazil

Co-authors: *Andreisa Castro, Jenaina Soares, Raphael Longuinhos, Rosane Schwan,*

This study optimized cultivation conditions for maximizing simultaneous lipid and carotenoid production by *Cystofilobasidium ferigula* CCMA 1623 in a crude glycerol medium. Research on this yeast is limited, especially concerning industrial metabolite production. Optimization was performed through response surface methodology using a central composite design to evaluate the effects of yeast extract concentration, temperature, and oxygen supplementation. Lipid analysis via gas chromatography-mass spectrometry (GC–MS) revealed a profile rich in palmitic, stearic, oleic, and linoleic acids, like those in vegetable oils and cocoa butter. The presence of carotenoids and lipids was qualitatively confirmed using comparative Raman spectroscopy, underscoring this technique's rapid, cost-effective, and non-destructive application for analyzing biological products. The total carotenoid extract demonstrated significant antioxidant potential in ABTS, FRAP, and DPPH assays. Under optimal conditions, yields reached 52.59% lipid content and 3.03 µg/mL carotenoid content, presenting promising prospects for the sustainable large-scale production of these valuable metabolites.

Abstract ID: 987 / Poster Number: 156.16P

Lipid nanoparticle-mediated CRISPR-Cas13a delivery into bacterial cells for the control of bacterial infection

Presenting author: Dr. Dajeong Kim, Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea

Co-authors: *Bookun Kim, Hwi Won Seo, Soohyun Lee, Kyuri Lee, Dongeun Yong, Yoon Kyung Park, Yujin Lee, Solip Lee, Do-Wan Kim, Choong-Min Ryu*

Lipid nanoparticles (LNPs) can assist in the delivery of nucleic acid inside animal cells, as demonstrated by their use in COVID-19 vaccine development. However, LNPs applicable to bacteria have not been reported. Here, the screening of 511 LNPs containing random combinations of different lipid components identified two LNPs, LNP 496 and LNP 470, that efficiently delivered plasmids into *Escherichia coli* BW25113. Since Gram-negative bacteria have lipid bilayers, the bacteria were pretreated with LNP-helper that weaken the bacterial membrane. The cationic lipid DOTAP improved delivery of LNP-encapsulated plasmid DNA when it was present at a molar ratio of 10–25 mol% in the LNP. LNP encapsulation of the Cas13a/gRNA expression vector controlled infection by a clinical *Escherichia* strain in *Galleria mellonela* larvae and mouse infection models when used in combination with non-cytotoxic concentrations of polymyxin B, a bacterial membrane disruptor. Together, the results show that LNPs could be useful as a delivery platform for agents that counteract pathogenic bacterial infections.

Abstract ID: 1146 / Poster Number: 283.17P

Lithium Bio-Recovery from discarded smartphones using Altiplanic Exiguobacterium strains

Presenting author: Dr. Juan Castro-Severyn, Universidad Catolica del Norte, Antofagasta, Chile; Universidad Andres Bello, Santiago, Chile

Co-authors: *Catalina Kusch, Andrea Jara, Andrea Avellaneda, Gabriel Kruger, Coral Pardo-Esté, Claudia P Saavedra, Francisco Remonsellez*

Salt flats are reserves of high-value elements such as lithium, a raw material used in rechargeable batteries and critical for the transition to electro-mobility. Although global projections estimate that by 2050 lithium demand will grow by 20-fold, the commonly used evaporitic technology for Li extraction has extensive environmental impacts, intensive use of water and technical limitations. Salt flats such as Salar de Huasco are known to hold great and uncharacterized microbial diversity adapted to thrive in high concentrations of different metal(oids). In poly-extreme environments biological trigger adaptations allow microbes to survive, which can be biotechnologically explored to develop cleaner recovery technologies. Such is the case of the *Exiguobacterium* genus with proven resistance to arsenic, chrome, mercury and the genetic potential to metabolize copper, cadmium, tellurite, cobalt, lead and nickel as well as the biosorption of highly valuable rare earth elements. We aimed to determine the capacity of three *Exiguobacterium* strains isolated from the Salar de Huasco to bio-recover lithium and cobalt from electronic waste. Also to characterize their functional potential and genetic repertoire. We found that the strains were able to biosorp ~90% of the lithium in a 3% solution of lithium carbonate extracted from the anode of cell phone batteries in less than 24 hours. Their genomes contain several mechanisms related to metal(oid)s transport and transformation, and different pathways associated with global stress response, EPS and biofilm formation. We highlight the microorganisms biotechnological potential for developing sustainable methods to recover high-value elements from secondary sources as electronic waste.

Abstract ID: 1433 / Poster Number: 348.16P

Little big heroes – the potential of soil bacteria against osmotic stress

Presenting author: Dr. Karolina Furtak, Institute of Soil Science and Plant Cultivation – State Research Institute, Pulawy, Poland

Co-authors: *Karolina Gawryjołek, Weronika Goraj, Beata Kowalska, Aleksandra Goszcz, Klaudia Dębiec-Andrzejewska*

Ongoing climate change is forcing the search for new adaptation solutions in agriculture. Osmotic stress caused by drought or salinity is one of the main abiotic stresses and directly affects plant growth and yields.

The aim of the study was to characterise bacterial strains isolated from soils subjected to excessive moisture stress and to assess their usefulness in agrobiotechnology.

Bacteria were isolated from soils subjected to simulated flood conditions. More than 80 strains were obtained. Selected strains were tested to determine their properties, *i.a*. ability to ammonification, solubilise phosphorus, produce osmoprotectants, exopolymers, indolyl-3-acetic acid, enzymatic activity, and metabolic potential. The sequencing was performed for taxonomic classification. The bacteria with the highest number of positive results were used in a pilot experiment with spring wheat to determine whether they can promote plant growth.

Results showed that some strains have the capacity for ammonification, phosphorus solubilisation, urease, gelatinase and esterase activity, production of phytohormones, osmoprotectants and exopolymers. Taxonomic classification allowed strains to be assigned to genera such as *i.a*. *Kocuria*, *Bacillus*, *Staphylococcus*, and *Rhodococcus*. In the experiment with wheat, it was reported that plant dry weight was up to 19.23% higher after the addition of bacteria compared to the control under saline conditions.

The study showed that the isolated bacteria possessed properties that enabled them to survive under osmotic stress conditions. In addition, they were found to have a positive effect on wheat.

Abstract ID: 1295 / Poster Number: 207.15P

Live cell imaging and quantification of mixed species biofilms as methods to analyze the dynamics of biofilm formation and gene expression

Presenting author: Dr. Ifey Alio, Universität Hamburg, Hamburg, Germany

Co-authors: *Raphael Moll, Tim Hoffmann, Calvin Tu, Roland Thünauer, Wolfgang Streit*

Introduction: Pathogenic multispecies biofilms play a crucial role in the persistence and virulence of several bacterial infections, complicating treatment methods due to their strong resistance to antimicrobial therapies.

Objective: Our goal is to employ advanced live cell imaging and quantification techniques to enhance our understanding of the time-dependent and spatial dynamics involved in the formation of mixed species biofilms with the following pathogens; *P. aeruginosa*, *S. maltophilia* and *S. aureus*. We further wanted to explore the localization and timing of gene expression within the different layers of these biofilms.

Methods: Using high-resolution microscopy combined with fluorescent reporter fusion constructs, we were able to visualize and quantify the development stages and architectural organization of biofilms composed of key pathogenic species.

Findings: This approach enabled the detailed observation of initial adhesion, microcolony formation, maturation, and dispersion phases in mixed species biofilms in real-time. Importantly, we could mapped the spatial and temporal expression of certain genes associated with antibiotic resistance, virulence factors, and respiration within these biofilms.

Conclusions: The data obtained in this study offer novel insights into the sequential and spatial organization of pathogens in a mixed species biofilm model. These findings improve our understanding of biofilm physiology and pathogenesis, providing potential targets for therapeutic strategies and disruption of biofilm development.

Abstract ID: 3061 / Poster Number: 434.15P

Live cell transcript measurement using in vivo mRNA sensors

Presenting author: Dr. Güler Demirbas Uzel, Technische Universität Wien, Vienna, Austria; Technische Universität Wien, Vienna, Austria

Co-authors: *Matthias G. Stieger*

Introduction: The majority of citric acid is produced through large-scale submerged fermentations with Aspergillus *niger*, which efficiently converts carbohydrates into organic acid with high yields and concentrations. Fungal mycelia develop from individual conidia, each possessing unique genetic and metabolic properties. To analyze the cellular factors critical for citric acid production, we will utilize intracellular RNA-based sensors that allow real-time in vivo measurements at the single-cell level. These live-cell sensors will enable the detection and monitoring of mRNA dynamics in A. *niger* during the process of citric acid production.

Objective: This study focused on developing live-cell mRNA sensors to investigate transcriptional dynamics in A. *niger* during citric acid production. Specifically, we target the mRNA of the citrate exporter gene, cexA, to determine its spatial distribution.

Method: Using the Pepper aptamer approach, synthetic transcripts containing the aptamers will be designed and integrated into the genome. Fluorescence microscopy and plate reader analyses, employing the fluorophore HBC, provide insights into mRNA localization and expression dynamics under varying cultivation conditions. mRNA sensors will be developed to allow real-time measurement of live cell transcripts to provide valuable insights into gene expression dynamics and regulatory processes within fungal tissue**.**

Finding and Conclusions: This work pioneers the application of live-cell mRNA sensors in filamentous fungi, enabling the real-time analysis of transcriptional heterogeneity and its impact on metabolic processes.

Abstract ID: 2591

Longitudinal dynamics of resistome and pathobiome in farmers, livestock and environment in aquaculture floating farms

Presenting author: Mr. Son Hoang Tran, University of Montpellier, Montpellier, France; University of Science and Technology of Hanoi, Hanoi, Vietnam

Co-authors: *Jean-Christophe AUGUET, Quang Huy NGUYEN, Anne-Laure BANULS, Restrepo-ortiz CLAUDIA, Gwenn PULLIAT, Troussellier MARC, Yvan BETTAREL, Thierry BOUVIER, Trung Du HOANG, Christian CULAS, Sylvain THERY, Kinh Hanh NGUYEN, Thi Kieu Oanh NGUYEN, Sebastien VILLEGER, Juliette HAYER, Constantin de Magny GUILLAUME, Fabien RIEUVILLENEUVE, Ramsès Djidjou DEMASSE, Sylvain GODERUIL, Thi Mien PHAM, Dinh Quang VU*

As Earth's population continues to grow rapidly, the demand for increased agricultural productivity rises accordingly. While agricultural advancements have consistently aimed to improve human well-being, they often come with unintended consequences for the health of humans, animals, and the environment. Aquaculture is one of the most fast-expanding sector of animal agriculture. Similarly to inland livestock farming, aquaculture farming may exposes workers to occupational hazards that directly affect the health of half a billion individuals and may play a critical role in the dissemination of antimicrobial resistance. Despite its global public health significance, the impact of these exposures on the structure and function of farmers' native microbiomes, particularly in terms of pathogens and ARGs exposure, remains largely unexplored.

To address this issue, we carried out an 18-month longitudinal survey (covering both the dry and rainy seasons) examining the faecal and skin microbiota of aquaculture farmers within the Nha Trang Bay, Vietnam. To examine the connectivity between compartments, we compared the microbiota of farmers with those of fish livestocks and water samples using a combination of phenotypic and genomic approaches.

Results from amplicon sequence variants (ASVs) analysis revealed the most dominant PPB lineages belonging to the *Bacillaceae* and *Lactobacillaceae* families across all samples. Both results from metabarcoding and bacterial isolation showed a potential circulation of PPB with 16.5% of the total PPB ASVs and 7% of isolated PPB shared between the three compartments. These shared PPB mainly belong to the *Bacteroides, Prevotella, Klebsiella,* and *Vibrio* genera. On the other hand, metagenomic analysis

detected a total of 1216 ARGs from 26 classes with 315 ARGs shared between the compartments, also indicating an active circulation of ARGs. The relative abundance of shared resistome reflected the main classes of antibiotic used on farms with a dominance of ARGs related to tetracycline, beta-lactams, macrolide-lincosamide-streptogramin, and multidrug resistance, which together accounted for 86.5% and 16.9% of the shared and total ARGs, respectively. In agreement, high phenotypic resistance to beta-lactams (penicillins, cephalosporins, monobactams) were observed. The composition of the pathobiome and resistome across the three compartments showed no seasonal variation, suggesting strong temporal stability.

Overall, our study demonstrates the interconnectedness of farmers, livestock and environment pathobiomes and resitomes. Our results also indicate that aquaculture farming may play a critical role in the dissemination of antimicrobial resistance, with potential consequences on human and livestock health.

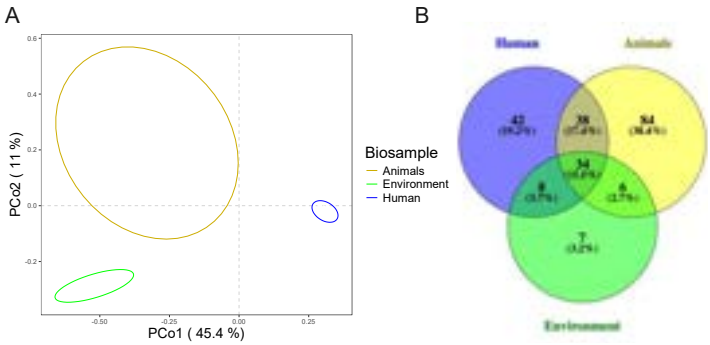


Figure 1: Resistome composition between the human, animal and envisonment compartments based on metagenomic analysis. A: PCoA plot with Bray-Curtis distances. B: Venn diagram of ARGs detected in each compartment.

Abstract ID: 884 / Poster Number: 348.15F

Longitudinal study of archaeal, bacterial and micro-eukaryotes communities from an unconfined aquifer in southern Quebec (Canada)

Presenting author: Karine Villeneuve, PhD, University of Quebec in Montreal, Montréal, Canada

Co-authors: *Cassandre Lazar*

Subsurface environments host an impressive diversity of microorganisms from all three domains of life. Until recently, these diverse communities were thought to be relatively more stable than their surface counterparts. However, recent studies have revealed dynamic temporal patterns of community succession driven by periodic disturbances in the form of groundwater recharge. Yet, most of these studies focused solely on bacterial communities and therefore response of archaeal and micro-eukaryote microbial communities to events of groundwater recharge remains poorly understood. In this study, we aimed to assess the temporal shifts in community composition of bacteria, archaea, and micro-eukaryote from an unconfined granular aquifer of the Vaudreuil-Soulanges region in southern Quebec (Canada). Groundwater samples were collected every 14 days from November 2021 to October 2022 through three piezometers installed 3 meters apart from each other. Microbes were identified using 16S and 18S rRNA gene sequencing. Results suggest distinct linear time-dissimilarity relationship between the different domains. While the archaeal communities were mostly stable across time, the micro-eukaryotes communities were more dissimilar from one sampling to another. Furthermore, our results revealed that communities of micro-eukaryotes sampled pre-groundwater recharge and during groundwater recharge were not significantly distinct ($p > 0.05$) as opposed to both groups of prokaryotes whose communities were significantly distinct between both periods. These findings support the importance of including all microbial domains in longitudinal analyses and highlights the knowledge gap surrounding micro-eukaryotes in aquifers.

Abstract ID: 1848 / Poster Number: 113.16P

Long-read metagenomics of Shanghai pet dogs captures global pet dog gut microbial diversity and recovers hundreds of bacterial species as high-quality MAGs

Presenting author: Anna Cusco, PhD, Fudan University, Shanghai, China

Co-authors: *Yiqian Duan, Alexei Chklovski, Fernando Gil, Shaojun Pan, Ulrike Löber, Sofia K. Forslund, Xing-Ming Zhao, Luis Pedro Coelho*

Introduction: Pet dogs interact closely with their humans, sharing spaces and even microbes. Understanding dog microbiomes can provide insights into animal and household health. However, most of the dog gut microbiome studies focus on colony animals and rely on short-read sequencing (16S rRNA or shotgun metagenomics), limiting our understanding of the microbiomes of pet dogs.

Objectives: Using long-read metagenomics sequencing, we aim to characterize the gut microbiome of Shanghai pet dogs, and to recover high-quality metagenomes assembled genomes (MAGs).

Methods: We collected faecal samples from a cohort of 50 pet dogs in Shanghai, alongside dog-associated metadata through a detailed questionnaire (>80 questions). For each sample, we obtained a minimum of: 20Gb of long-read metagenomics data (Oxford Nanopore Technologies) to assemble the metagenomes; and 20Gb of short-read metagenomics data (Illumina) to polish the assemblies.

Findings: Using long-read metagenomics, we assembled 2,676 MAGs from the gut microbiomes of Shanghai pet dogs, representing 320 distinct bacterial species (Figure 1). The most recovered MAGs belonged to the Bacillota_A phylum, with *Blautia hansenii*, *Faecalimonas umbilicata*, and *Ruminococcus_B gnavus* present in nearly all dogs in the cohort. We mapped external pet dog datasets from diverse geographies (USA, Germany, South Africa) to our MAG collection, achieving mapping rates of >90%, which suggests that our MAG collection captures the majority of pet dog gut microbes.

For species with publicly available genome assemblies, we selected reference genomes from RefSeq or GenBank and compared them to the species-level representative MAGs in our dataset. Our representative MAGs were significantly more contiguous and typically contained a greater number of ribosomal genes, tRNAs, and mobile genetic elements, demonstrating the advantages of long-read sequencing for genome reconstruction.

Long reads also enable the precise localization of antimicrobial resistance genes (ARGs) within their genomic contexts. Notably, we identified multi-drug resistant bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*. Further investigation is needed to evaluate their pathogenicity, zoonotic potential, and the distribution of ARGs in plasmids and other genomic regions.

Conclusions: Long-read metagenomics allowed the assembly of highly contiguous, high-quality MAGs from the gut microbiomes of dogs. The high mapping rates from external datasets suggest that our Shanghai-based MAG collection is broadly representative of the pet dog gut microbial diversity.

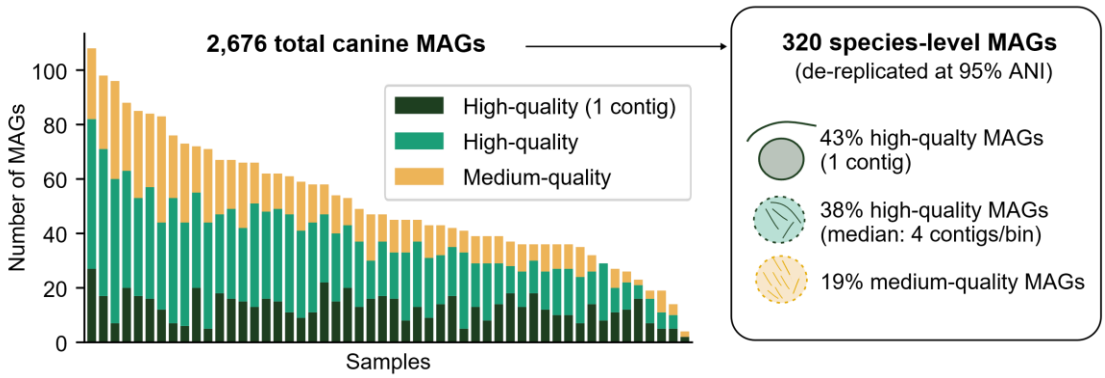


Figure 1. MAG catalogue of Shanghai pet dogs. The catalogue has a total of 2,676 canine MAGs, from which can be obtained 320 species-level MAGs (dereplicated at 95% ANI). Stacked bar plots show the total number of MAGs per sample, which are coloured by their quality.

Abstract ID: 2024 / Poster Number: 307.17P

Long-term adaptive laboratory evolution of Saccharomyces cerevisiae for high-titer lactic acid production at low pH

Presenting author: Dr. Martin Altvater, BOKU University, Vienna, Austria

Co-authors: *Irene Tomico-Cuenca, Michael Sauer, Diethard Mattanovich*

Introduction: Lactic acid (LA) is a versatile molecule with a wide range of applications in the polymer, food, chemical, pharmaceutical, and cosmetics industries. The growing demand for LA, particularly as a building block for the biodegradable polymer polylactic acid, emphasizes its industrial importance. Traditionally, LA is produced via microbial fermentation using lactic acid bacteria. Despite their natural LA production capabilities, these bacteria suffer from high sensitivity to the low pH produced during acid production. To counteract the acidity, neutralizing agents are added during fermentation, resulting in unwanted and costly waste by-product formation during downstream processing. To overcome these drawbacks, yeasts have emerged as promising alternative production hosts due to their higher tolerance to low pH and simpler media requirements, lowering costs and facilitating product purification.

Methods: In our study, we use *Saccharomyces cerevisiae* as an alternative LA production host. Unable to produce LA naturally, we genetically engineered the LA production pathway into this new yeast production strain. To enhance its robustness, adaptive laboratory evolution (ALE) was employed in shake flasks over a period of three years. This approach involved iterative cycles of selection under progressively more stringent conditions, such as high LA titers and low pH.

Findings: The evolved strains exhibited enhanced robustness at low pH and high LA concentrations, eventually increasing lactic acid production by up to 200%.

Conclusion: Overall, our approach proves the great potential of ALE in developing powerful microbial cell factories for organic acid production.

Abstract ID: 2226 / Poster Number: 017.15P

Long-term effects of ash-fertilization on the composition and functional gene pool of soil microbiome in drained peatland forests

Presenting author: Mx Matilda Kattilakoski, University of Helsinki, Helsinki, Finland

Co-authors: *Jenni Hultman, Krista Peltoniemi, Paavo Ojanen, Päivi Väänänen*

Finland aims to increase the use of wood ash in fertilizing drained peatland forests to improve annual carbon capture in the land use sector (1). While wood ash increases tree growth, its effects on the soil greenhouse gas (GHG) cycle and soil microbial communities are not fully understood (2, 3). To gain a comprehensive understanding of the overall GHG balance of a forest, it is crucial to understand the role of the soil microbiome.

We investigate the impact of ash-fertilization on soil microbial communities and their functional gene pool, as changes in these can impact GHG cycling, carbon storage, and overall biodiversity in drained peatland forests.

The experiment consists of seven ash-fertilized peatland forest sites in Finland, representing three drained peatland forest site types with different nutrient levels. Each site has an ash-fertilized plot and an adjacent unfertilized control plot. The plots have been fertilized (5-10 tons of ash per hectare) at different times, the oldest in 1979 and the most recent in 2014. DNA was extracted from a total of 168 soil samples, followed by metagenomic sequencing and analysis.

Our findings reveal that location and site type influence microbial community composition more than ash treatment. This indicates that microbial communities in drained peatland forest soils are diverse and vary between forests. To further understand impacts on the GHG cycle, we are analyzing genes related to GHG cycling. These analyses will show how ash-fertilization affects the functional potential of microbial communities in these soils.

Abstract ID: 2819 / Poster Number: 256.15P

Long-Term Effects of Biochar on Vineyard Soil: a mesocosm experiment

Presenting author: Dr. Elisabetta Toni, Unoversity of Florence, Florence, Italy

Co-authors: *Matteo Daghio, Francesco Primo Vaccari, Alessadra Lagomarsino, Roberta Pastorelli, Carlo Viti*

Applying biochar is important in mitigating greenhouse gas (GHG) emissions. Several studies have shown that adding biochar to soil can reduce methane, nitrous oxide, and carbon dioxide emissions. Additionally, its application stimulates soil microbial activity. Previous studies have also shown that biochar can alter the structure of microbial communities. However, there is little published data on the long-term behavior of biochar in soil. This study aims to assess the effect of long-term application of biochar on GHG emissions. Untreated vineyard soil, vineyard soil with a single application of biochar at a rate of 22 t ha-1 in May 2009, and vineyard soil with two applications of biochar at 22 t ha-1 each in May 2009 and February 2010 were used to set up mesocosms in 1000 cm^3 polyethylene jars, each filled with 0.25 kg of dry soil. Mesocosms were unsupplemented or supplemented with digestate or urea. The GHGs production was measured 10 times in 21 days using a portable DX4040 (FTIR) Gas Analyzer (Gasmet Technology Oy, Helsinki, Finland). A soil sample was taken at the beginning and end of incubation to evaluate the composition and diversity of microbial communities. DNA was extracted from 0.5 g of soil. To assess bacterial activity, RT-qPCR was conducted. Data analyses showed that soil with a double dose of biochar showed lower cumulative CO_2 and N_2O emissions rates and significant differences in the microbial community. Biochar amended on soil long-term retains its GHG mitigation properties and modulates microbial community.

Abstract ID: 3665

Long-term hypoxia drives chronic infection adaptation of cystic fibrosis lung pathogens and alters the abundance of orthologous proteins in different species

Presenting author: Dr. Joanna Drabinska, School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland

Co-authors: *Ciaran J. Carey, Niamh Duggan, Carlos Vivas Rodriguez, Lucia O'Connor, Siobhán McClean*

Introduction: Opportunistic bacterial infections are an increasing threat, especially for immunocompromised individuals such as people with cystic fibrosis (CF), with a track record in developing collaborations contributing to morbidity and mortality[1]. *Pseudomonas aeruginosa* (Pa), *Burkholderia cenocepacia* (Bc), and *Mycobacterium abscessus* (Mab) cause chronic lung infections, and have been extensively studied in this context[2], but the mechanism(s) driving their adaptation towards chronic colonisation in the CF lung is not fully understood.

Objective(s): Hypoxia is an important environmental pressure present in the CF lung, therefore we investigated the adaptations of pathogens to long-term hypoxia, to explore whether it drives the development of persistence in CF patients.

Methods: We examined how early CF isolates of the three species adapted to 6% oxygen over extended periods, using an experimental evolution approach followed by proteome analysis (Pa, Bc, Mab) and phenotypic characterisation (Bc, Mab) of the adapted cultures.

Findings: We identified changes in hypoxia adapted cultures that had been previously associated with chronic infection isolates: increased biofilm production, antibiotic and stress resistance, and decreased siderophore production in Pa[3]; increased oxidative stress resistance, host-cell attachment, and virulence in Bc; increased oxidative stress tolerance, intracellular survival and immune evasion in Mab. However, interspecies comparisons of proteomics data were challenging due to inconsistent functional annotation and varying protein IDs. To address this, we used OrthoFinder to develop an orthology-based tool for interspecies comparisons of proteomic datasets, enabling faster analysis with minimal bioinformatics expertise. Consequently, we identified 11 orthologs that were altered in all three pathogens, including five proteins previously identified as novel drug targets. Among the 11 were: GlpK, shown to contribute to bacterial fitness, regulatory Rph RNAse, and stress resistance-associated Qor quinone

oxidoreductase. Moreover, the abundance of universal stress proteins, histone-like proteins and Histidine Triad superfamily proteins was altered across all three pathogens.

Conclusion: Overall, we demonstrate that long-term hypoxia exposure contributes to multiple changes seen in CF chronic infections and that hypoxia adaptation of bacteria is likely to affect treatment outcomes (Fig.1).

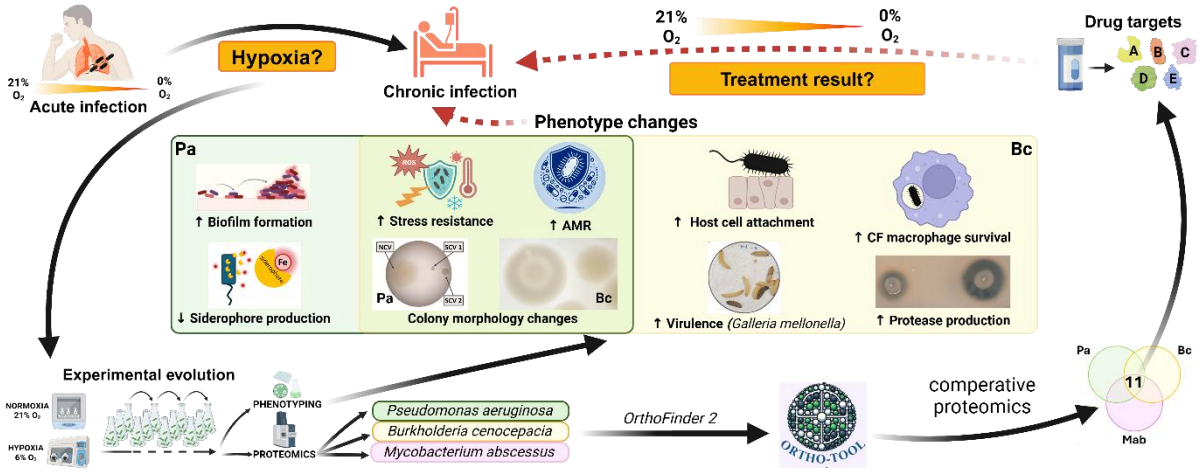


Figure 1: Graphical abstract showcasing our methods and key results, including a developed tool for orthology comparisons of proteomic datasets; five proteins commonly altered by long-term hypoxia in Pa, Bc and Mab which are drug targets, common and distinct changes of phenotype in Pa and Bc, which lead to a conclusion that hypoxia contributes to chronic infection adaptation and may affect treatment efficiency.

Abstract ID: 3124 / Poster Number: 008.17S

Looking for manganese-oxidizing autotrophs in microbial biofilms from historic mining sites of Upper Franconia

Presenting author: Dr. Dimitri Meier, University of Bayreuth, Bayreuth Centre for Ecology and Environmental Research, Bayreuth, Germany

Co-authors: *David Burger, Moritz Kallmeier, Felix Beulig, Martin Obst, Tillmann Lueders*

Introduction & Objectives: Redox cycling of iron (Fe) and manganese (Mn) can be an important source of energy for microorganisms in sediments and subsurface habitats. While Fe oxidation is well known to support autotrophic growth in various microorganisms and environments, the first chemolithoautotrophic Mn-oxidizing organism, *Candidatus* Manganitrophus, was only recently enriched from tap water[1]. However, it is reasonable to assume that autotrophic Mn-oxidation occurs in a wider range of taxa. Truly estimating the distribution and importance of chemolitho(auto)trophic Mn-oxidation remains a major challenge for environmental microbiology. Here, we report on massive, asphalt-like microbial biofilms from three historic mining sites in Northern Bavaria, which may well represent a natural habitat for autotrophic Mn-oxidizers.

Methods: We analyzed the chemical composition of the water seeping through the biofilms and the elemental composition of the biofilms and generated enrichments of Mn oxidizers. Furthermore, we quantified the microbial biomass by fluorescence microscopy, characterized the community composition of various Mn-accumulating and non-Mn-accumulating biofilms by 16S rRNA gene sequencing and analyzed the metabolic potential of metagenome-assembled genomes from biofilms and run-off water.

Findings: The biofilms were highly enriched in Mn oxides, with Mn making up to 40% of the biofilms' dry weight. Mn depletion was observed from the water while flowing through the biofilms. Identification of Mn(II), Mn(IV) and the labile Mn(III) in microbial cells via scanning transmission X-ray microscopy coupled to near-edge x-ray absorption fine structure spectroscopy suggested an ongoing biological Mn oxidation in situ. Cell numbers and morphologies, as well as taxonomic composition of the microbial communities differed between different types of biofilms and different mining sites, with multiple taxa being specifically enriched in Mn-accumulating biofilms. In lab cultures, we were able to reproduce the enrichment of *Manganitrophaceae* organisms on insoluble $MnCO_3$ substrate[1,2], though its relative abundance in natural biofilms was low. Nearly half

of the MAGs reconstructed from metagenomes encoded multi-heme cytochromes indicative of extracellular electron transfer, a necessary capability for electron exchange with a mineral surface. Most of the potential autotrophs MAGs contained multi-heme cytochromes and homologs of iron-oxidation genes *cyc2* or *pioA*. These potential Mn-oxidizing autotrophs belonged to multiple distant taxonomic groups like Gammaproteobacteria, *Manganitrophaceae* (*Nitrospirota*), *Acidobacterota* and a yet uncharacterized bacterial phylum, and constituted up to 23% of the community. Microcosms experiments aimed at measuring the fixation of $^{13}CO_2$, Mn-oxidation activity, and protein expression are currently ongoing.

Conclusion: Chemical analysis and metabolic potential of abundant microbiota support our hypothesis that metal-cycling microorganisms may be the major primary producers in the studied mine biofilms. Based on phylogenetic affiliation of the MAGs to genomes from lakes, rivers, groundwater, and soil, these peculiar biofilms can be seen as a natural enrichment of Mn-cycling freshwater microorganisms and will help us understand the relevance of microbial Mn cycling in freshwater ecosystems.

References: 1. Yu, H. *et al*. *Nature* 583, 453–458 (2020), 2. Yu, H. *et al*. *MBio* 13, e0342121 (2022)

Abstract ID: 2327 / Poster Number: 234.15P

Loss of the PLP homeostasis protein, YggS, results in conditional lethality of a metJ mutant in Salmonella enterica

Presenting author: Ms. Kailey Ezekiel, University of Georgia, Athens, United States

Co-authors: *Diana Downs*

Pyridoxal 5'-phosphate (PLP) is the active form of vitamin B_6 and a critical cofactor in numerous enzymes in all domains. Conserved PLP homeostasis proteins (PLPHP) influence B_6 homeostasis, though no clear biochemical mechanism of these proteins has been defined. YggS is a PLPHP homolog in *Salmonella enterica;* loss of *yggS* causes multiple subtle phenotypes. A lesion in *yggS* prevents a strain lacking the transcriptional repressor, MetJ, from growing with acetate as the sole carbon source. The *yggS* and *metJ* single mutants had no detectable growth defect on acetate. Loss of *metJ* is known to cause derepression of genes involved in methionine metabolism. Biochemical genetic approaches applied to this system have clarified the cellular role of YggS. The synthetic lethality on acetate is suppressed with serine, lysine or diaminopimelic acid (DAP) supplementation. Spontaneous suppressors contain mutations that compromise the homoserine dehydrogenase activity of the bifunctional enzymes MetL, or ThrA. These data suggested a working model where increased flux to lysine, and/or DAP, via aspartate semialdehyde is critical for growth of a *yggS metJ* mutant on acetate. Involvement of YggS in B_6 homeostasis suggests a model where a PLP-dependent enzyme in this pathway is compromised in a *yggS* mutant. Genetic and biochemical approaches, in combination with knowledge of the MetJ regulon, will be used to test and refine this model. The results to be discussed provide new insights into the role of YggS not only in maintaining B_6 homeostasis but ensuring stability of the metabolic network in *S. enterica*.

Abstract ID: 3104 / Poster Number: 449.15P

Macroalgae-Associated Actinomycetota: A Genomic Approach to Natural Product Discovery

Presenting author: Ms. Gabriela Alves Moreira, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal; I3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal; ICBAS – School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal

Co-authors: *Mariana Girão, Nádia Eusébio, Marta V. Mendes, Maria F. Carvalho*

Symbiotic Actinomycetota are a group of Gram-positive bacteria that remain underexplored despite having a metabolic potential comparable to their free-living counterparts.

This work aimed to investigate the biosynthetic potential of 12 actinobacterial isolates obtained from two Portuguese marine macroalgae, *Codium tomentosum* and *Chondrus crispus*, through the analysis of their genomes. Total DNA from the 12 isolates was sequenced using Illumina 2×250 bp paired-end technology and generated 14 *de novo* genome assemblies. Phylogenomic analysis confirmed previous 16S rRNA-based identification showing that they belong to the genera *Actinoalloteichus*, *Cellulosimicrobium*, *Kocuria* or *Streptomyces*. Genome mining using antiSMASH identified over 300 biosynthetic gene clusters (BGCs) across 19 metabolite classes. Some of the identified BGCs presented partial or no similarity to known clusters, while others were fully matched, indicating their potential to produce both known and novel compounds. For non-*Streptomyces* strains, 55% of the BGCs had no or limited similarity to known clusters (0-20%) and for *Streptomyces* strains this percentage was 36%. Conversely, 38% of *Streptomyces* BGCs and 11% of non-*Streptomyces* BGCs exhibited high similarity (81–100%) to known clusters. Within the clusters with high similarity are conserved metabolites, siderophores, pigments, and antimicrobials, among others.

The results of this analysis confirm the production of relevant natural compounds by macroalgae-associated Actinomycetota and emphasize the potential for discovery of new compounds, mainly in non-*Streptomyces* strains.

Abstract ID: 1853

Mak toxins as the ultimate weapons of Vibrio cholerae against ciliated protozoa

Presenting author: Mr. Jonah Moon, University of Technology Sydney, Sydney, Australia

Co-authors: *Gustavo Espinoza-Vergara, Mozammel Hoque, Parisa Noorian, Joyce To, Scott A. Rice, Diane McDougald*

Vibrio cholerae is the responsible agent of the acute diarrhoeal disease cholera. Previous work has shown that *V. cholerae* possesses several grazing resistance mechanisms against protozoa. For example, *V. cholerae* survives intracellularly and escapes as viable cells inside protozoan expelled food vacuoles (EFVs). We previously reported that *V. cholerae* encased in EFVs are hyper infectious, establishing an important link between anti-protozoal strategies and bacterial virulence. However, the molecular mechanisms behind grazing resistance remain poorly understood. To address this gap in knowledge, this work used the novel single cell RNA-transcriptomics of *V. cholerae* in coincubation experiments with the ciliated protozoan *Tetrahymena pyriformis*.

Recent results captured a total of 5,344 bacterial cells with heterogeneous gene expression. Cells with the same pattern of gene expression were grouped, resulting in eleven clusters of cells with a unique gene expression profile. Genes encoding outer membrane proteins, F1F0-Na+/H+ ATPase, metabolites and toxins showed differential expression among the clusters. Furthermore, the motility-associated killing factor (Mak) toxins (*makA, makB* and *makC*) were differentially expressed. A *V. cholerae* Δ*makA* strain was not capable of killing *T. pyriformis* and showed reduced survival inside EFVs compared to the wild type. Our findings reveal new insights into the grazing resistance mechanisms of *V. cholerae*, identifies factors associated with the survival of *V. cholerae* within EFVs and more broadly, highlights the connection between antiprotozoal and virulence factors displayed by pathogenic bacteria.

Abstract ID: 2021 / Poster Number: 015.15P

Making the difference?! Methodological considerations, challenges and future perspectives for the state-specific extraction of environmental DNA

Presenting author: Ms. Julia Zöhrer, Universität Innsbruck, Innsbruck, Austria

Co-authors: *Judith Ascher-Jenull, Andreas Otto Wagner*

The total environmental DNA (eDNA) pool is composed of intracellular (iDNA) and extracellular DNA (exDNA). While the former is present within structurally intact cells, the latter is located outside thereof in the extracellular environment. Compared to the directly extracted eDNA, the separation and differential analysis of iDNA and exDNA reveals more profound insights into the composition of either currently prevalent microbial communities being involved in biogeochemical cycles or past assemblages, respectively. Starting in the 1980s, a multitude of methodological approaches for the separation of exDNA and iDNA from environmental samples has been published. Even though many of them are conceptually similar, methodological biases are hardly acknowledged. Here, we categorize the major differences between these protocols and present a methodological approach to assess their impact on molecular microbial community studies. Based on single-gene deletion mutants of *Escherichia coli* and *Bacillus subtilis*, we developed spike-and-recovery controls for the extraction of eDNA simultaneously accounting for both the different eDNA states (iDNA, exDNA) as well as their bacterial origin (gram-negative, gram-positive) in a single experiment. The proposed approach was proven successful within different environments including soil, sediment, compost and sludge from anaerobic digestion. Hence, it emphasizes its potential to better understand the methodological ambiguities related to the separation of iDNA and exDNA, and thus facilitating the validation and further optimization of such protocols.

Abstract ID: 3572 / Poster Number: 177.16P

MALDI in Clinical Diagnostics: A Comparison of the Microflex LT and EXS2600 Systems in Practice

Presenting author: Dr. Ewelina Sibińska, Nicolaus Copernicus University, Toruń, Poland

Co-authors: *Adrian Arendowski, Piotr Fijałkowski, Dorota Gabryś, Paweł Pomastowski*

Introduction: Matrix-Assisted Laser Desorption/Ionization (MALDI TOF MS) has revolutionized clinical microbiology by offering a rapid and reliable proteomic approach for microorganism identification, replacing traditional biochemical methods.

Objective: This study evaluates the performance of two commercial systems, Microflex LT Biotyper and EXS2600 Ex-Accuspec, in rapid identifying urinary microbiome from patients with prostate cancer.

Method: A total of 1979 urinary isolates were analyzed using the direct extraction method on both MALDI TOF MS systems. Identification was evaluated at the genus and species levels, with a focus on consistency between devices and the quality of spectral scoring for gram-negative and gram-positive bacteria.

Findings: Biotyper and Ex-Accuspec systems identified 95.6 % and 92.4 % of isolates to at least the genus level, respectively. Results for 89.5 % of spectra were consistent across both systems. Gram-negative bacteria achieved the highest score values and species-level identification rates in both devices.

Conclusion: The results confirm that both the BioTyper and EXS2600 effectively provide rapid and accurate identification of clinical isolates, essential for timely diagnosis and treatment. Validating new mass spectrometers like the EXS series requires thorough analysis of factors such as matrix selection, sample preparation, and ongoing database updates. These findings highlight the need for continuous improvements in mass spectrometry methods and databases to enhance microbiological diagnostics.

Abstract ID: 4144

MALDI-ToF mass spectrometry, Fourier Transform Infrared Spectroscopy and Artificial Intelligence: new tools for the Microbiology Laboratory

Presenting author: Valeria Fox, Multimodal Laboratory Medicine, Bambino Gesù Children's Hospital, Rome, Italy

Co-authors: *Gianluca Vrenna, Martina Rossitto, Serena Raimondi, Marco Cristiano, Gianluca Foglietta, Carlo Federico Perno, Paola Bernaschi*

In recent years, clinical microbiology has undergone a series of transformations driven by emerging technologies and computational tools. Traditional identification methods, while still essential, are no longer sufficient to address complex clinical questions about microbial behavior, such as virulence, resistance, and epidemiological patterns. In this context, we present our experience combining MALDI-ToF mass spectrometry, Fourier-transform infrared spectroscopy (FTIR), and artificial intelligence (AI) to develop predictive models that support timely and informed clinical decision-making.

The objective of our work is to move beyond microbial identification and provide rapid insights into pathogen characteristics that directly impact patient care. Our method involves the integration of spectral data (from MALDI-ToF and FTIR) with whole genome sequencing (WGS), used as a reference standard for model training and validation. Machine learning algorithms are then applied to build predictive models capable of inferring features such as resistance mechanisms, virulence profiles, or clonality from new clinical isolates.

We present a series of practical applications developed at our hospital, including the use of FTIR to distinguish between persistent and newly acquired *Pseudomonas aeruginosa* strains in cystic fibrosis (CF) patients—allowing clinicians to select the most appropriate treatment within hours. We also report the development of an FTIR-based model to predict *Streptococcus pyogenes emm*-types, a tool with particular relevance in light of recent increases in invasive infections caused by the M1UK sub-lineage. Our results show high accuracy and promising clinical utility, reducing the need for time-consuming sequencing approaches.

In addition, we are currently integrating longitudinal genomic and spectral data to assess the prognostic significance of *P. aeruginosa* clones in CF, and extending our models to predict resistance markers such as VIM, OXA-23, and KPC directly from FTIR and MALDI

spectra. Ongoing efforts also include the development of MALDI-ToF-based models to discriminate individual species in polymicrobial cultures.

In conclusion, our approach demonstrates how integrating spectral technologies with AI enables microbiology laboratories to deliver actionable insights in clinically relevant timeframes. These innovations support faster diagnoses, tailored therapies, and ultimately better outcomes for patients.

Abstract ID: 3195

Manipulating bacterial metabolism to overcome antimicrobial resistance

Presenting author: Dr. Merve Zeden, University of Galway, Galway, Ireland

Co-authors: *Aaron C. Nolan, Jessica B. Kelly, James P. O'Gara*

Introduction: Antibiotics have long represented a successful form of therapy in medicine. However, infections caused by antimicrobial resistant (AMR) pathogens are on the rise, causing significant burden on healthcare worldwide. Therefore, finding novel, innovative and sustainable ways to preserve the efficacy of currently licensed antimicrobial drugs is a central part of efforts to address the AMR crisis. Central metabolic pathways control virulence and antibiotic resistance, and constitute potential targets for antibacterial drugs.

Objective(s): The overarching objective of this project is to evaluate the hypothesis that manipulating the metabolism of *Staphylococcus aureus* with the use of purine nucleosides, and anti-sense oligonucleotides (ASOs) have therapeutic potential as inhibitors of growth and resistance in *S. aureus* and will re-sensitize Methicillin Resistant *S. aureus* (MRSA) to antibiotics. This multidisciplinary project aims to enable re-purposing of antibiotics as part of efforts to overcome resistance in *S. aureus*, whilst also investigating the mechanism of purine and cell-penetrating oligopeptide (CPP) conjugated ASO uptake into *S. aureus* cells.

Methodology: MRSA cells were grown with exogenous purine nucleoside supplementation in combination with antibiotics, MICs and synergy experiments were carried out. Cells were examined using transmission electron microscopy to investigate physiological changes. Kill curve assays were carried out to determine the efficacy of combination treatments on cell viability. Metabolomics and proteomics approaches were used to determine the molecular level details and identify mechanism of action. To deliver ASOs into cells, CPPs were synthesised using Fmoc solid-phase peptide synthesis with specific linkers. Confocal microscopy and flow cytometry are used to evaluate the incorporation of fluorescently labelled CPPs into cells of *S. aureus*. Conjugation experiments are undertaken to establish optimal click-chemistry.

Findings: Exogenous guanosine and xanthosine, which are fluxed through the GTP branch of purine biosynthesis were shown to significantly reduce MRSA beta-lactam resistance (Figure 1). Mutations that interfere with *de novo* purine synthesis (*pur* operon),

transport (NupG, PbuG, PbuX) and the salvage pathway (DeoD2, Hpt) increased beta-lactam resistance in MRSA strain JE2. The purine-derived signalling molecule c-di-AMP levels were altered in Guanosine + Oxacillin treated cells, which underwent morphological changes, with decreased viability, integrity, and thinner cell walls. Thus, we identified that purine availability can control antibiotic susceptibility and the MRSA resistance to beta-lactam antibiotics can be manipulated with the use of purine nucleosides. In addition, we have identified CPPs that penetrate *S. aureus* cells efficiently, which represents an ideal cargo molecule for the delivery of ASOs into cells.

Conclusion: Uncovering the capacity and physiological impact of purine nucleosides and ASOs on metabolism, growth and virulence of *S. aureus* represents an exciting research avenue to be explored. These novel antimicrobial agents will be used in new drug combinations with antibiotic potentiators currently under investigation in our laboratory, to enhance MRSA eradication.

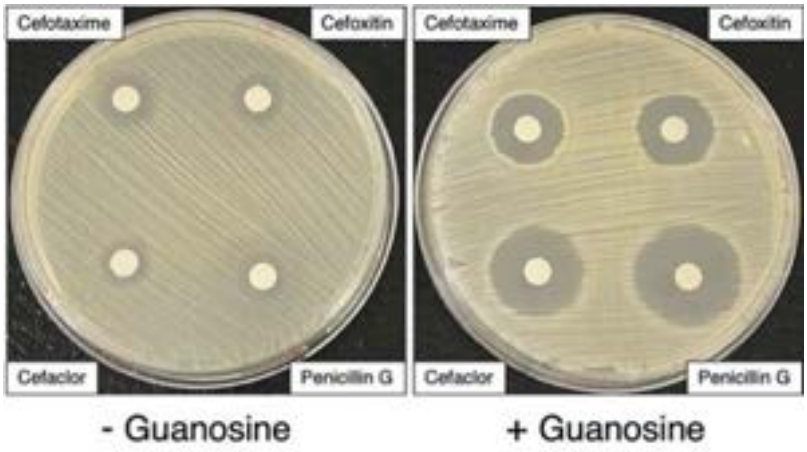


Figure 1: Exogenous guanosine increases the susceptibility of MRSA to beta-lactam antibiotics. (A) Susceptibility of JE2 to cefotaxime (top left), cefoxitin (top right), cefaclor (bottom left) and penicillin G (bottom right) Mueller-Hinton 2% NaCl agar (MHA) (-Guanosine) (left), or MHA supplemented with 0.2 g/l guanosine (+Guanosine) (right).

Abstract ID: 1142 / Poster Number: 444.15P

Mapping the Lineage-Specificity of Propanediol Fermentation in Limosilactobacillus reuteri and Assessing 1,2-Propanediol Competition Between Bovine-Lineage Limosilactobacillus reuteri and Salmonella Dublin

Presenting author: Mr. Martin Moran, The University of British Columbia, Vancouver, Canada

Co-authors: *Michael Gänzle, Nilusha Malmuthuge, Leluo Guan*

Microbiota encoding the propanediol utilization (*pdu*) operon ferments 1,2-propanediol, a gut metabolite that often provides a competitive growth advantage *in vivo*. It is conserved among *Salmonella* Dublin, a cattle-adapted pathogen, and is encoded by some of the host-adapted lineages of *Lm. reuteri*, a gut commensal. This study aimed to a) characterize the lineage-specific distribution of *pduCDE* in *Lm. reuteri* genomes, and b) assess 1,2-propanediol competition between cattle-adapted *Lm. reuteri* and *S*. Dublin *in vitro*. Three calf-isolates of *Lm. reuteri* (L8, L11, and L12) were either monocultured or co-cultured with *S*. Dublin in Mueller-Hinton and de Man, Rogosa, Sharpe media with and without 50 mM 1,2-propanediol. Viable plate counts were determined after anaerobic incubation (37°C, 24 h), and data were analyzed using Kruskal-Wallis and Dunn's tests. Analysis of published genomes (n=380) revealed five novel *Lm. reuteri* lineages (XI-XV) based on average nucleotide identity and phylogeny. While 52.6% of genomes encoded *pduCDE* with a lineage-specific distribution, genomes of calf and cattle isolates (lineage II) encoded *pduCDE* at 46.2% and 90.9%, respectively, revealing age-dependent variation in bovine isolates. 1,2-propanediol displayed a strain-specific effect on *Lm. reuteri* as the growth of L8 and L11 were unaffected ($p>0.1$), but that of L12 was enhanced ($p<0.005$). All calf-derived *Lm. reuteri* inhibited *S*. Dublin in co-cultures by decreasing the pH of cultures. Furthermore, co-culturing enhanced the growth of L8 and L11 ($p<0.005$), suggesting that these two strains are highly competitive. These findings demonstrate the diversity of *Lm. reuteri* and the complexity of interbacterial interactions, warranting future research.

Abstract ID: 3602 / Poster Number: 446.16P

Mass Spectrometry in the Biotechnology degree: a monitored practical training

Presenting author: Dr. Carlos Barreiro, Universidad de León, León, Spain

Co-authors: *Elías R. Olivera, Ana Ibañez*

The introduction of Mass Spectrometry in the biological world in 1990s gave rise to a methodological journey that is currently reaching unimaginable heights. The identification of microorganisms in clinical (human and animal) and environmental samples, as well as the detection of antibiotic resistance using the MALDI-Biotyper system is one of the most popular applications, nowadays.

The MALDI-Biotyper requires specific and expensive equipment, as well as a high technical level of the operator. It is in this last part where undergraduate teaching plays a relevant role and it is the core subjects of the mark 'Proteomics and Protein Engineering' (fourth year, Biotechnology Degree, Universidad de León). Thus, it was proposed to introduce at a practical level microbial taxonomy by mass spectrometry for university students, carrying out the identification of different microorganisms by means of Mass Spectrometry.

A simple multi-response survey is done after and before of the practical teaching, on different aspects of MALDI-Biotyper Mass Spectrometry, in order to monitor the evolution of the students.

Abstract ID: 1488 / Poster Number: 242.17P

Materials for Cultivating Microbial Dark Matter of Environmental Microbiomes

Presenting author: Mrs. Laura Meisch, Karlsruhe Institute of Technology, Karlsruhe, Germany

Co-authors: *Marta Velaz Martín, Anne-Kristin Kaster, Kersten S. Rabe, Christof M. Niemeyer*

Microorganisms exist in immense variety on earth, thriving in a wide range of habitats and often forming biofilm communities. However, studying these microbial communities in laboratory settings is challenging, since the environmental conditions are hard to mimic.

Using a macroporous elastomeric silicone matrix, the MESIF chip, to cultivate complex environmental microbiomes aids the transition from the environment to the laboratory. To better understand microbial adhesion, biofilm formation, and the composition of the cultivated communities, the surface characteristics of the MESIF matrix were modified through chemical alterations or functionalization with biomolecules. These surface modifications were validated using fluorescent dyes, fluorescent oligonucleotides, and proteins. After characterization of the surface properties, the MESIF matrix was integrated into the chip system, to investigate the influence of the different surface properties on microbial growth and biofilm formation.

Initial growth studies using model organisms equipped with plasmids expressing fluorescent proteins revealed distinct biofilm formation patterns. Additionally, the surface modifications impacted the colonization of microbiomes from environmental habitats, leading to unique microbial compositions and enrichments within the MESIF matrix as shown by detailed bioinformatic analysis. In conclusion, reproducible surface modification techniques were successfully developed for MESIF materials. These modifications enabled the enrichment of different microbial consortia, facilitating the systematic exploration of environmental microbiomes.

Abstract ID: 3563 / Poster Number: 092.16P

Maternal Obesity and Early-Life Microbiota: Implications on Immune Cell Activation

Presenting author: Ms. Maria Inês Magalhães, ICBAS – School of Medicine and Biomedical Sciences, Porto, Portugal

Co-authors: *Maria João Azevedo, Flávia Castro, Maria José Oliveira, Benedita Sampaio-Maia, Ângela M. Costa*

Background: Early-life gut microbiota plays a vital role in the development of the child's immune system and is modifiable by external factors such as delivery mode and maternal microbiota. Any disruption to its composition can impair the maturation of the immune system, potentially causing future health issues. Considering the role of mother, as one of the main sources of microorganisms to the offspring, it is crucial to assess whether maternal health can influence the gut microbiota of the infant and, thus, the immune response.

Objective: The study aims to understand the impact of maternal obesity on child's gut microbiota immunostimulatory potential.

Methods and Results: For that, faecal samples from infants of healthy and obese mothers were collected at one month, six months and one-year post-partum. Microbiota was separated and inactivated, and used to stimulate dendritic and T cells isolated from healthy blood donors. A total of 16 pairs of children from healthy vs obese mothers were evaluated.

Flow cytometry analysis revealed that infant gut microbiota induced the activation of both DC and T cells, assessed by the expression of the activation markers CD86, CD40, HLA-DR and CD69. However, no significant differences in the activation levels of DCs and T cells were observed between infants of obese and lean mothers over time.

Conclusions: These findings demonstrate that infant gut microbiota can stimulate innate and adaptive immune cells, regardless of maternal obesity. Further studies are needed to explore whether maternal obesity influences other aspects of immune priming in offspring.

Abstract ID: 3592 / Poster Number: 395.17P

Mechanical properties of Schizophyllum commune mycelium materials: sustainable alternatives for diverse applications

Presenting author: Ms. Anna Bogomolova, Utrecht University, Utrecht, Netherlands

Co-authors: *Antonio d'Errico, Han A. B. Wösten*

Fungi-derived alternatives to conventional materials, such as leather, have great potential for sustainable economy. *Schizophyllum commune* is widely studied for mycelium materials due to short life cycle, well-characterised cell wall structure and fully sequenced, modifiable genome. Materials can be produced from both monokaryons and dikaryons of *S. commune*. Monokaryons, which lack fruiting body formation, yield more homogeneous materials but are more susceptible to mutations affecting phenotype and material production. Dikaryons, with two nuclei, mitigate these effects and produce more stable phenotypes.

This study compared biomass production and material properties of one *S. commune* monokaryon and four dikaryons. Strains were grown in liquid shaken conditions across three media varying in nitrogen source and phosphate buffer concentration. Biomass yields ranged from 1.48–7.71 g L^{-1} for dikaryons and 3.11–9.31 g L^{-1} for the monokaryon. While the monokaryon materials exhibited an ultimate tensile strength (σ) of 4.62–7.77 MPa, a Young's modulus (E) of 0.38–0.64 GPa and elongation at break (ε) of 1.51–1.93%, most dikaryons significantly outperformed them, with σ of 19.55–47.29 MPa, E of 0.69–2.06 GPa, and ε of 2.09–7.92%. Remarkably, dikaryons 139 and 351 produced materials with σ of 47.29 and 47.16 MPa, the strongest untreated pure mycelium materials reported to date. Cell wall composition analysis revealed that high material strength correlated with percentage of KOH-soluble cell wall components (R^2= 0.64).

These findings categorise monokaryon materials as natural foams and dikaryon materials as thermoplastics demonstrating the potential of *S. commune* dikaryons for diverse sustainable applications.

Abstract ID: 587

Mechanism of bacterial predation via ixotrophy

Presenting author: Dr. Yun-Wei Lien, University of Vienna, Vienna, Austria

Co-authors: *Davide Amendola, Kang Soo Lee, Nina Bartlau, Jingwei Xu, Go Furusawa, Martin Polz, Roman Stocker, Gregor Weiss, Martin Pilhofer*

Ixotrophy is a contact-dependent predatory strategy of filamentous bacteria in aquatic environments for which the molecular mechanism remains unknown. We show that predator-prey contact can be established by gliding motility or extracellular assemblages we call “grappling hooks.” Cryo–electron microscopy identified the grappling hooks as heptamers of a type IX secretion system substrate. After close predator-prey contact is established, cryo–electron tomography and functional assays showed that puncturing by a type VI secretion system mediated killing. Single-cell analyses with stable isotope–labeled prey revealed that prey components are taken up by the attacker. Depending on nutrient availability, insertion sequence elements toggle the activity of ixotrophy. A marine metagenomic time series shows coupled dynamics of ixotrophic bacteria and prey. We found that the mechanism of ixotrophy involves multiple cellular machineries, is conserved, and may shape microbial populations in the environment.

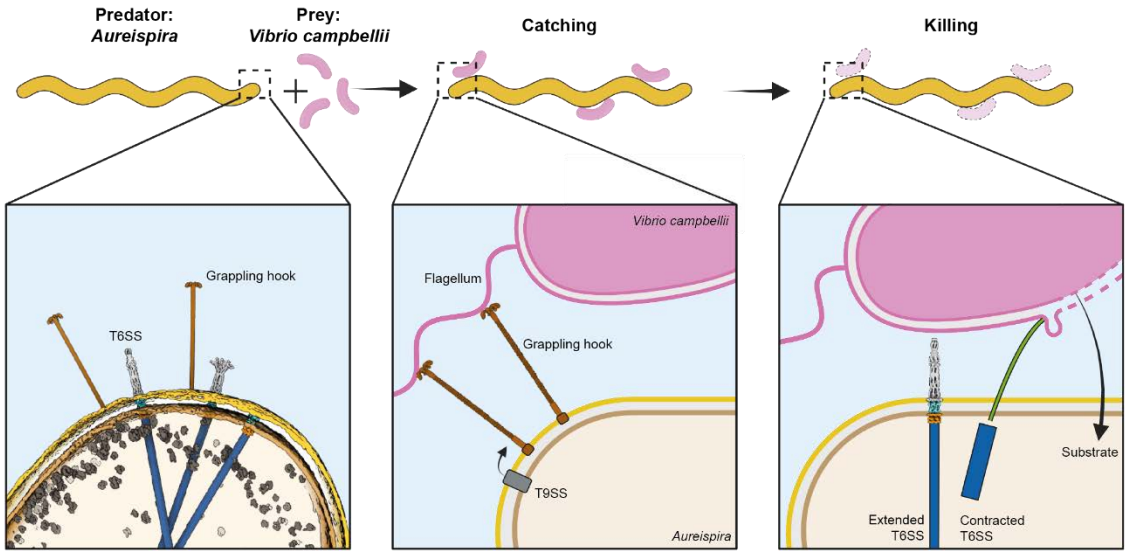


Figure 1: *Aureispira* predates through a two-step process: Cell-cell contact with prey is established using gliding motility (on solid surfaces) or T9SS-secreted grappling hooks (in liquid environments). This is followed by prey killing by means of a T6SS and uptake of substrates. [Figure partly created with BioRender.com]

Abstract ID: 210 / Poster Number: 405.15S

Mechanisms of Antagonism and Synergy in Phage and Macrolide Antibiotic Treatments Against Acinetobacter baumannii Biofilms

Presenting author: Dr. Jinling Xue, Technical University of Munich, Munich, Germany

Co-authors: *Jinlong Ru*

Introduction: The effectiveness of phage therapy in treating biofilm-related infections, such as those caused by multidrug-resistant Acinetobacter baumannii, varies widely due to our limited understanding of phage-host and phage-antibiotic interactions.

Objectives: we aim to reveal the mechanisms of phages' distinct behaviors when treated together with macrolides or alone, from molecular and transcriptional levels, and possibly contribute to the design of future phage formulas in clinical settings

Methods: This study employs integrative biological, genetic, and transcriptional analyses to explore these interactions between phage-host and phage-phage.

Findings: Phage vB_AbM_HMGU encodes multiple ribosomal and transcription regulatory proteins that counteract the translation inhibition caused by macrolide antibiotics, thereby enhancing biofilm eradication. In contrast, phage vB_AbA_HMGU, lacking these crucial regulatory genes, shows significantly reduced propagation in the presence of macrolides. Furthermore, we identified phage-induced mutations affecting bacterial receptor functions and morphology and decreased expression of toxic and drug-resistant genes, which reduce bacterial virulence and restore antibiotic susceptibility. Prophages released during these processes also contribute to reduced phage resistance. Additionally, identifying a depolymerase gene in phage and significantly reducing bacterial mutations accounts for the significant biofilm degradation.

Conclusions: These results highlight the potential of phage cocktails as an effective strategy against biofilm infections, providing valuable insights for designing phage-based biofilm treatments.

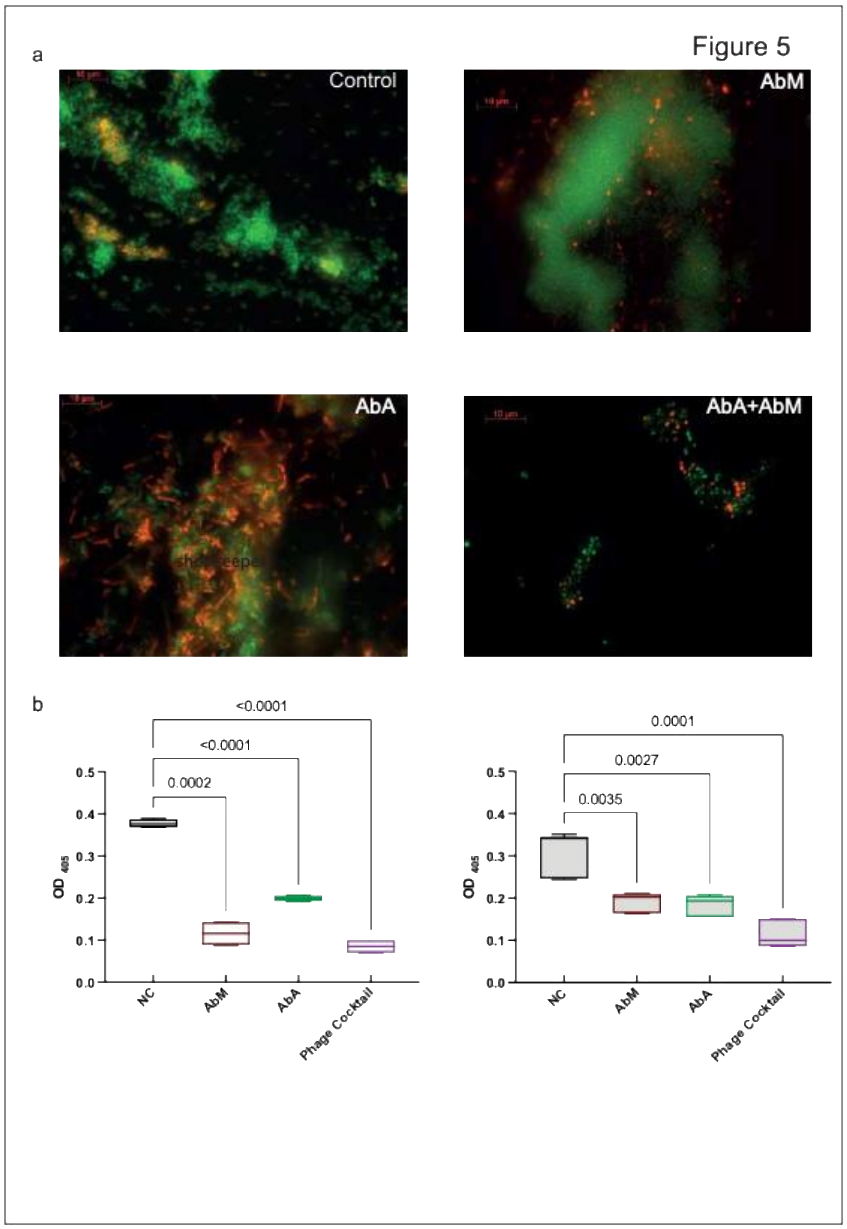


Figure 1: The biofilm prevention and removal effects of phage and cocktail treatment. (a). the fluorescent images live/dead staining on biofilm structure from negative control, treated with phage AbA, phage AbM, and phage cocktail (AbA+AbM, MOI=1). (b). The biofilm prevention (left) and removal (right) effects of phage AbM, phage AbA, and phage cocktail.

Abstract ID: 2039 / Poster Number: 126.16P

Mechanisms of Interaction Between Attenuated Salmonella Derby and Human Innate Immunity

Presenting author: Laura Mazzera, PhD, Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna B. Ubertini, Brescia, Italy

Co-authors: *Melissa Berni, Martina Tambassi, Ilaria Menozzi, Alessandra Dodi, Chiara Bracchi, Marina Morganti, Erika Scaltriti, Stefano Pongolini*

Introduction: *Salmonella enterica* serovar Derby is commonly found in pigs, but it is rarely responsible for human disease, likely due to its ability to persist as an asymptomatic colonizer in its host. The balance between the host immune response and the ability of *Salmonella* to persist within macrophages dictates the outcome of infection and systemic dissemination and is finely regulated by SPI-2 effectors.

Objective: The aim of this work is to study the mechanisms by which *S*. Derby (SD), lacking several SPI-2 effectors, interact with human innate immunity compared to virulent *S*. Typhimurium (ST).

Method: We evaluated complement and neutrophil killing resistance of SD and ST. Macrophage cell death was examined by trypan blue staining, the macrophage phagocytotic activity was determined by bacterial count. *Salmonella* replication in macrophages was evaluated by fluorescence microscopy.

Findings: We observed that SD and ST showed little or no difference in resistance to complement, neutrophil killing, or in the number of bacteria phagocytosed by macrophages. Conversely, we observed that SD induced significantly lower macrophage cytotoxicity and more pronounced replication inside the *Salmonella*-containing vacuole compared to ST. By complementing SD with the SPI-2 effector GtgA, which inhibits the NF-kB pathway, we observed a reversion of the SD phenotype within macrophages to ST.

Conclusion: Despite lacking several SPI-2 effectors affecting its virulence, SD can replicate within human macrophages by activating the pro-survival functions of NF-kB, a condition favouring both host and bacterial survival. This interaction could be crucial for the persistence of the asymptomatic infection of SD.

Abstract ID: 2468 / Poster Number: 239.15P

Mechanisms of viral persistence in seasonally dry grassland soils

Presenting author: Dr. Lucie Jiraska, University of California, Davis, United States

Co-authors: *Christian Santos-Medellín, Hazel Anagu, Haley Alvarez Bolanos, Joanne Emerson*

Viruses are critical yet understudied components of soil microbiomes, influencing population dynamics, genome evolution, and nutrient cycling. In Californian grasslands, seasonal desiccation (summer) and re-wetting (winter) have shaped soil communities over centuries. While soil moisture fluctuations are known to influence microbial communities, the mechanisms enabling viral particle persistence during prolonged dry periods and their links to soil moisture levels remain unclear.

To assess the influence of soil moisture and temperature on formation of dry-season viral communities (seed banks), we studied the viral community persistence in periodically dry Californian grassland soils through temporal field sampling and laboratory microcosms experiments. Monthly sampling from four mounds and four valleys at Jepson Prairie Reserve revealed significant habitat-driven differences in viral communities, with mound and valley communities consistently distinct regardless of sampling time, soil moisture level or employed experimental temperature. Further analyses of field data revealed clear seasonal cycling, with viral communities showing greater similarity within dry and wet seasons and distinct differences between seasons (Figure 1A).

Wet-season soils were experimentally dried (December 2023–January 2025) for 30 days and subjected to 24-hour re-wetting (Figure 1B). Results from December 2023 and January 2024 (analyses from other time points are ongoing) show significant differences in viral communities in dried samples compared to field peak dry-season viral communities. Within habitats specific (mounds or valleys), predictable patterns in both dry and re-wet states were observed, possibly due to differential adaptation to annual hydrological regimes in each habitat.

These findings suggest viral community stability is governed by complex mechanisms beyond soil moisture alone. Habitat differences, potentially linked to soil chemistry, partially explain the distinct responses of mounds and valleys to drying and re-wetting under varying temperatures. Further analyses are underway to elucidate these processes.

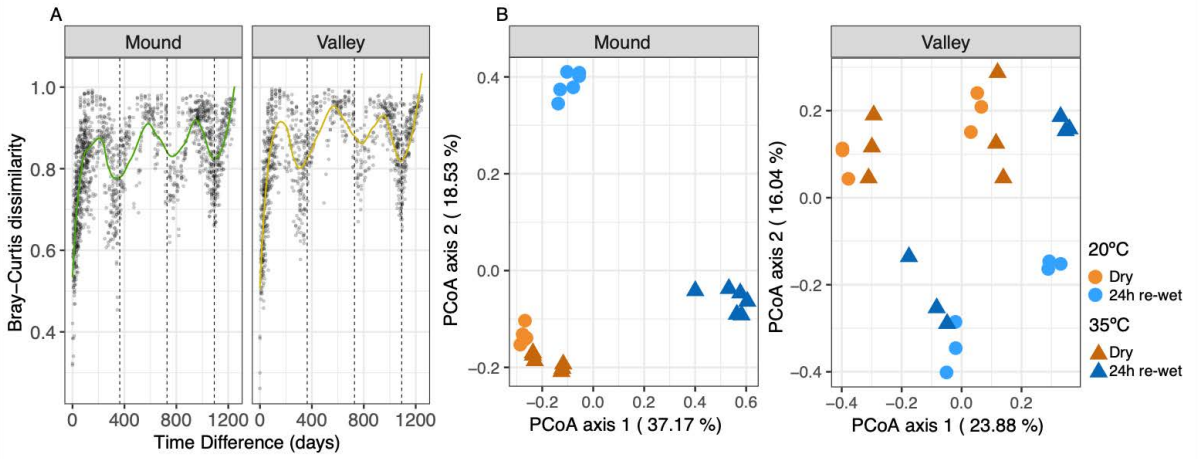


Figure 1. **A** – Pairwise Bray-Curtis dissimilarity of viral communities based on samples collected during monthly field sampling. Dotted lines mark 365 (1 year), 730 (2 years), and 1095 (3 years) days of temporal distance. The plot highlights annual cycles of viral communities, with maximum dissimilarity occurring ~150 days apart (opposite seasons) and minimum dissimilarity around 0 and ~350 days apart (same season). **B** – PCoA of viral communities from December 2023 and January 2024 lab microcosm experiments. The plot illustrates habitat-specific responses of viral communities to drying and re-wetting.

Abstract ID: 3163 / Poster Number: 283.16F

MEGAN7: Enhanced Optimization and Advanced Functionality for Metagenomic Analysis

Presenting author: Mr. Anupam Gautam, IBMI, University of Tuebingen, Tuebingen, Germany

Co-authors: *Daniel H. Huson*

Introduction: MEGAN is a widely used, user-friendly tool for metagenomic analysis, suitable for long and short read data, and remains the only tool with a GUI interface. MEGAN7 introduces optimized workflows and enhanced functionality. By utilizing smaller, clustered reference databases, MEGAN7 improves computational efficiency while maintaining high-quality taxonomic and functional assignments, making it a scalable solution for diverse datasets.

Objective: This study presents current work on MEGAN7, a major update of our MEGAN software, and highlights the impact of utilizing smaller reference databases on the computational efficiency and effectiveness of metagenomic sequencing data analysis, as integrated into MEGAN7.

Method: Metagenomic analysis was conducted on short and long reads from ten diverse datasets. Reads were aligned to various resolutions of the UniRef database (100%, 90%, and 50%) and clustered NCBI-nr databases (90% and 50% identity) using DIAMOND. Taxonomic and functional binning of the aligned reads was carried out using MEGAN7.

Findings: Smaller reference databases, particularly at 90% and 50% identity, significantly accelerated processing times while maintaining high-quality alignment and assignment rates. The integration of DIAMOND's clustering capabilities further enhanced efficiency, demonstrating improved performance across all downsized databases. MEGAN7 achieved good and agreeable assignment rates for both taxonomic and functional binning, even with reduced database sizes.

Conclusion: These findings illustrate that downsizing reference databases effectively reduces the computational burden of metagenomic analysis without compromising result quality. The incorporation of DIAMOND's clustering features offers additional efficiency gains. With these optimized workflows, MEGAN7 presents a scalable and efficient tool for metagenomic data analysis, offering enhanced functionality for diverse datasets.

Abstract ID: 436 / Poster Number: 188.16P

Meta-analysis on the Global Prevalence of Arcobacter in food-producing animals and humans

Presenting author: Dr. Yanlin Niu, Beijing Center for Disease Prevention and Control, Beijing, China

Co-authors: *Xiaochen Ma, Penghang Zhang*

Introduction: The genus Arcobacter has been associated with illnesses in both animals and humans, where Arcobacter butzleri, Arcobacter cryaerophilus, and Arcobacter skirrowii have been linked to numerous cases of gastrointestinal diseases in humans. Despite a few localized reports of Arcobacter infection, there is a lack of comprehensive data indicating the global burden of Arcobacter infection.

Objective: This meta-analysis aimed to determine the pooled prevalence of Arcobacter from various sources worldwide.

Method: We conducted a thorough literature search of Scopus, PubMed, and ScienceDirect databases to identify studies published from 1992 to 2022 on Arcobacter prevalence in humans and food-producing animals. Multilevel random-effect meta-analysis models were applied to estimate the mean incidence of Arcobacter and to compare different factors potentially associated with incidence outcomes.

Findings: Seventy-five articles were included in the meta-analysis. The pooled prevalence of Arcobacter spp. from different sources was 21.9% (95% CI: 18.0%-26.1%), and the mean prevalence of A. butzleri, A. cryaerophilus, and A. skirrowii was 15.1%, 2.8%, and 0.1%, respectively. The prevalence of Arcobacter spp. was lowest in humans (1.8%; 95% CI: 0.7%-3.3%) and highest in broilers (38.8%; 95% CI: 28.0%-50.1%). Animal food products with the highest prevalence of Arcobacter spp. were carcasses or parts of carcasses (28.6%; 95% CI: 23.7%-33.7%).

Conclusion: This meta-analysis concludes that A. butzleri is the most prevalent Arcobacter species worldwide, with broilers, as well as seafood, being the primary hosts of Arcobacter spp. We recommend developing appropriate prevention strategies and conducting further local in-depth studies to establish the actual epidemiological burden of Arcobacter.

Abstract ID: 1045 / Poster Number: 149.16P

Metabolic capability of neonatal gut microbiotas to metabolise human milk oligosaccharides: implications for personalised infant nutrition

Presenting author: Emilia Hick, PhD, Institute of Dairy Products of Asturias (IPLA – CSIC), Oviedo, Spain

Co-authors: *Alejandra Rey-Mariño, Marta Suárez, Laura Mantecón, Jose David Herrero-Morín, Gonzalo Solís, Silvia Arboleya, Miguel Gueimonde*

Introduction: Breastfeeding constitutes the gold standard for infant nutrition, providing bioactive components such as human milk oligosaccharides (HMOs) that support gut microbiota and immune development. Nevertheless, when breastfeeding is not possible, infant formulas enriched with HMOs, including 2'-fucosyllactose (2'FL) and 3'-fucosyllactose (3'FL), are used. These HMOs, predominant in secretor and non-secretor mothers respectively, play prebiotic and immunomodulatory roles. However, it remains unclear whether all infants benefit equally from these oligosaccharides.

Objective: To evaluate the capability of different neonatal microbiotas to metabolise 2'FL and 3'FL.

Method: Faecal samples from 66 infants aged 1 to 3 months were independently cultured with 2'FL or 3'FL under anaerobic conditions at 37ºC for 24 hours. Parameters assessed included changes in pH, levels of 2'FL and 3'FL, sugars, organic acids (via HPLC), and microbiota composition (via metataxonomics) at baseline and after incubation.

Findings: Fermentation of 2'FL and 3'FL varied among faecal cultures, allowing categorise microbiotas as fast, slow, or non-degraders. Approximately 70% of microbiotas metabolised 2'FL, whereas only 40% were able to degrade 3'FL. These metabolic patterns were associated with differences in neonatal gut microbiota composition.

Conclusion: This study identifies phenotypes based on the microbiota's ability to metabolise 2'FL and 3'FL, linking them to specific microbial profiles. These findings highlight the potential for personalised nutritional strategies to optimise HMO supplementation and meet individual infant needs. Further research is required to understand the factors driving these metabolic variations.

Abstract ID: 2567 / Poster Number: 280.16F

Metabolic pathway analysis of Acinetobacter sp. Tol 5 utilizing diverse non-sugar substrates

Presenting author: Prof. Doc. Katsutoshi Hori, Nagoya University, Nagoya, Japan

Co-authors: *Shori Inoue, Shogo Yoshimoto*

The highly adhesive bacterium *Acinetobacter* sp. Tol 5 efficiently metabolizes diverse non-sugar substrates. Utilizing its adhesiveness, we developed a highly efficient cell immobilization method. This advancement enables the development of efficient bioprocesses for converting various chemicals into valuable materials using this bacterium. To achieve this, it is essential to elucidate the metabolic pathways of this bacterium for representative carbon sources.

Objective: The objectives of this study are the genome-based construction of the metabolic pathways of *Acinetobacter* sp. Tol 5 and the comparative analysis of transcriptomes from different carbon sources.

Methods: Deduced metabolic pathways of Tol 5 were constructed based on its whole genomic sequence (AP024708 for the chromosome and AP024709 for the plasmid), which includes 4402 coding sequences. The reconstruction was further informed by pathway data from related species, including *A. baumannii*, *A. baylyi*, and *Pseudomonas putida*. Transcriptome analysis was conducted using cells immobilized on polyurethane foam during the logarithmic growth phase with substrates such as lactate, ethanol, toluene, hexadecane, succinate, and phenol.

Findings: The basic metabolic pathways of Tol 5 from representative non-sugar carbon sources were visualized. Transcriptome analysis revealed that the expression of genes encoding enzymes involved in both upper and central metabolic pathways was also altered depending on the carbon sources.

Conclusion: We successfully constructed a metabolic map of Tol 5 and identified pathways induced or enhanced by different carbon sources. These findings establish Tol 5 as a platform strain for bioproduction from various non-sugar substrates.

Abstract ID: 1071

Metabolic plasticity in freshwater diazotrophs: Drivers and controls of mixotrophic N_2 fixation

Presenting author: Mrs. Carla Villamarin, Ben-Gurion University of the Negev, Sde Boqer, Israel

Co-authors: *Hagar Siebner, Melany Ruiz-Urigüen, Edo Bar-Zeev*

Mixotrophic N_2 fixation, the ability of cyanobacterial diazotrophs to fix N_2 using phototrophic and heterotrophic energy sources, has been sparsely reported from marine environments. However, the ubiquity and drivers of freshwater mixotrophic diazotrophs remain unknown. This study investigates mixotrophic diazotrophy (i.e., abundance, N_2 fixation rates, and diversity) in the Jordan River and examines the factors driving planktonic diazotrophs to shift between phototrophic and heterotrophic energy sources. This goal was achieved using lit and darken freshwater mesocosms that were amended with increasing concentrations of DOC. New insights into the metabolic plasticity controlling diazotrophy in the Jordan River were gained by measuring N_2 fixation rates, total bacteria and diazotroph abundance, community structure of N_2 fixers and nutrient stoichiometry. Average N_2 fixation rates in the lit mesocosms were 1.5 nmol N L^{-1} d^{-1}, while dark rates were 60% lower. Nonetheless, increasing concentrations of DOC enhanced dark N_2 fixation rates by 190%, indicating that N_2 fixation persists under dark conditions and is enhanced with increasing DOC availability. Diazotroph abundance in the lit mesocosms was $1.95x10^7$ cells L^{-1}, only 3% higher than in dark conditions, with the proportion of diazotrophs to total bacteria only 5% higher in lit mesocosms. DOC additions slightly increased the number of diazotrophs (1%), yet drastically increased bacterial abundance (1000%), reducing their ratio by 91%, likely due to competitive interactions over phosphorus. Community structure of potentially active diazotrophs revealed a higher proportion of cyanobacteria in lit than in dark mesocosms. Still, cyanobacterial diazotrophs remained active under dark conditions, representing more than 40% of the active community, strongly indicating mixotrophic N_2 fixation. Additionally, a mismatch between phototrophic bacterial abundance and activity further supports the occurrence of mixotrophic N_2 fixation in the Jordan River. This study provides the first evidence of mixotrophic N_2 fixation in freshwater ecosystems, demonstrating the metabolic plasticity of phototrophic diazotrophs in response to changes in nutrient stoichiometry. This flexibility may significantly impact nitrogen cycling in freshwater environments, particularly under their immense environmental heterogeneity.

Abstract ID: 2529 / Poster Number: 140.15P

Metabolic versatility of acetogenic microbial community from deep granitic subsurface of Deccan Traps, India

Presenting author: Ms. Swatilekha Sarkar, Indian Institute of Technology Kharagpur, Kharagpur, India

Co-authors: *Debarshi Mukherjee, Swatilekha Sarkar*

Introduction: Understanding the carbon and energy metabolism within the energy starved deep biosphere hosted by Archean granitoid remains one of major research gaps in geomicrobiology. Acetogenesis, that allows fixation of CO_2 via energy efficient Wood-Ljungdahl pathway (WLP) is considered to be the driver of the primary production in these systems, though the nature and characteristics of these organisms remained poorly characterized. This study elucidates the physiological and metabolic strategies underpinning carbon cycling and ecosystem functioning in the hot, energy-starved deep (1679–2912 mbs) subsurface underneath the Deccan Traps (India).

Objective: To elucidate the mechanistic, metabolic, and genomic characteristics of acetogenic microorganisms inhabiting the Archaean granitic basement of Deccan traps, India.

Method: A culturomics approach is used to reactivate acetogens from granitoid samples through high temperature, anaerobic incubations. H_2 and CO_2 utilization, metabolite production, qPCR, and 16S-rRNA metataxonomy were done, followed by metagenomic analyses.

Findings: The acetogenic characteristics of the microbial cultures were corroborated through qPCR, clone library of functional genes, amplicon sequencing, metagenomic profiling and metabolites production (acetate). The cultures demonstrated preference for nitrate and sulfate as electron acceptors. Metagenomic insights revealed the presence of genes associated with thermotolerance and WLP, alongside the CBB and the rTCA cycle.

Conclusion: This study provides the insight into acetogenesis of deep subsurface environment of Deccan Traps, India. Results suggested these diverse microbes can mediate carbon fixation through WLP alongside rTCA and CBB cycle. Ability to utilize diverse sources and sinks along with the help of various energy conservation systems helps them thrive at the thermodynamic limits of life.

Abstract ID: 1686

Metagenomic Analysis of Microbial Bioplastic Degradation in Forest Soil: A Family VIII Esterase with Dual Roles in Bioplastic Degradation and Antibiotic Resistance

Presenting author: Dr. Harry Lerner, University of Konstanz, Konstanz, Germany; KU Leuven, Leuven, Belgium

Co-authors: *Stefan Mecking, David Schleheck*

Microorganisms found in the plastisphere are frequently associated with plastic degradation, however, studies have also suggested that the plastisphere predominantly consists of organisms simply making use of aa additional colonizable surface. Interestingly, it has been observed that the plastisphere exhibits an unusually high occurrence of antibiotic resistance genes (ARGs), suggesting plastic debris as a potential for vector antibiotic resistant microorganisms. The enrichment of ARGs has been primarily attributed to a high frequency of horizontal gene transfer in plastisphere biofilms, for lack of a better explanation. This study offers an additional hypothesis.

In this study, we investigated microbial communities associated with the degradation of aliphatic long-chain polyester (LCPE) bioplastics in forest soil. Using 16S rDNA and metagenome sequencing, we were able to identify putative degrader organisms as well as enzymes likely involved in the extracellular depolymerization of the LCPE bioplastics. Custom bioinformatics pipelines revealed several novel candidate plastic depolymerases (Fig. 1a). One enzyme was identified as a family-VIII type esterase with structural similarity to type C β-lactamases. Structural modelling and substrate docking analysis revealed catalytically favourable binding of both LCPE, as well as β-lactam antibiotics in the active site (Fig.1b). Heterologous expression and in vitro testing confirmed the predicted functionality of this enzyme in a rare dual function.

This study thereby not only identifies a novel polyesterase but also reveals a rarely reported overlap in the substrate spectrum of plastic depolymerases and antibiotic-degrading enzymes, which may contribute to the frequently observed enrichment of ARGs in the plastisphere. These findings provide new insights into the enrichment of ARGs in plastisphere biofilms and underscore the ecological and biotechnological significance of microbial adaptation in plastic-impacted environments.

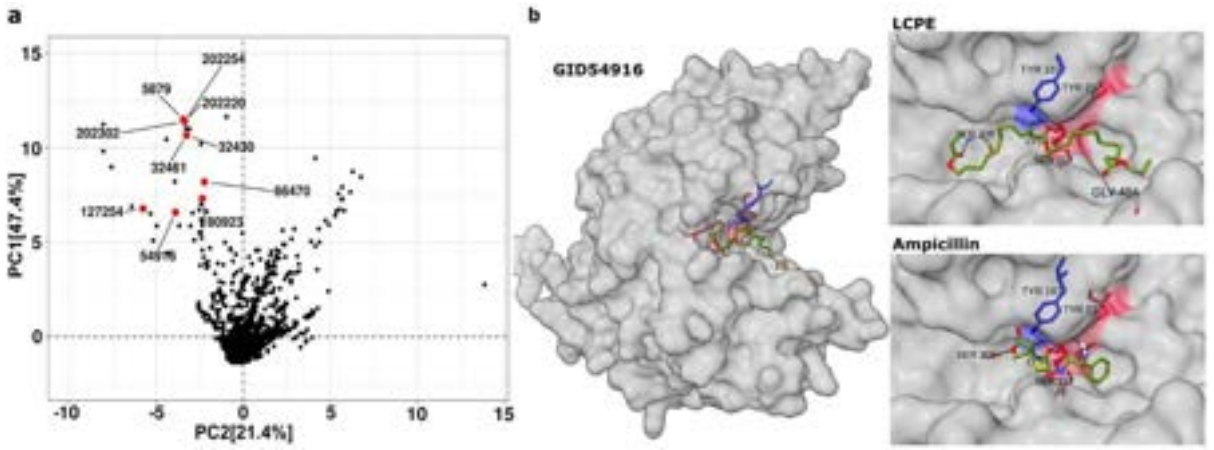


Figure 1. (a) Volcano plot based on principal component analysis (PCA) illustrating the clustering of proteins. The ten highest-scoring matches are highlighted and labelled with their respective gene IDs (GID). Percentages on the axes indicate the variance explained by each principal component. (b) AlphaFold-predicted surface structure of GID54916, including a close-up view of the bound ligands, LCPE and Ampicillin. The catalytic triad residues (Ser117, Lys120, and Tyr232) are highlighted in red, with Tyr187 shown in blue. Ampicillin and the LCPE dimer bound to the enzyme surface are shown in green, with red sticks representing the oxygen atoms of ester bonds. Potential hydrogen bonds between the ligands and catalytic residues are depicted as black dotted lines.

Abstract ID: 3537 / Poster Number: 117.15F

Metagenomic analysis of sessile and planktonic microbial communities in a simulated aquifer system

Presenting author: Mr. Nicolas Ortiz, Université du Quebec à Montréal, Montreal, Canada

Co-authors: *Cassandre Lazar, Maikel Rosabal*

Aquifers are vital resources for drinking water, with approximately one-third of the global population relying on groundwater for domestic use. Microorganisms in subsurface ecosystems play crucial roles in recycling organic matter, degrading contaminants, and improving water quality. However, functional insights into biofilm microbial communities in aquifers remain limited, primarily due to the challenges associated with core subsurface sampling.

To address this gap, our objectives were to (I) investigate the functional traits of sessile (biofilm) and planktonic microbial communities and (II) compare their functional profiles across summer and winter seasons. We employed a bioreactor setup simulating an aquifer system to study both communities simultaneously and used metagenomic methods to characterize their functional traits.

Our findings revealed that community type significantly influenced gene relative abundance, with planktonic communities showing a higher abundance of hydrocarbon degradation genes compared to sessile ones, while sessile communities exhibited greater abundance in metal resistance genes. The seasonal factor had not effect on overall gene abundance; however, in specific pathways such as sulfur metabolism, planktonic communities showed higher gene abundance in summer compared to winter.

Our results suggest that although functional richness was similar in both communities, sessile and planktonic communities adopt distinct metabolic strategies to thrive under different lifestyles. Furthermore, we believe this project will serve as a foundation for future work to characterize microbial communities in other aquifers in Canada. This study highlights the potential of subsurface microbial communities to recycle organic matter and metabolize pollutants, contributing to improved water and soil quality.

Abstract ID: 3492

Metagenomic analysis of urban wastewater resistome and mobilome: Implications for Reclaimed Water Safety in Agriculture

Presenting author: Dr. Jessica Subirats Medina, Institute for Environmental Diagnosis and Water Studies (IDAEA-CSIC), Barcelona, Spain

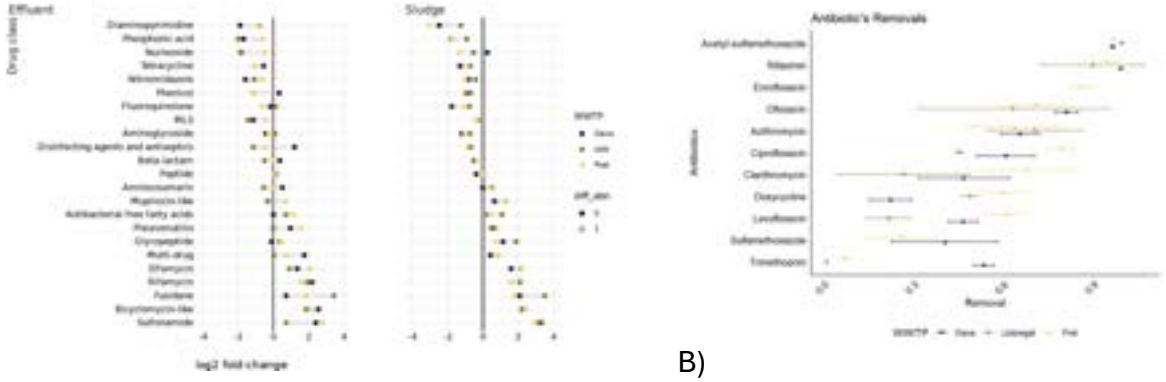
Co-authors: *Anna Marizzi, Liam Brown*

Introduction: The use of reclaimed wastewater for agricultural irrigation has become an increasingly important practice, particularly in areas facing water scarcity. This approach not only provides a sustainable water source but also contributes to the conservation of freshwater resources. Reclaimed wastewater, especially when subjected to advanced treatment processes, can be a valuable resource for irrigation, allowing for the continued growth of crops while mitigating water shortages. However, despite its potential benefits, the practice raises concerns regarding the presence of contaminants that may persist even after treatment, such as pathogenic microorganisms, pharmaceuticals, and antibiotic resistance genes (ARGs). These contaminants can be introduced into the soil and accumulate in crops, presenting risks to both environmental health and food safety.

The Objectives: We aimed to i) examine the removal efficiency of resistome and antibiotic load across the three different WWTPs and decipher the contributing factors; ii) to analyse the abundance of clinically relevant β-lactam, as well as the presence of β-lactam-resistant Gram-negative bacteria of clinical importance in the WWTP.

Material and Methods: Influent, effluent, and sludge samples were collected from three municipal WWTPs in Barcelona, Spain, once per week over three consecutive weeks in February 2024. These WWTPs treat both domestic wastewater and pre-treated industrial water from the metropolitan area. Metagenomic analyses were performed on all sample types to characterize the resistome and mobilome. DNA was extracted from each sample, and high-throughput sequencing was used to identify ARGs, MGEs, and the overall bacterial community. Additionally, the antibiotic load in the samples was measured using UHPLC-qTOF.

A)



B)

Figure 1: A) Removal efficiency of ARGs by target drug in the Gava, Llobregat and Prat WWTPs. Negative fold-change, removal; positive-fold change, enrichment. B) Antibiotic removal by antibiotic family in the Gava, Llobregat and Prat WWTPs.

Results and conclusions: The results showed that Spanish WWTPs exhibited high levels of ARGs, with an efficiency of 2.34–2.43 log reduction in ARGs and a 50–99% removal of antibiotics. Effluent samples were ranked at moderate levels (Level 2 and Level 3) in the exposure prioritization scheme based on total ARG abundance. Notably, ARGs associated with anthropogenic activities, high mobility, and potential pathogenicity were found to be present and even enriched in the reclaimed water, highlighting the need for enhanced risk management. Furthermore, source tracking of the effluent resistome and structural equation modelling were conducted to investigate the differences in ARG abundance and diversity across the various sample types. The findings of this study, along with the recommendation for a comprehensive exposure assessment, will support decision-making in resistome management at WWTPs, aiming to reduce ARG and antibiotic-resistant bacteria (ARB) contamination in receiving environments.

Abstract ID: 2718 / Poster Number: 059.15P

Metagenomic insight into airborne pathogenic microorganisms community and antibiotic resistomes in Belgrade during spring season

Presenting author: Mr. Stefan Stanovcic, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

Co-authors: *Danka Matijasevic, Milka Malesevic, Lazar Gardijan, Nemanja Kljajevic, Katarina Novovic*

Introduction: In recent years, it has been recognized that air is an important transmission route for antibiotic resistance genes (ARGs). In addition, airborne pathogens could be part of particle matter ($PM_{2.5}$), which is an indicator of air pollution. The intertwining of these two serious health threats poses an additional risk to public health.

Objective: To examine the abundance and diversity of pathogens and resistomes in air samples collected during the spring season of 2024 at two locations in the Belgrade metropolitan area selected as highly polluted during the heating season.

Method: Outdoor air was sampled at two locations (Borca and Zemun Ugronovacka) using polyethersulfone membrane filters (air volume of 30 m^3). Collected DNA samples were subjected to shotgun metagenome sequencing (Illumina Novaseq X plus) and bioinformatic analysis (Novogene, UK). Pathogens and ARGs were annotated using PHI and CARD databases, respectively.

Findings: Both airborne metagenomes showed similar patterns of significant human and animal pathogens (Salmonella enterica, Pseudomonas aeruginosa, Staphylococcus aureus, Mycobacterium tuberculosis, Escherichia coli, Acinetobacter baumannii), and plant pathogens (Xanthomonas oryzae, Fusarium graminearum, Xanthomonas campestris, Erwinia amylovora). The most abundant ARG in the airborne metagenomes was bla_{TEM}, but genes conferring resistance to aminoglycosides (aac(2')-Ic), beta-lactams ($bla_{OXA-129}$, blaC, cepA, cfxA2), fluoroquinolones (mfpA), fosfomycin (murA), macrolides (ermF, mphE), tetracyclines (tet(Q)), vancomycin (vanH, vanT, vanY, vanW) and disinfectants (cepA, qacG) were also detected. Moreover, the gene encoding multidrug resistant efflux pump adeF was present in both metagenomes.

Conclusion: This study revealed that Belgrade air could represent a reservoir for pathogenic microorganisms and antimicrobial resistance.

Abstract ID: 2562 / Poster Number: 394.16P

Metagenomic insights and identification of novel endophytes from Ophiorrhiza mungos L. for uncovering camptothecin production potential

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Co-authors: *Arya Nikathil Pradeep, Binoy Ambika Manirajan, Linu Mathew, Remakanthan Appukuttan, Indu C Nair, Sebastian Franz Bender, Marcel Van der Heijden, Radhakrishnan Edayileveetil Krishnankutty*

Introduction: The medicinal plant *Ophiorrhiza mungos L.*, found in the Western Ghats of India, is remarkable due to its bioactive metabolites and is known for the synthesis of camptothecin (CPT), an anticancerous compound. The plant hosts diverse endophytic microorganisms that play a crucial role in CPT biosynthesis, and identifying the microbial communities and isolating the potential endophytic microbes will be important to use as an alternative source for CPT production in the pharmaceutical sector.

Objectives: The combined approach of metagenomics and laboratory cultivation to investigate the diversity of the endophytic microbiota associated with different plant parts of *O. mungos*.

Method: Tissues from plant parts (roots, stem and leaves) were used for the 16S and ITS metagenomic analysis. The tissues were also used to isolate endophytic bacterial and fungal strains associated with CPT production, which were evaluated by HPLC and LC-MS/MS analysis.

Findings: The study revealed the keystone taxa associated with *O. mungos* were identified from the metagenomic analysis. From our study, 17 isolates were identified with CPT biosynthesis potential was quantitively evaluated by HPLC and LC-MS/MS analysis using m/z values of 349 and a quantifier value of m/z 305.10 as confirmatory markers for CPT production. The major taxa identified with CPT production were *Bacillus* sp. and *Aspergillus* sp.

Conclusion: The study provides the potential novel endophytic microbes with high-yield, reliable and economically viable alternative sources for global pharmaceutical demands. Also, the metagenomic approach will be beneficial to understand the potential role of endophytes in CPT synthesis.

Abstract ID: 940

Metagenomic insights into the functional responses of pond microbial communities to chemical pollution

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Co-authors: *Lynn Govaert, Hans-Peter Grossart, Ahmed Abdelfattah, Stephanie Spahr, Luis Alberto Macías-Pérez, Phetcharin Bartel*

Urbanization comprises several significant and intensive anthropogenic activities, threatening global biodiversity and functioning of natural ecosystems. Urban ponds, which serve as a hotspot for biodiversity and perform various ecosystem services, are typically exposed to large numbers of organic contaminants potentially affecting the overall aquatic biodiversity. In particular, microbial communities, key players of biogeochemical processes in aquatic ecosystems, are indicators of chemical pollution with pollution-sensitive and less sensitive species. Here, I will present the impact of chemical pollution on the diversity and functional composition of microbial communities along an urbanization gradient in the Berlin-Brandenburg area, and their potential to degrade organic contaminants. Water samples from 40 ponds along the urbanization gradient were collected for extracting environmental DNA. Shotgun metagenomics was used to recover high-quality metagenome-assembled genomes (MAGs) of rare and abundant taxa as well as to annotate genes which are involved in the biodegradation of target organic contaminants. We have also set-up a microcosm experiment using microbial inocula of selected ponds to analyse the potential of pond bacterial communities to degrade organic contaminants and to answer whether exposure to these contaminants result in specific adaptation of bacterial communities to chemical pollution. The results enable us to better understand how microbial community composition responds to chemical pollution and shed light on the resilience and adaptability of pond ecosystems to environmental stressors.

Abstract ID: 3780 / Poster Number: 094.16P

Metagenomic Insights into the Gut Microbiome of Colorectal Cancer Patients in Jordan: A Comprehensive Analysis

Presenting author: Assoc. Prof. Loai Alanagreh, Zarqa University, Zarqa, Jordan; The Hashemite University, Zarqa, Jordan

Introduction: Colorectal cancer (CRC) is a major global public health concern, with microbial dysbiosis and associated metabolites contributing to its onset and progression. Despite being the second-leading cause of death related to cancer in Jordan, there has been little research on the microbiome changes linked with CRC in the Jordanian population.

Objective: Examine microbial composition of CRC patients in the Jordanian population.

Methods: We employed 16S rRNA sequencing targeting the V3-V4 region of 50 CRC patients using the iSeq 100 platform and compare it with 98 healthy individuals from the GutFeelingKB database.

Findings: Our findings indicated that colorectal cancer (CRC) samples had higher proportions of *Firmicutes*, with 64% in cancer compared to 22% in healthy samples (P-value < 0.05). Specifically, *Enterococcus* was 7.55% vs. 0.05% in healthy, and *Streptococcus* was 22.8% vs. 0.23% in healthy (both P-value < 0.05). In contrast, healthy samples were enriched with *Roseburia* (1.83% vs. 0%) and *Rumminococcus* (4.0% vs. 1.45%). CRC samples also showed increased levels of *Proteobacteria*, such as *Klebsiella* (4.17% vs. 0.03%) and *Escherichia* (3.33% vs. 1.96%) (P-value < 0.05). *Prevotella* was more abundant in cancer samples (2.80% vs. 0.53%), while *Bacteroides* was significantly lower (4.38% vs. 65.57%).

Conclusion: This study reveals the microbiome composition in colorectal cancer (CRC) patients in Jordan, identifying unique microbial profiles associated with the disease. These profiles may serve as potential biomarkers or treatment targets, emphasizing the gut microbiome's role in colorectal cancer's pathophysiology.

Abstract ID: 1113 / Poster Number: 193.15P

Metagenomic Insights into the Microbial Diversity and Biosynthetic Potential of Baltic Sea Sediments

Presenting author: Ms. Elaine Romines, Nicolaus Copernicus University, Toruń, Poland

Co-authors: *Anastasia Ilvytska, Patrycja Golińska*

The Baltic Sea has a unique brackish environment that harbors diverse and complex microbial communities within its sediments. Marine microorganisms are known to play critical roles in nutrient cycling and biogeochemical processes through specialized natural products synthesized in metabolic pathways that essentially impact the overall productivity of a marine environment. Such specialized metabolites produced by iron-reducing and -oxidizing bacteria are key natural products, mediating iron cycling and influencing carbon (CH_4, CO_2, organic carbon) and nitrogen dynamics, as well as driving processes like iron-dependent anaerobic methane oxidation (Fe-AOM) in low-salinity coastal sediments. We studied eight Baltic Sea sediment samples, using a combination of culture-dependent and metagenomic analyses. Over 300 actinomycete strains were isolated from these samples and identified based on 16S rRNA gene sequence. Chrome Azurol S (CAS) assay demonstrated the iron-chelating activity of Gram-positive strains, confirming the production of siderophores. Shotgun metagenomic analysis revealed the microbial communities thriving in the brackish environment and their role in iron cycling. The reconstruction of the metagenome-assembled genomes (MAGs) revealed interesting and uncultivated species that had not yet been described. Diverse and potentially novel secondary metabolite biosynthetic gene clusters (smBGCs) were mined depicting the biosynthetic potential of uncultured bacteria from brackish environments. Our findings highlight the Baltic Sea sediments as a reservoir of natural products with novel biomedical and biotechnological applications, providing a deeper understanding of microbial contributions to iron cycling and their broader ecological significance.

Abstract ID: 2641 / Poster Number: 001.15P

Metagenomic profiling of tomato rhizosphere under water stress conditions

Presenting author: Ilaria Larini, PhD, University of Trento, Trento, Italy

Co-authors: *Sara Cremonesi, Cristina Sudiro, Gerardo Puopolo, Nicola Segata, Michele Perazzolli*

Soil microbiome can contribute to plant growth and tolerance to abiotic stress. In particular, rhizosphere-associated microorganisms can mitigate water stress in the host plant. Tomato is one of the most important crops worldwide, but scarce information is available on the possible contribution of rhizosphere microbiome in mitigating water stress. This study aimed to understand the role of tomato genotypes and growth conditions in shaping rhizosphere microbial communities and the microbial functions possibly implicated in plant stress mitigation. A total of 215 tomato genotypes derived from a MAGIC population was screened under controlled conditions; 10 susceptible and 10 tolerant genotypes were then selected according to wilting symptoms, shoot dry weight, and plant dry weight. Rhizosphere samples were collected from well-watered and water-stressed plants at five days of recovery with four replicates; DNA was extracted and subjected to shotgun sequencing with the Illumina platform. Bioinformatic pipelines were used, and statistical correlation analyses are ongoing. Preliminary analysis revealed Proteobacteria, Actinobacteria, and Verrucomicrobia as dominant phyla possibly involved in soil nutrient cycling and organic matter degradation. Metagenomic analysis of the rhizosphere will clarify the effect of tomato genotypes for selecting beneficial microbial communities and identify microbial gene functions possibly associated with water stress tolerance and plant growth promotion.

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Abstract ID: 701 / Poster Number: 019.17F

Metagenomic recovery of novel bacterial symbionts from nudibranchs treated by different preservation methods

Presenting author: Ms. Dafne Porcel Sanchis, Institute for Integrative Systems Biology, Paterna, Spain

Co-authors: *Carles Galià Camps, Marta Pola, Mária Džunková*

Soft-bodied marine organisms, such as sponges and nudibranchs, produce bioactive molecules to deter predators. In our previous studies, we demonstrated that some species of nudibranchs harbour symbiotic microbes from novel taxonomic lineages, that contain novel biosynthetic gene clusters (BGCs) responsible for nudibranch chemical defense. Our strategy to detect symbiotic bacteria is to perform metagenomic sequencing of ultra-small samples precisely dissected from mantles from several nudibranchs of the same species. There are about 3,000 nudibranch species worldwide, and only a minuscule portion has been analyzed for presence of symbiotic microbes. Nudibranchs are often collected in remote areas and cannot be processed at the sampling sites. Therefore, specimen preservation is necessary, however, it is yet unknown how tissue preservation methods influence metagenomic recovery of symbiotic bacteria.

This study aims to identify novel bacterial groups with pharmacological potential in the mantle microbiome of nudibranchs from Spain and Timor Leste. The specimens were treated by different preservation **Methods:** dried, frozen, preserved in ethanol, RNAlater or kept at 4°C. Samples were subjected to shotgun sequencing, assembled by SPAdes, and DevTools was used to integrate binning results from various programs. BGCs were further analyzed using AntiSMASH.

Our results led to the recovery of novel metagenome assembled genomes (MAGs), exhibiting distinctive metabolic potential compared to free-living marine taxa. The MAGs were analyzed across the distinct preservation methods, which helps to design of the most suitable sample collection approach in future studies.

Abstract ID: 295 / Poster Number: 030.16P

Metagenomics driven discovery of gluten degrading enzymes for Celiac disease therapy

Presenting author: Bhagyashree Karmarkar, PhD, National Center for Cell Science, PUNE, India

Co-authors: *Dhiraj Dhotre*

Introduction: Celiac disease (CeD) is experiencing a notable surge in incidence across India. While the genetic predisposition—carrying the HLA-DQ2/DQ8 gene—plays a role, not all genetically susceptible individuals develop the disease, pointing to other critical factors in CeD pathogenesis. Emerging research suggests that differences in gut microbiota may be key, with certain microbes in healthy individuals potentially transforming gluten, the primary environmental trigger, into a non-immunogenic form.

Objective: This study aimed to mine metagenomic data for gluten-degrading enzymes, screen candidates in silico, and validate their potential in a Celiac disease cell line model.

Method: Bioinformatic analysis of metagenomic data was used to reconstruct genes encoding gluten degrading enzymes. In-silico filtering was done via docking and MD simulation. Peptidases predicted to degrade gluten to a non-immunogenic form were cloned, purified and characterized. CaCo-2 cell line was used to determine immunogenicity of degradation products via qPCR for ZO-1 and Occludin, ELISA for cytokine estimation and confocal microscopy.

Findings: We have identified and validated two enzymes that significantly decrease the immunogenicity of 33-mer gliadin and an 11-mer epitope at a ratio of 1:250 and 1:500 micromoles. Decrease in immunogenicity was determined via qPCR, ELISA and confocal microscopy of CaCo-2 cell culture. These enzymes operate at a pH range of 2-6 which makes them functional in the human stomach.

Conclusion: The validated enzymes show potential as therapeutic candidates for CeD. The next phase will evaluate their efficacy in preclinical mouse models, aiming to develop a therapeutic solution for CeD.

Abstract ID: 3787 / Poster Number: 245.16P

Metal Ions as Local Bioactive Agents: Optimizing Antimicrobial and Antitumor Activity

Presenting author: Andrea Osmokrovic, University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia

Co-authors: *Vesna Lazic, Zeljko Zizak, Bojana Obradovic*

Antimicrobial resistance remains a global public health challenge that disproportionately affects cancer patients compared to the general population. This study aimed to develop a composite material for topical application, designed to address the unique challenges associated with malignant wounds. Upon exposure to physiological fluids, this composite releases bioactive agents, specifically Zn^{2+} ions and activated charcoal particles, to provide localized therapeutic effects. The composite material was comprehensively characterized *in vitro*, with a particular focus on its antimicrobial and antitumor properties. Antimicrobial activity was evaluated against several pathogens from the ESKAPE group in both planktonic and biofilm forms. Antitumor properties were assessed using two cancer cell lines: the malignant melanoma line Fem-X (a model for primary skin cancer) and the breast cancer line MDA-MB-453 (a model for metastatic skin cancer). Even thought, the composite material demonstrated excellent antibacterial activity, Zn^{2+} ions showed selective effects against resistant bacterial strains. Additionally, Zn^{2+} ions, at antimicrobial concentrations, exhibited cytotoxic activity against both cancer cell lines. These findings suggest that this composite has potential for simultaneously addressing antimicrobial resistance and targeting specific malignant cells.

Abstract ID: 1962 / Poster Number: 060.16P

Metaproteomic profiling of sarcopenia: uncovering microbial protein dysregulation in muscle loss

Presenting author: Laia Domingo-Costa, PhD, Institute for Integrative Systems Biology (I2SysBio), Valencia, Spain; Foundation for the Promotion of Sanitary and Biomedical Research (FISABIO), Valencia, Spain

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An increase in life expectancy does not necessarily implies an increase in the quality of life. Consequently, there is an evident necessity to promote healthy aging regarding independence and functional autonomy. Sarcopenia is a disease associated with ageing that is characterized by the accelerated loss of skeletal muscle and deterioration of muscle quality. In its more severe form, this results in limitations of physical performance. Aging is associated with reduced microbiota biodiversity and increased interindividual variability, phenomena that may have great relevance to skeletal muscle function and mass.The present study examined the gut metaproteome from a sub-group of the Seniors-ENRICA-2 cohort (Madrid, Spain). The study comprised 216 elder individuals, 86 of whom were diagnosed with sarcopenia. A differential expression analysis was conducted to ascertain the presence of any significant changes in gut microbial protein expression between sarcopenic and non-sarcopenic subjects. In addition, a taxonomic analysis was performed in order to study the microbial composition. A substantial decline in Mucin-2 was evident in subjects exhibiting sarcopenia, accompanied by a reduction in human antimicrobial proteins, such as Neutrophil defensin 1 and Lysozyme C. This decline has the potential to disrupt gut bacterial homeostasis. Furthermore, a considerable increase in the abundance of regulators of toxic substances, including Bacteroides fragilis toxin, was observed.We have evidence, then, of the existence of functional alterations of the gut microbiota associated with the development of sarcopenia.

Abstract ID: 3463 / Poster Number: 293.15P

Metatranscriptomics of microbial processes driving greenhouse gas emissions in tropical soils

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Introduction: In tropical peatland ecosystems, carbon and nitrogen cycles are vital for sustaining biodiversity, productivity, and greenhouse gas (GHG) balance. Microbial-driven biogeochemical processes regulate the distribution and quantities of carbon and nitrogen, but there is limited understanding of the active microbial mechanisms responsible for methane (CH_4) and nitrous oxide (N_2O) emissions.

Objectives: This study aimed to uncover the key active microbial processes in soils that regulate gaseous fluxes (CH_4, N_2O, and N_2) in tropical peatlands.

Methods: In 2023 and 2024, peat samples from soil columns (0–10 cm) were collected from tropical forested and open peatlands in Peru, Congo, and Uganda. In situ measurements of soil CH_4 and N_2O emissions, potential N_2 emissions, and physicochemical parameters were conducted. Metatranscriptomic analysis of these soil samples was performed and linked to the other measurements.

Findings and Conclusions: Agricultural fields exhibited the highest N_2O emissions, primarily driven by denitrifying and nitrifying microbial processes, although microbes mediating dissimilatory nitrate reduction to ammonium also contributed. The highest CH_4 emissions came from the wet Ugandan open peatlands controlled by the methanogens. Microbial activities varied significantly among peatlands in different countries, and distinct patterns were observed between open and forested peatlands.

Abstract ID: 2859 / Poster Number: 388.15P

Methanotrophy dominated symbiosis in novel species Gigantidas niobengalensis from the cold seeps of K-G basin

Presenting author: Ms. Nitisha Sangodkar, CSIR-National Institute of Oceanography, Dona Paula, India; Go; University, Taleigao Plateau, India

Co-authors: *Maria Judith Gonsalves, Delcy Nazareth*

Bathymodiolus mussels are prominent invertebrates at cold seeps and hydrothermal vents, are well-known for hosting symbiotic microbes within their gill tissues. These symbionts enable mussels to adapt to extreme environments by facilitating carbon fixation. This study aimed to characterize the microbial communities associated with the gills of the novel bathymodioline mussel *Gigantidas niobengalensis* discovered at a cold seep site in the K-G basin. The methane oxidation rates of the gill tissue samples under aerobic and anaerobic conditions were measured using gas chromatography and metagenomic analysis was employed to determine microbial community composition and functional pathways. Culturable methanotrophs in the gill tissues averaged 3.4 ± 0.9 × 10^4 CFU g^{-1}**,** with methane oxidation rates of 1790.8 ± 21.9 nM d^{-1} g^{-1} under aerobic conditions and 1900.5 ± 1.0 nM d^{-1} g^{-1} under anaerobic conditions. Metagenomic analysis revealed the dominance of methanotrophs, comprising over 55% bacterial and 28% archaeal methanotrophs, with prevalent phyla including Proteobacteria, Firmicutes, Bacteroidetes, Verrucomicrobia, Actinobacteria, Euryarchaeota, and Crenarchaeota. Key taxa, including *Methylococcales sp.* (54.1%), *Verrucomicrobiaceae sp.* (0.4%), *Prevotella sp.* (3.1%), *Pseudomonas sp.* (1.3%), and ANME groups (28.2%), were linked to functional pathways of methane metabolism (20%) and carbon fixation (22%). The dominance of methanotrophs and their functional contributions highlight their role as chemosynthetic symbionts, enabling *G. niobengalensis* to adapt to cold seep ecosystem. These findings advance our understanding of microbial symbiosis in deep-sea mussels and their role in methane cycling within extreme environments.

Abstract ID: 2679 / Poster Number: 371.16P

Methodological improvement for virus and bacteria analysis

Presenting author: Ms. Gemma Saucedo Pagès, Aigües de Barcelona, Barcelona, Spain; University of Barcelona, Barcelona, Spain

Co-authors: *Ane López Saiz, Anna Pinar Méndez, Carles Vilaró Juanuix, Belen Galofré Porcar, Albert Carcereny Sánchez, David García Pedemonte, Raquel Casas Mangas, Anicet R. Blanc, Rosa Mª Pintó Solé, Albert Bosch*

Introduction: Aigües de Barcelona (AB) is the entity responsible for the management of the complete water cycle, encompassing water treatment, distribution, wastewater treatment and reclaimed processes within the urban center of Barcelona and its surrounding metropolitan region. AB provides essential water services to a population exceeding 3 million inhabitants.

Objective: In addition to adhering to the regulatory parameters established by the Spanish RD3/2003 to ensure water quality, AB has been conducting comprehensive microbiological analyses of viruses and bacteria in the drinking water treatment plant (DWTP), distribution network (DN) and wastewater and reclaimed water systems (RW). This extensive research initiative has been carried out in collaboration with the University of Barcelona (UB) for more than 3 decades.

Method: Samples were taken from the DWTP, DN and RW monthly. The primary objective has been to develop and optimize a method for concentrating large volumes of water and detecting emerging pathogens. This goal was successfully achieved through the implementation of Rexeed® filters, which possess the capacity to filter up to 1000 L of water, depending on the specific water matrix composition. Following the filtration process, the eluted volume, typically ranging from 400 to 700 mL, undergoes a secondary concentration step. In adherence to a policy of continuous improvement, the protocol was modified in 2024. The previously employed Centricon® plus centrifuge method for this secondary concentration has been replaced with the Innovaprep Concentrating Pipette® (CP).

Findings: Upon concentration, samples underwent analysis for diverse bacterial species (encompassing total coliforms, *E. coli, Campylobacter sp, Salmonella sp*) and viral agents (including Adenoviruses, Enteroviruses, Hepatitis A, Norovirus GI and GII, somatic coliphages and CrAssphage). Results demonstrated significant microbial reduction during potable water treatment processes (exceeding 5 log units for total coliforms or

CrAssphage). Infrequent positive detections in treated water remained below regulatory thresholds, while most analyses yielded negative results. Reclaimed water exhibited substantial reduction for all parameters (up to 7 log units). Comparative analysis of concentration techniques (Centricon vs. CP) for RT-qPCR was conducted. Higher results were obtained using CP for Noroviruses GI and II (excluding carbon filtration), while Hepatitis A remained undetected independently of the method. Enteroviruses were detected once in raw water. Adenoviruses demonstrated enhanced recovery with Centricon methodology.

Conclusion: The adoption of the CP® technology represents a significant advancement in the protocol, potentially offering improved recovery rates and reduced processing times compared to the previous centrifugation-based approach. Methodological refinement aims to enhance the efficiency and sensitivity of the pathogen detection process, particularly for emerging microbial threats in various water matrices. Long-term collaborative research effort between AB and UB represents a proactive approach to water quality management, extending beyond regulatory requirements to enhance the understanding of microbial dynamics in urban water systems and further safeguard public health. The efficacy of treatment protocols is assured to maintain potable water quality assurance. These methodologies encompass a range of physical, chemical, and biological interventions designed to eliminate contaminants and pathogens. This comprehensive approach to water treatment and management provides a high degree of confidence in the reliability and safety of the resulting drinking water supply. Through these integrated water management practices, AB plays a crucial role in ensuring water security, environmental sustainability, and public health for the Barcelona metropolitan area.

Abstract ID: 1969 / Poster Number: 469.15P

Microalgae Bioflocculation with White Rot Fungi after Wastewater Treatment

Presenting author: Ms. Anna Civzele, Riga Technical University, Riga, Latvia

Co-authors: *Linda Mezule*

Microalgae have long been considered a promising renewable biomass source for various industries, particularly for wastewater treatment due to their ability to efficiently remove nutrients like nitrogen and phosphorus, along with other pollutants. However, the commercialization of microalgae-based technologies remains limited by the challenge of efficient biomass harvesting. Therefore, the study aims to explore the fungal-assisted bioflocculation as a sustainable and effective solution for microalgae harvesting while enabling biomass valorisation.

The study investigates the use of the white rot fungus *Irpex lacteus* for microalgae harvesting after wastewater treatment, conducting experiments at batch and reactor scale. Batch-scale tests optimized parameters such as fungi-to-algae dry mass ratio, stirring rate, pH, and temperature. Reactor-scale experiments assessed the scalability and applicability of the process under semi-realistic conditions. The study also involves the investigation of the mechanisms of fungal-algal floc formation, including enzymatic analysis and microscopy examinations.

Reactor-scale bioflocculation resulted in over 95% *Tetradesmus obliquus* and almost 85% *Chlorella vulgaris* harvesting efficiency from secondary wastewater within less than 24 hours, demonstrating the method's scalability and industrial applicability. The study also reveals that the successful fungal-assisted harvesting process is accompanied by the enzymatic hydrolysis of microalgae cells, suggesting a potentially harmful relationship with the fungi rather than symbiosis. Enzymatic analysis indicated significant production of lignocellulolytic enzymes by *I. lacteus*, highlighting the potential for co-production of value-added products.

Therefore, the study demonstrates the dual benefits of fungal-assisted bioflocculation: efficient microalgae harvesting and fungal enzyme production, broadening the applicability of the resulting biomass in biotechnological applications.

Abstract ID: 1138

MICRO-B: a novel dynamic model of the human gut microbiota

Presenting author: Dr. Marco Calvigioni, University of Pisa, Pisa, Italy

Co-authors: *Costanza Daddi, Virginia Rossi, Diletta Mazzantini, Francesco Celandroni, Giovanni Vozzi, Emilia Ghelardi*

Introduction: Clinical studies and animal models were traditionally used to investigate intestinal microbial populations in healthy and pathological conditions. To overcome the main limitations of working with humans and animals, several *in vitro* models simulating *in vivo* intestinal conditions were developed and validated in recent years. Despite their countless potential applications, their exploitation in gut microbiota research is still limited.

Objectives: In this study, we present a newly designed *in vitro* model, named MICRO-B, for culturing the gut microbiota for clinical research. The evolution of the model will be described starting from preliminary stages in static conditions to its most recent implementation with flow and peristalsis.

Methods: MICRO-B was designed as a portable, easy-to-assemble, millifluidic device. Gelatin electrospun membranes were used as scaffolds for microbial adhesion within the model and inoculated with fecal microbiota suspensions. Membranes were assessed for their suitability in supporting microbial growth and adhesion by crystal violet assay and scanning and confocal laser microscopy. The composition of the consortia grown on the scaffolds was evaluated by 16S rRNA gene-targeting Real-Time PCR and amplicon sequencing. A mucin coating was added to the membranes to mimic the intestinal mucosa, and the microbial composition on the mucus-coated scaffolds was investigated. The clinical applicability of the platform was questioned by simulating a gastrointestinal infection by *Bacillus cereus* and observing its effects on the gut microbiota. The model was then implemented with a constant medium flow and peristalsis-like movements to simulate dynamic *in vivo* conditions. The ability to form biofilms and the microbial composition of the microbiota cultured in MICRO-B were evaluated over time under static conditions, flow or peristalsis only, and combining both flow and peristalsis. B-group vitamins and short-chain fatty acids produced by the cultured microbiota were also quantified in culture supernatants.

Findings: Scaffolds well sustained microbial growth and adhesion, maintaining the overall biodiversity and richness of the fecal communities. Due to the reticular nature of the scaffolds, microbial communities self-organized on the membranes in 3D long-lasting biofilms, detected within the entire width of the membranes. Coating the scaffolds with mucus was demonstrated effective in selectively enriching the gut communities in mucin-binding microbes, including *Akkermansia*, *Bifidobacterium*, *Faecalibacterium*, and *Lactobacillus*. The inoculation of enteropathogenic *B. cereus* in the system caused substantial alterations of the gut microbiota, increasing levels of *Bifidobacterium*, *Clostridium*, and *Mitsuokella* and reducing those of *Pseudomonadota*, *Akkermansia*, and *Lactobacillus*. When flow and peristalsis were implemented in the dynamic model, differences in biofilm formation and microbial composition were pointed out. *Actinomycetota* and *Pseudomonadota* were more abundant in the case of peristalsis and flow only, respectively, after 72h of incubation. *Bacillus*, *Clostridium*, *Enterococcus*, *Escherichia-Shigella*, *Roseburia*, and *Staphylococcus* were found more abundant on the scaffolds when flow alone or in combination with peristalsis was present. While vitamin B_8 was produced with no differences among the four conditions, vitamin B_9 was significantly more abundant in the presence of flow and/or peristalsis. Acetic and propionic acids were less abundant in the presence of flow, whereas levels of butyric acid were not affected by this condition.

Conclusions: The model based on electrospun gelatin membranes resulted in a suitable platform for culturing the gut microbiota, demonstrating physiological fluctuations in biofilm formation, microbial composition, and metabolite secretion in response to the different imposed conditions. The platform is progressively getting closer to the *in vivo* intestinal conditions and will be useful for clinical applications due to its reliability and extreme versatility. The use of *in vitro* models, including MICRO-B, may be helpful in the future for a better comprehension of the gut microbiota in health and disease, thus opening new perspectives for targeted preventive and therapeutic strategies to manage diseases.

Abstract ID: 2903 / Poster Number: 264.16S

Microbes on the Go: Ready-to-Eat Foods as vehicles of live microorganisms for the gut in Urban Lifestyles

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Co-authors: *Beatrice Pizzelli, Robin Duncan, Elena Pierallini, Fabio Angelini, Giorgio Gargari, Simone Guglielmetti*

The Microbial Deprivation Hypothesis posits that early-life exposure to harmless microorganisms is essential for proper immune system development, reducing the risk of autoimmune and allergic diseases. Industrialization has profoundly altered microbial exposure by reducing contact with rural environments, animals, and diverse microorganisms, compounded by improved sanitation practices. Furthermore, diet significantly influences intestinal microbial diversity. While the role of fermented foods in enhancing gut microbial diversity is well-established, limited research has explored the contribution of unfermented foods, such as raw vegetables.

To address this gap, we analyzed the microbial diversity of ready-to-eat (RTE) rocket salad (RS) from different cultivation systems and assessed the survival of RS-associated lactic acid bacteria (LAB) during gastrointestinal (GI) transit.

Our results showed similar bacterial loads among land-based cultivation methods, while vertically farmed RS exhibited much lower bacterial loads. Notably, viable LAB were detected exclusively in land-based RS samples (2–5 $\log_{10}$ CFU/g) and were absent in vertically farmed RS. Taxonomic analyses identified *Leuconostoc* and *Weissella* as the dominant LAB genera in RS. Metataxonomic profiling revealed higher evenness and richness in land-farmed RS compared to vertically farmed RS, with β-diversity analysis indicating reduced inter-sample variability in land-farmed RS.

To determine whether RS-associated LAB could survive GI transit, human intervention studies were conducted. Volunteers consumed RS with varying LAB loads: high-load RS (4.1 $\log_{10}$ CFU/g, primarily *Weissella* spp.), low-load RS (2.3 $\log_{10}$ CFU/g, primarily *Leuconostoc* spp.), and washed RS (no detectable LAB). Viable *Weissella* spp. were recovered in fecal samples only after high-load RS consumption, while no RS-associated LAB were detected following low-load or washed RS consumption.

Simulated GI transit experiments were performed to investigate potential genus-specific differences in LAB survival. Strains of *Weissella* and *Leuconostoc* isolated from

RS exhibited comparable survival capacities, with RS providing a protective effect that enhanced their resistance to GI conditions.

To expand the concept that raw RTE foods can be important sources of live microorganisms for the human gut microbiota, simulated GI transit experiments were extended to RTE foods included in a nutritionally balanced daily meal plan. This plan was designed according to the Italian Recommended Intake Levels of Nutrients and energy (LARN) guidelines for a 70 kg, 1.7 m tall, middle-aged man with a physical activity level (LAF) of 1.45, providing approximately 2300 kcal/day. Only industrial RTE foods available at the local supermarkets were included, such as mixed vegetable and fruit salads, sushi boxes, poké bowls, and tartare.

This study demonstrates that a nutritionally balanced diet composed exclusively of industrial RTE foods can deliver significant quantities of microorganisms capable of surviving GI transit, thereby potentially enriching the gut microbiome. These findings challenge common misconceptions about industrial ready-to-eat foods and emphasize their role in promoting microbial diversity, particularly in urbanized, on-the-go lifestyles.

Abstract ID: 3546

Microbial Alchemy: How Invertebrate Gut Microbiomes Transform Bioplastics for Host Benefits

Presenting author: Prof. Doc. Marcus A. Horn, Leibniz University Hannover, Hannover, Germany

Co-authors: *Linda Hink, Stephan Rohrbach, Nadine Rüppel, Joey Rehkopf, Anja Holzinger, Alfons Weig, Tobias Sandfeld, Andreas Schramm, Heike Feldhaar*

Microplastic is essentially everywhere in the environment including soils with largely unknown consequences for environmental and human health. Soil dwelling invertebrates like earthworms and wood lice are abundant invertebrates that accelerate elemental cycles, structure soils, impact soil greenhouse gas source and sink functions, and represent an important trophic link in food webs. Earthworms and wood lice ingest large amounts of soil and microplastics. Digestion of food largely depends on their gut microbiomes that produce short chain fatty acids nurturing the host. Despite the important function of the invertebrates' gut microbiomes, impacts of microplastics on gut microbiome structures, activities, and consequences for the hosts are essentially unknown. Thus, we fed the model earthworm *Eisenia fetida* and the model wood louse *Porceli scaber* with soil in the presence and absence of biodegradable and conventional microplastic. Life history parameters of invertebrates were unaffected by conventional and moderately enhanced by biodegradable microplastic. 16S rRNA and 16S rRNA gene qPCR as well as amplicon sequencing demonstrated significant impacts of the microplastic on the gut microbiomes. Gut short chain fatty acid profiles along with hydrogen and pH microsensor analyses indicated an impact on gut fermentation activities. Interestingly, hydrogen and lactic acid concentrations were increased upon feeding with biodegradable poly-L-lactic acid microplastic, suggesting hydrolysis and utilization of biodegradable microplastic by the gut microbiome. ^{13}C-stable isotope tracing with ^{13}C-poly-L-lactic acid substantiated such a conclusion. While a direct resorption of lactic acid by the earthworm *E. fetida* was suggested, the data indicated a fermentation of lactic acid by *Enterobacteriaceae* inside the gut of *P. scaber* (Fig. 1). The combined data indicate that (i) microplastics impacts invertebrate gut microbiomes, (ii) biodegradable microplastic is degraded inside invertebrate guts, and (iii) benefits for the host occur due to increased short chain fatty acids that can be used for host nutrition. Biodegradable is thus a more eco-friendly material than conventional plastics.

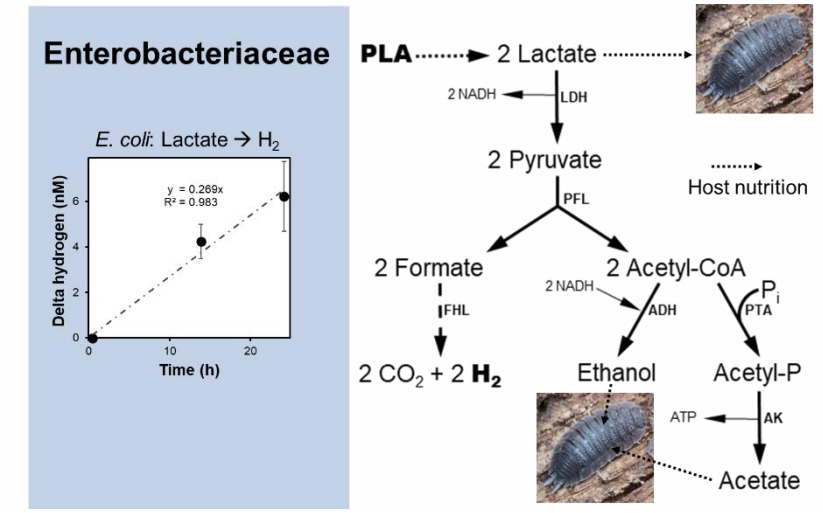


Figure 1: *Enterobacteriaceae* were ubiquitous in gut contents of the wood louse *P. scaber*. Lactate fed, washed cells of *Escherichia coli* as a representative member of *Enterobacteriaceae* converted lactate to molecular hydrogen (left shaded area), suggesting a potential mixed acid fermentation based mechanism for poly-L-lactic acid (PLA) stimulated hydrogen production in *P. scaber* guts upon PLA ingestion. LDH, lactate dehydrogenase; PFL, pyruvate formate lyase; FHL, formate hydrogen lyase; ADH, alcohol dehydrogenase; PTA, phospho trans acetylase; AK, acetate kinase.

Abstract ID: 2504

Microbial Carbon and Nitrogen Cycles in Belowground and Aboveground of Congo Basin Peatland Forests and Grazed Savannas

Presenting author: Dr. Mikk Espenberg, University of Tartu, Tartu, Estonia

Co-authors: *Laura Kuusemets, Jaan Pärn, Kaido Soosaar, Gaël Bouka, Joël Loumeto, Ülo Mander*

Introduction: In the peatland ecosystems of the Congo Basin, carbon and nitrogen cycles are essential for maintaining biodiversity, productivity, and greenhouse gas balance. These peatlands serve as major carbon sinks and significantly influence nutrient availability, which in turn affects plant growth, soil fertility, and microbial activities crucial for ecosystem health. Microbial-driven biogeochemical processes govern the distribution and amounts of carbon and nitrogen, yet limited understanding of the microbial mechanisms behind methane (CH_4) and nitrous oxide (N_2O) emissions—especially the roles of aboveground microbes in these forests—hinders accurate assessments of tropical peatlands' climate change impact.

Objectives: This study aimed to identify key microbial processes in soils and plants that control gaseous fluxes (CH_4, N_2O, and N_2) in the Congo Basin's tropical peatlands.

Methods: In March (dry season) and November (wet season) 2024, peat samples from soil columns (0–10 cm and 10–20 cm) and plant leaves (*Raphia hookeri* (palm), *Macaranga spinosa* (tree), *Guibourtia demeusei* (tree)) were collected along a tropical peatland forest transect and a grazed alluvial savanna. Quantitative real-time PCR and sequencing were used to assess bacterial and archaeal community abundance and structure. Functional genes related to the nitrogen cycle, including those involved in prokaryotic and fungal denitrification (*nirK*, *nirS*, *nosZI*, *nosZII*, fungal *nirK*), nitrification (bacterial, archaeal, and COMAMMOX *amoA*), dissimilatory nitrate reduction to ammonium (*nrfA*), and N fixation (*nifH*), were analyzed. Additionally, methane cycle-related processes such as nitrate/nitrite-dependent anaerobic CH_4 oxidation (n-DAMO-specific 16S rRNA), methanotrophy (*pmoA*), and methanogenesis (*mcrA*) were studied. In situ measurements of soil CH_4 and N_2O emissions, potential N_2 emissions, and physicochemical parameters were also taken.

Findings and conclusions: Total carbon and nitrogen concentrations, including nitrate and ammonium, were higher in forest soils, indicating more active nutrient cycling. This

was reflected in soil gas emissions, with elevated N_2O emissions from forests and significant CH_4 emissions from certain forest areas with high soil moisture. All sites showed low potential N_2 emissions. Soil microbial communities were influenced by pH, which was notably higher in savannas (around 5) compared to forests (around 3.5). Preliminary gene analysis revealed abundant archaeal nitrifiers and denitrifiers in forest soils, with bacterial nitrifiers absent. Fungal denitrifiers were present in both ecosystems. Among aboveground microbial activity, *Raphia hookeri* leaves hosted the most active microbial community, while *Guibourtia demeusei* leaves had relatively low microbial activity in nutrient cycles.

Abstract ID: 660 / Poster Number: 336.15S

Microbial chemosynthetic communities flourish in a sea cave ecosystem

Presenting author: Dr. Francesco Ricci, Monash University, Clayton, Australia

Co-authors: *Pok Man Leung, Tess Hutchinson, Thanh Nguyen-Dinh, Jialing Zeng, Tent Jirapanjawat, Vera Eate, Wei Wen Wong, Perran Cook, Chris Greening*

Chemosynthesis, an ancient metabolic process, harnesses energy from reduced chemical compounds to sustain microbial growth and survival[1]. While traditionally associated with deep ocean ecosystems, recent studies highlight its widespread occurrence in diverse marine environments, including shallow waters[1]. In euphotic zones, life is predominantly supported by photosynthesis[2]; however, permanently dark habitats, such as marine caves, may harbour thriving chemosynthetic communities. In this study, we investigated a marine cave at a depth of 35 meters to test the hypothesis that chemosynthesis sustains microbial life in these unique ecosystems. Short-read metagenomic analyses of sediment samples revealed a decline in the abundance of photosynthetic marker genes in the inner cave (Fig. 1A), coupled with enrichment of chemosynthetic genes encoding enzymes involved in the oxidation of reduced compounds such as ammonium, sulfide, thiosulfate and trace gases (Fig. 1B). These patterns were also reflected in the recovered 130 archaeal and bacterial genomes. Radioisotope analyses demonstrated higher rates of $^{14}CO_2$ incorporation in the inner cave compared to the entrance (Fig. 1C), alongside increased O_2 consumption (entrance: 0.098 μmol L^{-1} h^{-1}; inner: 0.157 μmol L^{-1} h^{-1}; Fig. 1D). These findings suggest that chemoautotrophic and anaplerotic processes enhance baseline CO_2 assimilation in the inner cave, shaping its distinctive biogeochemical and ecological dynamics. Altogether, the synergy between low hydrodynamic energy and perpetual darkness of sea caves fosters conditions for chemosynthetic microbial communities to flourish, resembling ecosystems in the deep sea.

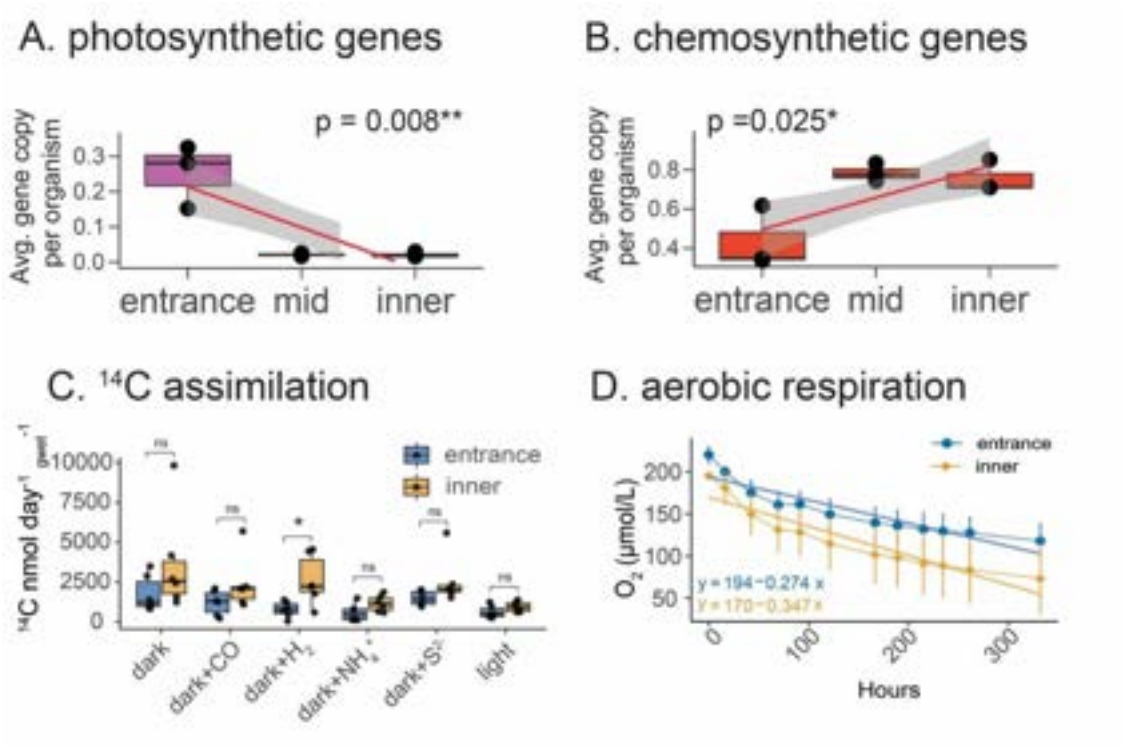


Figure 1 | Gene Abundance and Activity in Sea Cave Environments. (A) Average gene copy numbers per organism for photosynthesis marker genes (*psaA* and *psbA*). **(B)** Average gene copy numbers per organism for chemosynthesis marker genes (*cooS, coxL, NiFe G1* and *G2, amoA, nxrA, fcc, sqr,* and *soxB*). **(C)** Comparison of $^{14}CO_2$ incorporation rates between entrance and inner sea cave sediments supplemented with different energy sources. **(D)** Oxygen consumption rates in sea cave entrance and inner sediments.

Abstract ID: 2856 / Poster Number: 077.15P

Microbial communities from a Pyrenean ice cave

Presenting author: Mr. Víctor Muñoz, Centre for Astrobiology (INTA-CSIC), Torrejón de Ardoz, Spain

Co-authors: *Miguel Bartolomé, M. Cinta Osácar, Reyes Giménez, Eva Garcia, Ana Moreno, Cristina Cid*

Introduction: In Southern Europe the last deposits of fossil ice are located inside ice caves, where ice bodies are protected against climatic perturbations. These ice caves are barely explored due to their difficult accessibility. In this work, we studied the microbial ecosystem of the Devaux ice cave, located on the French-Spanish border in the Pyrenean mountain range. We can describe two differentiated zones in this cave: In the entrance, temperature oscillations are wider and is crossed by a river. Inside the galleries, temperature oscillations are almost absent and below 0º throughout the year. These galleries are occupied by massive fossil ice bodies. In the Devaux cave, cryogenic cave minerals like calcite and pyrite has been documented. Implications of microbial metabolism in biomineralization processes is under current investigation. Microbial communities that inhabit this cave are exposed to extreme conditions such as cold temperature and nutrient scarcity, but depending on the sample type (Liquid water, recent ice or fossil ice), the ecological niche can be different. The interest of this ecosystem is also based on the fact that microorganisms are almost the only living beings that maintain biogeochemical cycles active. In this work, we explored the taxonomic composition, diversity and metabolic potential of microbial communities that inhabits the Devaux ice cave, contributing to the description of a threatened ecosystem.

Objectives:

1. Reveal the taxonomic composition and diversity of microbial communities.

2. Compare microbial community structure in different water-related ecological niches

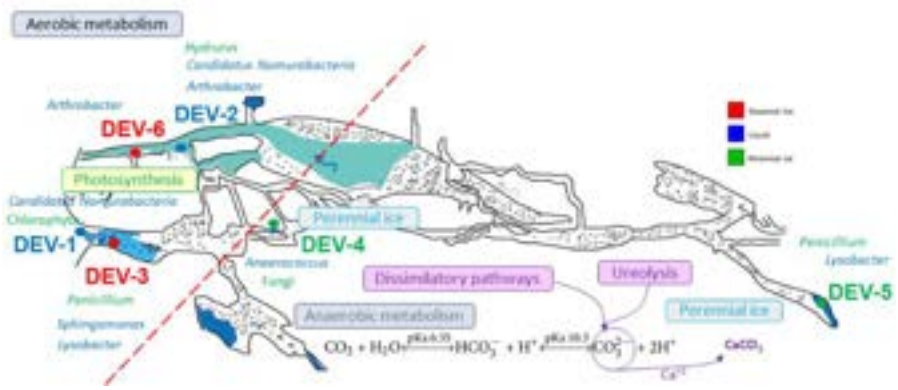
3. Explain the interaction between microbial communities and their surroundings, focusing on the C, N and S cycles and their relationship with mineral precipitation processes.

Method: The taxonomic composition of microbial communities, as well as their diversity and spatial distribution is based on 16S/18S rRNA profiling while metabolic potential of fossil samples is inferred from shotgun metagenomics. Chemical composition of samples was analysed by ion chromatography and ICP-mass spectrometry.

Finding: Proteobacteria, Actinobacteriota and Patesibacteria were the most abundant phyla between bacteria while Opisthokonta and Archaeplastide dominated the eukaryotic communities. There were differences in the taxonomic composition depending on the sample type. Composition of samples from the same niche showed similar diversity and predicted functions. Deeper analysis of functional features relative to biogeochemical cycles in perennial ice samples were inferred from metagenomics. There were found several enzymes that play key roles in carbon, nitrogen, and sulfur cycles. The relationship of this metabolic pathways with biomineralization processes were also explored, highlighting the relevance of microbial communities on the geologic evolution of the environment.

Conclusion: Microbial communities from this ice cave are differentially distributed across the diverse samples. These communities are mainly composed of bacteria, but the presence of eukaryotes archaea and viruses, was also detected. These microbial communities could be responsible of the formation of unique biomineral structures detected.

Graphical Abstract including cave structure and main taxonomic results:



Abstract ID: 2542 / Poster Number: 367.16P

Microbial Communities in Potato Rhizospheres: A Path to Sustainable Crop Protection and Growth

Presenting author: DEIHAPHISHISHA KHARPRAN, PhD, NORTH EASTERN HILL UNIVERSITY(NEHU), SHILLONG, India

Co-authors: *SANTA RAM JOSHI*

Introduction: The rhizosphere, the soil region surrounding plant roots, is home to diverse microorganisms that influence plant health and growth. These microbes enhance nutrient uptake, promote growth and provide protection against pathogens, making the rhizosphere an essential focus for sustainable agriculture. This study explores the microbial diversity in potato rhizospheres, aiming to identify microorganisms with plant growth-promoting (PGP) traits and biocontrol potential.

Objective: The main objective of this study was to isolate and characterize bacteria and fungi from rhizospheric soil of potato fields, identifying potential PGP microorganisms and biocontrol agents. The study also evaluated the antagonistic effects of these microorganisms on *Rhizoctonia solani*, a key pathogen in potato production.

Method: Soil samples were collected from potato fields; bacterial and fungal isolates were obtained. These isolates were identified using morphological, biochemical, and molecular techniques. The isolates were screened for key PGP traits, such as phosphate solubilization, indole-3-acetic acid (IAA) production and siderophore production. To assess biocontrol potential, antagonistic activity against *R. solani* was tested.

Findings: A diverse range of bacterial and fungal isolates were obtained, several of which exhibited significant plant growth-promoting traits. Some isolates showed strong antagonistic activity against *R. solani*, indicating their potential as biocontrol agents.

Conclusion: The isolation of beneficial microorganisms with PGP traits and biocontrol properties suggests they could be valuable for enhancing potato growth and reducing dependency on chemical fertilizers. Further research and field trials are necessary to optimize their use in agriculture.

Abstract ID: 3189 / Poster Number: 274.15P

Microbial communities of the seasonal snowpack in the Swiss Alps

Presenting author: Ms. Anastasiia Kosolapova, École polytechnique fédérale de Lausanne (EPFL), Sion, Switzerland

Co-authors: *Romain Castro, Ianina Altshuler*

Snow is an interface between the ground and the atmosphere, accumulating dust, biological particles and microorganisms. The microorganisms can originate both from local sources and transport over long distances, depending on seasonal and meteorological conditions. The origin of microorganisms in the atmosphere varies greatly with geography and might include different aquatic, terrestrial, animal, and plant surface sources. As adjacent alpine valleys are affected by the same atmospheric events, the variations in community composition of the surface snow come from variations in short-distance transport and snow accumulation patterns, with differences in elevation and terrain features.

The study's objective is to assess microbial community composition in the snowpack of adjacent alpine valleys and distinguish potential ecological factors shaping microbial communities composition of snowpack in the Swiss Alps.

To achieve this, we collected snow samples from 19 sites located in three valleys in the Swiss Alps (elevations between 1798 and 2578 m.a.s.l.). Sampling took place on April 18-19, 2023. For each site, we collected the surface snow and mixed underlying bulk snow (15 cm) in triplicates. Snowpack microbial community structure and diversity were assessed using full-length 16S rRNA sequencing.

We demonstrated that cold-adapted genera were among the most abundant in microbial communities within seasonal snow including Hymenobacter (10.67%), Sphingomonas (4%), and Cryobacterium (2.16%). Community diversity decreases with snow depth, with surface snow communities showing greater similarity between sites compared to more dissimilar bulk snow communities. Once deposited, snow microbial communities undergo post-depositional selection, which drives communities apart and reduces their diversity.

Abstract ID: 2787 / Poster Number: 060.15P

Microbial Community Dynamics in Lake Dziani Dzaha: The Role of Seismic Disturbances

Presenting author: Dr. Hélène Agogué, LIENSs, UMR7266, La Rochelle Université – CNRS, LA ROCHELLE, France

Co-authors: *Cécile Bernard, Marc Troussellier, Adrien Vigneron, Lilian A. Cloarec, Arthur Escalas, Maxime Bruto, Philippe M. Oger, Sébastien Nicolas, Sébastien Duperron, Sébastien Halary, Théotime Roussel, Charlotte Duval, Nicolas Villeriot, Claire Carré, Patrice Got, Cécile Roques, Gérard Sarrazin, Alexis Groleau, Didier Jézéquel, Christophe Leboulanger, Magali Ader, Mylène Hugoni*

Lake Dziani Dzaha, located on Petite Terre Island (Mayotte archipelago), is a thalassohaline ecosystem with unique geochemical signatures, including growing stromatolites, strongly depleted carbon and nitrogen isotopes, steep oxygen and sulfur gradients, and high temperatures. These features make it a potential modern analog for Proterozoic lakes. In From 2018 to 2020, the Comoros archipelago experienced a major seismic swarm, including a volcanic event.

We monitored the microbial community of Lake Dziani Dzaha over an 8-year period, which included this significant seismic event. Before the event, a diverse panoply of microorganisms, resembling those in hypersaline environments like soda lakes, was identified. This included primary producers *Picocystis salinarum* (a picoeukaryote) and *Limnospira platensis* (a cyanobacterium), various Archaea such as *Woesearchaeota*, and heterotrophic bacteria. Vertical stratification of microbial assemblages was driven by salinity, oxygen, and sulfur concentrations.

Following the seismic event, changes in environmental conditions—such as a gradual decline in salinity and pH, depletion of sulfides and methane, and increased oxygen penetration—altered the microbial community structure. We observed a loss of microbial richness and the disappearance of stratification. Despite their resilience, significant changes of abundance and biomass of the two primary producers was observed. Major changes also occurred within the microbial community highlight the varying sensitivities of species to environmental changes following seismic disturbances.

These results suggest that seismic crises and their consequences played a major role in shaping microbial communities in early aquatic environments, possibly driving their diversification over geological timescales.

Abstract ID: 1669 / Poster Number: 043.15P

Microbial Community on Kiwifruit Surfaces is Influenced by Photoselective Nets

Presenting author: Prof. Paulo Fernandes, Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castel, Viana do Castelo, Portugal; Centro de Investigação e Desenvolvimento em Sistemas Agroalimentares e Sustentab, Viana do Castelo, Portugal

Co-authors: *Concha Cano-Diaz, Rui Pinto, Isabel Mourão, Luís Miguel Brito, Luísa Moura*

Understanding the effects of agricultural practices on fruit microbiota is essential for optimizing production, minimizing losses, and preserving quality during storage. The surface microbiota of fruits has influence in shelf life, and changes within these microbial communities can significantly influence fruit quality and postharvest durability. This study examined the impact of different colors of photoselective nets (pearl, grey, and yellow) on the kiwifruit microbiome. Metagenomic DNA sequencing was conducted using a paired-end Illumina platform, with amplicons generated from 16S and ITS primers, allowing for the characterization of taxonomic composition, functional profiles, and co-occurrence networks of bacteria and fungi. The absence of nets favored the proliferation of pathogens associated with postharvest rot, including *Alternaria*, *Didymella*, and *Cladosporium*. Furthermore, each type of net uniquely influenced the kiwifruit microbial community. Pearl nets enhanced bacterial richness and fungal diversity, while yellow nets promoted both the diversity and resilience of microbial communities. In contrast, grey nets resulted in a more uniform distribution of fungal species but produced less robust bacterial networks. The findings of this study contribute valuable insights for developing more effective strategies to control postharvest diseases and extend the shelf life of fruits such as kiwifruit.

Abstract ID: 266 / Poster Number: 335.15P

Microbial community shifts induced by selenite in bentonite: Harnessing bacterial potential for safer Deep Geological Repositories

Presenting author: Cristina Povedano-Priego, PhD, Department of Microbiology, Faculty of Sciences, University of Granada, Granada, Spain

Co-authors: *Fadwa Jroundi, Isabel Guerra-Tschuschke, Maria del Mar Abad-Ortega, Mohamed L. Merroun*

The Deep Geological Repository (DGR) is the preferred strategy for the safe storage and management of high-level radioactive waste. This approach employs a multi-barrier system, which includes sealing spent nuclear fuel within metal canisters, enclosing them in engineered barriers (compacted bentonite), and placing the entire structure deep within a stable geological formation. All projected scenarios for nuclear waste disposal indicate that the long-term release of radionuclides from the repository is probable. Se-79 is one of the most critical radionuclides in HLW. The toxicity of selenium is largely determined by its oxidation state, with selenium oxyanions—selenate [Se(VI)] and selenite [Se(IV)]—being the most toxic due to their high solubility and mobility, which can lead to detrimental environmental effects. Natural occurring microorganisms in bentonite could minimize the migration of radionuclides producing their immobilization through processes such as biomineralization, and bioreduction. Several bacteria can reduce Se(IV) to Se(0) and Se(-II) forms exhibiting low or negligible toxicity. Therefore, this study aims to describe the impact of microbial diversity in the immobilization of Se(IV) through its reduction and transformation in bentonites in a long-term experiment simulating the DGR conditions.

Water-saturated bentonite was treated with Se(IV), spiked with a bacterial consortium, and then incubated anaerobically for 4 years. DNA extractions and Next Generation 16S rRNA gene sequencing were performed to study the shifts in microbial diversity. Microscopic and spectroscopic techniques (VP-FESEM, STEM/HRTEM, EDX, Raman) were performed to characterize the Se(0) nanostructures (SeNS). The total DNA sequencing of the samples revealed the presence of Desulfobacterota in both the control and selenium-treated bentonite samples. Some bacteria within this phylum, such as Desulfovibrio desulfuricans, are sulfate-reducing bacteria (SRB) that can reduce selenite to Se(0). Macroscopically, orange and black precipitates were observed in the microcosms treated with Se(IV). These precipitates were analyzed using STEM, revealing

the presence of crystalline Se(0) nanostructures with various morphologies (nanospheres, hexagonal nanoparticles, and polycrystalline aggregates) primarily composed of Se and S. These nanoparticles were found both extra and intracellularly which indicates that they are biologically formed (Figure 1).

These results highlight the potential direct or indirect impact of bentonite bacterial communities on selenium immobilization. These communities exhibit great bacterial diversity, including SRB. Through a reduction process to Se(0), followed by biotransformation, selenium is detoxified, resulting in the formation of less soluble and more stable precipitates.

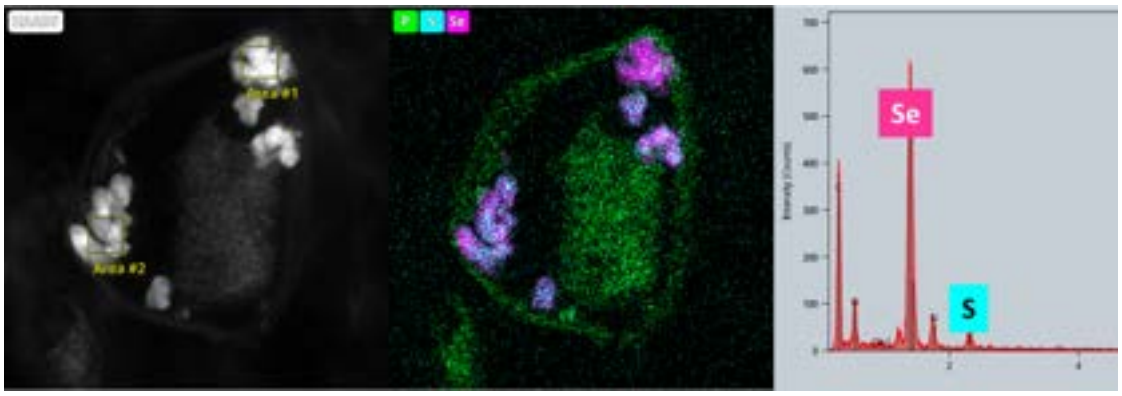


Figure 1: STEM image and EDX maps of a Se(0) nanocrystals composed of Se (pink), and S (blue). EDX spectrum from the selenium precipitates (right).

Abstract ID: 589 / Poster Number: 347.15F

Microbial contributions to metal accumulation in sunflower cultivated under climate changes

Presenting author: Dr. Pooja Sharma, Helmholtz Centre for Environmental Research UFZ, Leipzig, Germany

Co-authors: *Marie Muehe*

The climate change factors temperature, soil moisture, and atmospheric CO2 were shown to increase metal mobility in soils and disrupt microbial interactions with plants. Sunflower, a versatile crop used for food, feed, and bioenergy production, also shows promise for the phytoremediation of metals. However, the impact of climate change on microbe-sunflower interactions and its potential to alter plant metal accumulation remains underexplored. This study will investigate the response of different sunflower varieties grown on Cd-contaminated soils, focusing on metal accumulation in both the straw and edible parts of the plant. Determining parameters include shifts in rhizosphere microbiome profiles, root exudates, soil solution metabolites, and nutrient and metal mobility. These variables will be analyzed and correlated to better understand the underlying mechanisms. The study spans sampling at three-time points during critical growth stages within a single sunflower growth cycle: the vegetative, flowering, and harvest stages. By examining microbial community dynamics and geochemical changes across these stages, the study aims to elucidate how microbial interactions promote the release of root exudates and metabolites, which support plant growth and influence contaminant uptake. The findings will highlight the role of plant-microbe interactions in enhancing the uptake, transport, and detoxification of Cd. This research offers valuable insights into improving sunflower food quality or implementing phytoremediation strategies as part of sunflower-based cover cropping systems.

Abstract ID: 1485 / Poster Number: 213.15P

Microbial diversity in Parisian urban soils: the impact of tree species and soil characteristics

Presenting author: Mrs. Amandine Hecquet, Sorbonne University, Paris, France

Co-authors: *Séverine Planchais, Jean-Christophe Lata, Yoan Marcangeli, David Carmignac, Patricia Genet*

Soil is a key component of terrestrial ecosystems, providing a multitude of ecosystem services essential to mankind. It provides vital plant nutrients and usually hosts a rich microbial biodiversity. Soil micro-organisms interacts with plants, directly influencing their growth and development. Although the impact and feedbacks of plant and soil type on microbial communities' structure has been widely studied in crops or natural systems, this relationship remains poorly understood in urban environments.

This study aims to characterize overall microbial diversity in Parisian urban soils and identify specific patterns related to tree species and soil functioning to better understand their interactions. We sampled 48 urban soils from the Paris city (France), representing four distinct conditions: soil beneath Linden, Plane, Pagoda trees (most commonly planted species in Paris) of the same age category, and bare soils (where no trees have been grown). Samples were collected at two depths (10–20 cm and 30–40 cm) in triplicates. Total microbial diversity was quantified and sequenced (archaea, bacteria and fungi), and physico-chemical properties of these soils were analysed.

The results suggest that microbial diversity is influenced by the interactions with trees and that soil properties in urban environments may also play a role in these patterns. Some of these microorganisms likely adapted to urban conditions, could help newly planted trees in cities to face climate change and harsh environmental conditions (pollution, compaction...). This argues for better knowledge of microbial diversity in this context and its inclusion to help green space managers in tree plantation and urban soil management practices.

Abstract ID: 2459 / Poster Number: 057.15P

Microbial dynamics and methane metabolism in bubble emission sediments of the East Siberian Sea

Presenting author: Dr. Jehyun Jeon, Korea Polar Research Institute, Incheon, South Korea

Co-authors: *Ji-Hoon Kim, Dong-Hun Lee, Jong Kuk Hong, Yeonjin Choi, Yung Mi Lee*

Methane (CH4), a potent greenhouse gas, significantly contributes to global warming due to its high radiative forcing potential. The East Siberian Sea (ESS), with shallow waters and extensive submarine permafrost, is a critical methane emission region, yet microbial processes driving methane dynamics remain poorly understood. During Arctic cruises (ARA12C in 2021, ARA14C in 2023, and ARA15B in 2024), conducted by the Korean ice breaker Araon, submarine methane vents with high methane concentration ("methane hotspots") were found on the shallow continental shelf (50-60 m). To elucidate microbial contributions to methane cycling, microbial communities and metabolic pathways of anaerobic methanotrophic and methanogenic archaea and related microbial groups in combination with biogeochemical profiling of the sediments from methane hotspots were investigated. Our analysis revealed a steep gradient of CH4 and sulphate concentrations at the shallow depths (1-16 centimeters below seafloor), indicating the presence of sulphate-methane transition zone. Using metagenomic approaches, we identified a dominant of *Methanosarcinales* (Euryarchaeota) with operational taxonomic units (OTUs) primarily affiliated with anaerobic methanotrophic archaea (ANME-2a, 92%). Additionally, metagenome-assembled genomes (MAGs) of methanotrophic archaea ANME-2a, ANME-2c, ANME-3, and putative methanogen of the genus Methanococcoides were reconstructed. Their metabolic potential in methane metabolism, including methane oxidation and methanogenesis, was confirmed. These findings underscore the critical role of microbial communities in regulating methane emissions in this underexplored Arctic region, providing insights into their ecological and climatic importance of methane dynamics.

Abstract ID: 4027 / Poster Number: 304.16S

Microbial Electrochemical Synthesis of Carboxylates from Organic Waste: Microbial Selection and Functional Adaptation to hypersaline conditions

Presenting author: Prof. Theodore Bouchez, INRAE PROSE, Antony, France

Co-authors: *Louise Rigaud, Ariane Bize*

Introduction: Microbial electrochemical technologies (METs) represent a promising approach to valorizing organic waste by separating oxidation at the bioanode from bio-based chemical synthesis at the biocathode. The taxonomic composition of microbial communities directly influences the spectrum of multicarbon compounds synthesized. Electrolyte salinity is a key performance parameter, reducing internal resistance and optimizing energetic yields. Hypersaline conditions might also constitue a lever for steering microbial selection and metabolic functionality in METs.

Objective: This research aims to evaluate the selection, adaptation, and functional characterization of electrosynthetic microbial communities under hypersaline conditions. It focuses on the impact of contrasted electrolyte salinity on microbial assembly and metabolic specialization, targeting the efficient bioconversion of CO_2 into carboxylates.

Method: A microbial electrochemical system was designed with three compartments: (i) a bioanode for organic matter oxidation, (ii) a biocathode for electrotrophic CO_2 reduction, and (iii) an intermediate compartment for selective extraction and concentration of carboxylates via electrodialysis.Two inoculation strategies were compared in triplicated labscale reactors: hypersaline communities from salt marsh sediments and industrial digester sludge as a reference non-saline inoculum. The cathode inoculum was heat-treated to enrich homoacetogenic *Clostridia* while eliminating methanogenic archaea. The anode was configured as a flat carbon cloth to maximize electrocatalytic activity, while the cathode consisted of a granular carbon brush to enhance microbial adhesion and electron transfer.

Findings: Experiments demonstrated that the nature of salts in the electrolyte significantly influences catalytic performance. NaCl-based saline conditions enhanced electrochemical efficiency; achieving current densities up to 8 A/m^2 as daily averages with real biowaste hydrolysates as substrate and promoting carboxylate synthesis at the biocathode. 16S rDNA sequencing confirmed taxonomic shifts between conditions with

Alkalibaculm dominated cathodic communities versus *Sporomusa*dominated ones, under saline and non saline conditions, respectively. Our findings reveal how selective pressures exerted by ionic environment finaly translated into in contrasted microbial community and carboxylate electrosynthesis patterns. Shotgun metagenomics and metabolic modeling from metagenome-assembled genomes (MAGs) are underway to further resolve underlying metabolic processes and community interactions.

Conclusion: This study provides insights into microbial selection in bioelectrochemical systems, demonstrating that salinity-driven adaptation can be used to steer microbial community structure and associated metabolic pathways. Metabolic models of engineered hypersaline electrosynthesis communities are being designed to further optimize the efficiency of CO_2-to-carboxylate conversion.

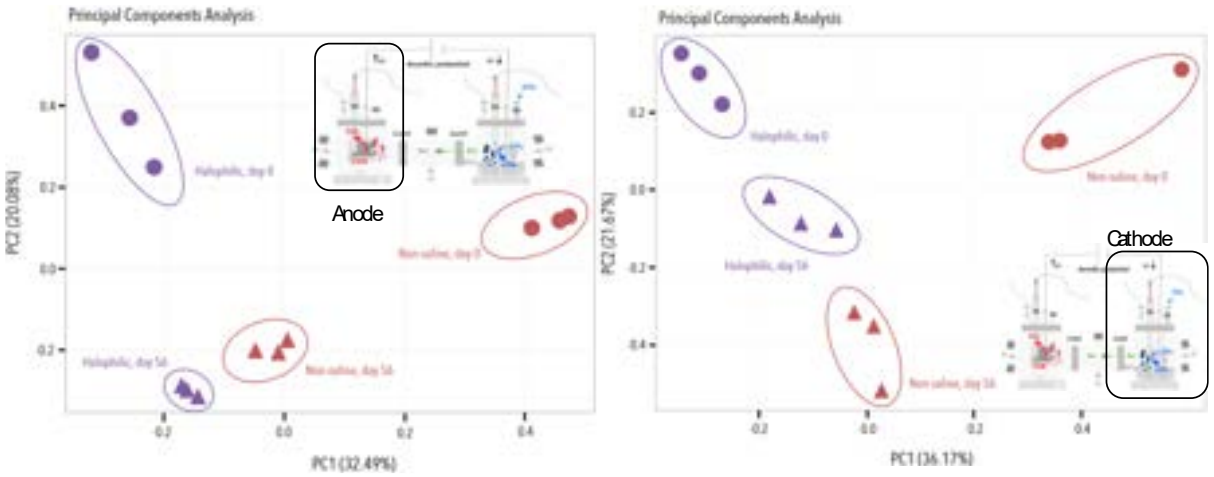


Figure 1: Principal Component Analysis depicting the evolution of halophilic (violet) and non saline (red) microbial community structures in the anodic (left figure) and cathodic (right figure) compartments monitored by 16S rDNA ribotag sequencing.

Abstract ID: 3359

Microbial Fe(III) reduction via novel flavin-based extracellular electron transfer pathway under oxic conditions by Gram positive Microbacterium deferre sp. nov. A1-JK

Presenting author: Jamie Lustermans, PhD, University of Antwerpen, Wilrijk, Belgium

Co-authors: *Naja Basu, Leonid Digel, Kartik Aiyer*

The *Microbacterium* genus includes metabolically highly versatile Gram positive bacteria that occur in a range of ecosystems. While interactions with other microbes are known (including pathogenic ones), thus far there are no indications for extracellular electron transfer (EET) in this genus. A novel species, *Microbacterium deferre*, was isolated from the oxic-anoxic interface, also known as the microaerophilic veil, of a freshwater sediment that is dominated by multicellular long-distance electron-transferring cable bacteria. We explored the metabolic versatility of *M. deferre* in microaerophilic environments with redox gradients that rapidly change through electric sulphide oxidation activity of the cable bacteria.

In this study, we investigated whether *M. deferre* A1-JK was able to perform EET, which could be used to survive the rapid movements of the oxic-anoxic interface. Combining cyclic voltammetry, differential pulse voltammetry, and chrono amperometry in three-electrode cells and physiological analyses with genomics, we found that *M. deferre* could transfer electrons to electrodes using flavins as intermediates. This is the first report of EET within the *Microbacterium* genus. Curiously, we found that oxygen reduction occurred simultaneously with Fe(III) reduction, breaking the conventional model, where Fe(III) reduction only happens after oxygen is depleted. Flavins were found to be secreted and subsequently used to reduce Fe(III) under anaerobic, microaerophilic and atmospheric conditions.

Gram positive bacteria, capable of flavin-based EET (FLEET), all use a highly similar pathway and protein machinery. Genome analysis revealed presence of FmnA, Ndh2, DmkAB, but lacking crucial elements EetAB, PplA of the FLEET machinery (Fig 1). Interestingly, it contained FccA, known from Gram negative *Shewanella oneidensis* as periplasmic electron-conduit in EET. FccA was localized to the cytoplasm in *M. deferre*, suggesting a novel metabolic FLEET pathway (Fig 1). In our model, FccA transports electrons from oxidation processes to the FLEET proteins DmkAB, Ndh2 and FmnA that

regulate the electron flow towards flavins. To facilitate quick flavin reduction, *M. deferre* likely has a periplasmic electron-conduit. The flavins then diffuse through the cell wall to reduce extracellular electron acceptors.

Our results indicate the presence of a novel EET pathway with a new protein. The strain is capable of performing an anaerobic process under fully oxic conditions, showing *M. deferre*'s metabolic versatility, which makes it interesting from a physiological and a bioengineering perspective.

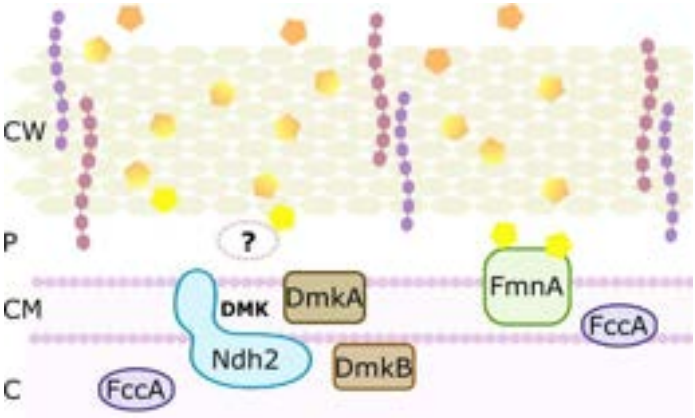


Figure 1: Potential EET pathway in M. deferre A1-JK. Pentagons: reduced flavins, ?: proposed protein, CW: cell wall, P: periplasm, CM: cytoplasmic membrane, C: cytoplasm, hexagonal strings represent teichoic acids.

Abstract ID: 3660 / Poster Number: 386.17P

Microbial Functionality and Cultivar-Dependent Effects of Liquid Vermicompost Extract in Chickpea

Presenting author: Maria Munoz Arbelaez, PhD, Scuola Superiore Sant'Anna, Pisa, Italy

Co-authors: *Gilbert Koskey, Paolo Barberi, Alessandra Turrini*

Chickpea (Cicer arietinum L.), a key legume in Mediterranean low-input systems, often faces productivity challenges due to nutrient limitations, water stress, and poor soil health. The role of soil microorganisms in mitigating these challenges is increasingly recognized. Liquid vermicompost extract (LVE), a biostimulant enriched with plant growth-promoting rhizobacteria (PGPR), offers a sustainable approach to enhance microbial functionality and soil fertility. These microorganisms facilitate crucial processes such as nitrogen fixation, phosphorus solubilization, and support beneficial interactions with arbuscular mycorrhizal fungi (AMF), contributing to improved plant health and resilience.

This study investigated the microbial-driven effects of LVE on nine chickpea cultivars, emphasizing plant growth, soil microbial activity, and nutrient cycling. Conducted at the Centre for Agri-Environmental Research 'Enrico Avanzi' in Italy, LVE was applied at critical growth stages from sowing to flowering. Results revealed significant cultivar-specific responses, highlighting the role of microbial interactions. Sultano demonstrated a 26% increase in biomass, while Pascia and Ares showed grain yield increases of 40% and 32%, respectively. Conversely, Vittoria and Reale experienced yield reductions (23% and 20%). LVE enhanced AMF colonization rates and microbial inoculation potential, showcasing its capacity to boost microbial diversity and soil functionality.

These findings underline the importance of utilizing microbial resources through biostimulants like LVE to optimize chickpea productivity and soil health. Further research is needed to elucidate the mechanisms driving these microbial interactions and to develop strategies for sustainable crop production.

Keywords: Chickpea, liquid vermicompost extract, plant growth, mycorrhizal potential, microbial activity

Abstract ID: 3405 / Poster Number: 135.16S

Microbial Insights Into Healthy Aging And Inflammation: Bridging Metagenomics and Experimental Evidence

Presenting author: Mr. Amir Nabinejad, European Institute of Oncology IRCCS (IEO), Milan, Italy; European School of Molecular Medicine (SEMM), Milan, Italy

Co-authors: *Elisa Piperni, Elisa Mattavelli, Farnaz Fazaeli, Davide Golzato, Liviana Ricci, Roberta Marconi, Francesco Asnicar, Andrea Baragetti, Alberico Luigi Catapano, Sabrina Tamburini, Nicola Segata*

The gut microbiome is a pivotal health ecosystem that modulates immune function, metabolism, and disease prevention by regulating inflammatory responses, producing metabolites, and maintaining intestinal barrier integrity across age-related conditions like cardiovascular disease, diabetes, and neurodegeneration. In addition, the rising elderly population underscores the urgency of studying age-related outcomes and exploring the gut microbiome signatures of aging. We enrolled 1,043 middle-aged to elderly participants (72 ± 11 years), integrating fecal metagenomics, quantitative blood metabolomics (250 biomarkers), cardiometabolic health assessments and microbial isolation to investigate how microbial composition could influence cardiometabolic health and inflammation in aging processes.

Our metagenomic analysis profiled a total of 2,655 species-level genome bins (SGBs), of which more than half were "unknown" SGBs (uSGBs) lacking reference genomes from isolates and representing uncharacterized microbes. Through Spearman's partial correlation analysis (adjusted for age, sex, BMI, and medication; $q < 0.2$) between microbiome and blood metabolomics, 94 of these SGBs negatively associated with GlycA (a systemic inflammation marker) including an unclassified *Clostridium* (SGB14252) and *Lachnospiraceae bacterium* (SGB4706). Instead, 23 SGBs were positively correlated, with the two strongest associations being with *Ruminococcus gnavus* and *one unknown Clostridiaceae* (SGB4746). Functional analysis of metagenomics-derived genes according to the KEGG orthology (KO) revealed that 941 gene families (~13% of the total KOs) correlated with GlycA (870 positives, 71 negatives; $q < 0.05$). Metabolism-related functions (442 KO), particularly carbohydrate metabolism pathways (124 KO) such as pyruvate and propanoate metabolism, were the most represented, highlighting a potential link between microbial metabolic activity and systemic inflammation.

To further characterize the unknown SGBs found to be linked with anti-inflammatory and health-associated phenotypes, we designed a cultivation-based strategy based on colony PCR relying on primers for over 80 species. These primers targeted unique marker genes within the SGBs of interest and were validated experimentally. Our protocol was applied to identify multiple colonies for SGBs of interest in each fecal sample and led to the identification of eight previously uncultured or uncharacterized microbes. We are currently performing combined computational prediction and *in-vitro* experiments to better elucidate their properties and potential translational significance.

These findings confirm the gut microbiome's crucial role in aging and inflammation, and identify specific microbial species and carbohydrate metabolism pathways associated with systemic inflammation. With the current phase of experimental characterization of some of the identified previously unknown microbes, we are generating promising insights into the complex interactions between microbial composition and age-related health processes.

Abstract ID: 2854 / Poster Number: 326.17P

Microbial interactions in mass-reared insects to promote host growth and health

Presenting author: Elena Crotti, PhD, University of Milan, Milano, Italy

Co-authors: *Elisa Bigarella, Dorra Hentati, Valentina Candian, Carlotta Savio, Gabriele Brenna, Costanza Jucker, Rosemarie Tedeschi*

Introduction: Microorganisms have established complex interactions with their insect hosts exerting a plethora of crucial functions for host biology, survival and immunity. In the recent years, insect farming has been promoted as an environmentally-friendly way to bioconvert agro-industrial by-products and to replace/complement traditional protein-based feed/food.

Objective: We aim to investigate the microbiota associated with mass-reared insects, i.e. the yellow mealworm (YM) *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and the black soldier fly (BSF) *Hermetia illucens* (L.) (Diptera: Stratiomyidae), to promote host growth and health, particularly when reared on different agro-industrial by-products.

Method: Besides insect microbiota characterization by 16S rRNA gene high-throughput sequencing, this multidisciplinary work evaluates insect performances on various by-products, with different nutrient compositions and under both healthy and diseased conditions.

Findings: Both species were able to grow on different locally available by-products (i.e. okara, brewer's spent grains, potato peels and potato selection waste for BSF, or mixes of wheat bran with brewer's spent grain or tomato peels for YM), though with varying degrees of efficiency. We then characterized BSF and YM gut microbiota through DNA-based techniques and cultivation approaches to gain an in-deep knowledge of the gut microbiota and to select potential bacterial probiotics for further insect dietary supplementation. Considering the possible involvement in immunity response and thus insect health, we characterized the hemolymph microbiota in YM to unveil interactions with the host and the hemocytes.

Conclusion: A better knowledge of microbial dynamics in mass-reared insects can promote the development of healthy production of insects as feed and food.

Abstract ID: 4152

Microbial life strategies across extreme environments

Presenting author: Dr. Claudia Coleine, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy

The Antarctic continent hosts some of the most extreme environmental conditions on Earth, where microbial life is forced to adapt to low temperatures, high UV radiation, desiccation, and oligotrophy. Among these niches, cryptoendolithic communities inhabiting translucent rocks in ice-free regions like the McMurdo Dry Valleys offer unique insights into the boundaries of life. This talk explores the adaptive strategies of these extremophiles, which include specialized pigmentation, metabolic flexibility, and community-level resilience. Drawing from recent metagenomic, metatranscriptomic, and cultivation-based studies, we illustrate how these microorganisms form highly structured consortia, often dominated by Actinobacteria, Cyanobacteria, and black fungi, optimized for survival under long-term environmental stress. Key mechanisms such as stress-induced dormancy, antioxidative defense systems, and rock-weathering capabilities reveal a complex ecological interplay shaped by geological and climatic constraints. These microbial ecosystems not only inform our understanding of terrestrial survival at the edge of habitability but also serve as analogs for potential life on Mars and other icy planets. Integrating geomicrobiology with functional omics provides a powerful framework for deciphering the signatures of microbial adaptation across geological time scales in the coldest desert on Earth.

Abstract ID: 3814 / Poster Number: 100.15S

Microbial monitoring in coral reef ecosystems: Associations between seawater gene content and water chemistry

Presenting author: Dr. Patrick Laffy, Australian Institute of Marine Science, Townsville, Australia; AIMS@JCU, James Cook University, Townsville, Australia

Co-authors: *Marko Terzin, Steven Robbins, Sara Bell, Kim-Anh Lê Cao, Renee Gruber, Pedro Frade, Michael Emslie, Philip Hugenholtz, Nicole Webster, David Bourne, Yun Kit Yeoh*

Introduction: Marine heat waves have impacted global coral reef ecosystems in recent years, leading to increased coral mortality, including on the Great Barrier Reef (GBR). As the frequency and severity of thermal stress events continues to impact reef composition, health and resilience, there is an increased need for rapid and biologically relevant monitoring metrics, and seawater microbes are showing potential as early warning indicators of reef resilience that may inform timely reef management strategies.

Objectives: This study characterized how pelagic reef microbes associate with seawater physico-chemical parameters (temperature, salinity, and nutrient concentrations) across seven GBR reef sectors. By exploring reef-associated seawater microbiomes in combination with existing reef monitoring activities, this study also aimed to identify whether any microbial taxa or functions can inform our understanding of reef health.

Method: Seawater samples were collected from 48 sites on the GBR for metagenomic DNA extraction and water quality evaluation across reef sectors as a part of the long-term monitoring program (LTMP) by the Australian Institute of Marine Science. The mixOmics R package was utilized to perform multivariate integrated gene-centric analyses, uncovering relationships between microbial communities, genes and physico-chemical parameters with adjustments for environmental variability.

Findings: Integrated gene-centric analyses utilizing 17 physico-chemical variables identified that microbial communities and functional gene profiles varied by season in concordance with changing nutrient concentrations and seawater physical parameters. Microbial functional genes demonstrated higher stable associations across sectors to seawater physico-chemical metrics compared to microbial taxa, with heterotrophic microbes (e.g., *Rhodospirillaceae*, *Burkholderiaceae*, and *Rhodobacteraceae*) consistently enriched in reefs with elevated dissolved organic and particulate carbon.

Strong associations of functional genes related to membrane transport, sugar utilization, and energy metabolism were also observed with elevated seawater nutrient dynamics.

Conclusion: By combining seawater microbial metagenomes, water chemistry and biological reef health monitoring data, stable taxonomic and functional microbial indicators were identified that inform metabolic changes within the reef water column. These indicator taxa and metabolism of the functional genes could reveal how coral reefs respond to stress events, uncovering the mechanisms involved in ecosystem resilience, informing future management efforts.

Abstract ID: 2369 / Poster Number: 314.17P

Microbial potential for biodegradation of toxic residual carbon in fly ash – isolation and identification and characterization of novel bacterial isolates

Presenting author: Prof. Łukasz Drewniak, University of Warsaw, Warsaw, Poland

Co-authors: *Mikolaj Iwan, Dawid Gmiter*

Fly ash, a by-product of fossil fuel combustion, generates 800 Mt annually, with most being landfilled due to the presence of toxic residual carbon, including polyaromatic hydrocarbons (PAHs) such as naphthalene, anthracene, fluoranthene, and phenanthrene. While microorganisms are known to degrade PAHs in polluted environments, their role in fly ash biotransformation remains underexplored. This study aimed to identify organic compounds in residual carbon and determine microorganisms capable of metabolizing these toxic components.

Fly ash samples from four power plants were characterized chemically, mineralogically, and texturally to identify unburnt organic components. GC-MS analyses confirmed the presence of key PAHs, providing a basis for isolating microorganisms able to colonize and degrade fly ash components. Using both direct isolation and pre-enrichment approaches, cultures were grown on minimal salt media (e.g., MSM, M9, Bushnell-Haas) supplemented with fly ash or individual PAHs as sole carbon sources. Following a four-week enrichment process, 500 isolates were screened for their colonization ability, PAH tolerance, and capacity to mineralize selected hydrocarbons.

Genomic analysis of 20 selected isolates identified high catabolic potential for degrading aliphatic and aromatic hydrocarbons and transforming heavy metals (Zn, Cd, Pb, Cu). The isolates included bacteria from the genera *Lysinibacillus* sp., *Brevundimonas* sp., *Microbacterium* sp., *Bacillus* sp., *Micrococcus* sp., and *Kocuria* sp.. These findings highlight the potential of microbial consortia for bioremediation and recycling of fly ash, offering sustainable solutions for reducing environmental impact.

Abstract ID: 3935

Microbial Powerhouses: Driving India's Sustainable Bioeconomy Revolution

Presenting author: Dr. Princy Hira, Maitreyi College, University of Delhi, New Delhi, India

Co-authors: *Rup Lal*

Introduction: Microbiology is a driving force behind India's transition to a sustainable and circular bioeconomy, addressing challenges related to energy, environment, and economic resilience (BioE3). The metabolic versatility of microorganisms enables eco-friendly biotechnological innovations that reduce dependence on fossil fuels and mitigate environmental degradation.

Objective(s)

This presentation aims to examine the role of microbial systems in advancing India's bioeconomy, focusing on indigenous microbial strains and their applications in biofuel production, biopolymer synthesis, and bioremediation. It highlights key microbial technologies that offer sustainable alternatives to resource-intensive industrial processes.

Method: The study explores microbial fermentation technologies for bioethanol and biogas production, microbial consortia for bioremediation of industrial and agricultural waste, and the use of extremophiles in high-stress industrial processes. Case studies of successful microbial interventions in India, such as biofertilizer development and eco-friendly wastewater treatment, are analyzed to demonstrate their impact.

Findings: Advances in microbial genomics, metagenomics, and synthetic biology are accelerating the discovery of novel microbial pathways, facilitating scalable biotechnological solutions. Integrating traditional microbiology with modern technologies enhances India's ability to harness microbial resources for sustainable development and economic growth.

Conclusion: Microbiology is a cornerstone of India's sustainable bioeconomy, offering innovative solutions to global challenges while reinforcing the country's leadership in microbial biotechnology. By leveraging its rich microbial diversity and cutting-edge research, India can drive innovation, promote sustainability, and play a pivotal role in shaping the future of the global bioeconomy.

Abstract ID: 1502 / Poster Number: 365.17P

Microbial Strategies for Tailored Metal Nanoparticle Production

Presenting author: Ana Lucía Campaña Perilla, PhD, University of Oslo, Oslo, Norway

Co-authors: *Athanasios Saragliad, Mohamed L. Merrun, Pavlo Mikheenko, Dirk Linke*

Bacteria possess sophisticated defense mechanisms that enable the reduction of toxic metal ions into nanoparticles (NPs), offering a sustainable alternative to conventional chemical and physical synthesis methods [1]. These microbial-metal interactions present exciting opportunities for eco-friendly production of precious metal NPs, including palladium, palladium-iron and other metal alloys at varying concentrations of gold, platinum, copper, silver, and selenium [2,3]. This study aims to optimize the biosynthesis of metal NPs for industrial-scale applications through genetic engineering and process refinement while elucidating the mechanisms underlying bacterial metal reduction. Wild-type and genetically engineered *Escherichia coli* strains were exposed to various metal ion solutions to facilitate NP formation. The resulting NPs were characterized using TEM, revealing sizes under 5 nm and distribution across cellular regions [4]. Additional advanced characterization techniques, including EDX, AFM, TGA, and FTIR, were employed to analyze NP composition, size distribution, morphology, and surface characteristics. Comparative analyses demonstrated that genetic modifications influenced metal uptake and enhanced the efficiency and specificity of NP synthesis. Catalytic activity tests, such as alkene hydrogenation and Suzuki coupling reactions, showed that biosynthesized palladium NPs exhibited catalytic performance comparable to commercial nanocatalysts. Efforts to purify NPs by removing residual biological molecules were evaluated, an essential step to ensure their compatibility for applications such as biomedicine. These findings highlight the potential of microbial systems as a sustainable platform for the synthesis of metal NPs with high catalytic efficiency, paving the way for scalable production and their use in diverse industries.

Abstract ID: 3679 / Poster Number: 406.16P

Microbial Synergies in Sustainable Horticulture: Advancing Peat-Free Substrates for Optimized Plant Growth

Presenting author: Ms. Katja Burow, Erfurt University of Applied Sciences, Erfurt-Kühnhausen, Germany

Co-authors: *Rhedia Proma, Neetu Neetu, Dalia Gaber, Sneha Sabu, Sarah Kühne, Julia Brandes, Philipp Franken*

The development of sustainable products, of optimized processes and of methods to conserve natural resources finds currently its way into many areas of research. In horticultural sciences, the development and improvement of peat-free substrates becomes increasingly important. Due to the environmental repercussions associated with peat extraction and its limited availability, a critical need has been prompted to explore sustainable alternatives. This study focuses on addressing challenges related to nitrogen (N) immobilization, substrate compaction and optimizing plant growth in cultivation systems that minimize or eliminate peat usage by using several groups of microorganisms.

First, the impact of various peat-reduced and peat-free substrates (steamed/ unsteamed), comprising among others of peat, green compost, wood fiber and digestate on growth of *Petunia exserta*, *Petunia hybrida* cv. 'Mitchell' and *Ocimum basilicum* were examined. The plants' phenotypic characteristics were observed, and the substrates were analyzed for their chemical properties.

After the culture period, the plants exhibited superior growth in sterile, peat-free substrates. In the subsequent phase, diverse microorganisms will be introduced to different substrate mixtures to augment substrate quality and assess their effects on plant growth development. Various plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF) – adapted to high phosphate concentrations, endophytic entomopathogenic fungi (EEMPF) and dark septate endophytes (DSEs) will be employed, leveraging their unique qualities for the improvement of peat-reduced/ peat-free substrates. DSEs are tolerant to abiotic stress and can mobilise N from organic resources. It could be proven that Petunia plants inoculated with DSEs showed significantly improvement plant growth under sterilized and peat free conditions. Potential representatives of EEMPF, colonizing plant tissues and protecting the plants against insect attacks, were investigated in terms of their impact against fungus gnats which poses a serious problem in pot cultures under glass and especially highly attracted to peat free substrates. Furthermore, PGPR are selected due to their ability to fix

atmospheric N, enhance nutrient uptake, and stimulate plant growth through hormone production and root colonization. Such mycorrhizal helper bacteria are able to form biofilms on AMF surfaces protecting them from mycophagous bacteria and enhances their vitality. For the final compilation of an effective microbial consortium additional studies are intended to further improve the microbial impact in peat-free substrates focusing on the understanding of interspecific interactions between different microorganisms. For this purpose, an *in vitro* system was modified, to investigate the effects of bacterial consortia aiming to determine the influence of individual bacterial strains and combinations of various bacteria on plant development. Initial results demonstrated an influence of the individual bacterial strains and their combinations on root growth in *Petunia hybrida* cv. 'Mitchell', particularly concerning root length and branching. Additionally, the endophytic properties of the used single bacterial strains and of bacterial consortia were further examined. The results indicated that bacteria within the consortium could colonize the plant roots and that certain bacteria could outcompete others in the colonization process. Moreover, bacterial strains not belonging to the inoculated consortium were re-isolated from the plant roots. Consequently, it is hypothesized that the pre-existing microbiota in plant seeds may represent an additional factor to consider when applying microbial consortia in peat-free substrates, particularly regarding influencing bacterial/fungal interactions. Following these results, further microbiome investigations are planned.

Abstract ID: 3377 / Poster Number: 291.15P

Microbial δ18O(PO_4^{3-}) as a Dynamic Indicator of Metabolic Responses to Environmental Stressors in Soil Ecosystems

Presenting author: Ms. M. Elena Evertz, ETH, Zurich, Switzerland

Co-authors: *Cheng Shi, Thomas Hofstetter, Federica Tamburini*

Human activities, such as urbanization and pollution, significantly impact soil microbiomes and alter their metabolic processes. These disruptions change microbial community composition and function, influencing ecosystem resilience.

Phosphate oxygen isotope ratios (δ18O(PO_4^{3-})) serve as a potential indicator of microbial metabolic shifts, as phosphoryl transfer is central to energy support, replication, signalling, and other fundamental processes. Pyrophosphatase has traditionally been considered the primary biological determinant of δ18O(PO_4^{3-}), resulting in isotopic equilibrium between oxygen in phosphate and water[1]. However, environmental studies frequently reveal

discrepancies from this assumption[2], suggesting unknown processes influences.

This study challenges the assumption that pyrophosphatase is the sole determinant

of δ18O(PO_4^{3-}) by investigating the role of other enzymes under stress conditions. Such work provides a new framework for understanding isotopic signatures as dynamic indicators of microbial metabolism. We conducted experiments on *Escherichia coli* and *Bacillus subtilis*, subjecting them to controlled variations in temperature and nutrient availability. Cytosolic phosphate was extracted and its δ18O(PO_4^{3-}) determined by isotope ratio analysis using mass spectrometry (IRMS). Metabolic fluxes of phosphometabolites were traced using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Preliminary findings suggest that environmental stress influences δ18O(PO_4^{3-}) beyond

pyrophosphatase-mediated equilibrium, likely due to shifts in metabolic

[1] Chang and Blake, “Precise Calibration of Equilibrium Oxygen Isotope Fractionations between Dissolved Phosphate and Water from 3 to 37°C.”

[2] Tamburini et al., “Oxygen Isotopes Unravel the Role of Microorganisms in Phosphate Cycling in Soils”; Tamburini et al., “Oxygen Isotopes for Unraveling Phosphorus Transformations in the Soil-Plant System”; Gross and Angert, “What Processes Control the Oxygen Isotopes of Soil Bio-Available Phosphate?”

regulation. By linking δ18O(PO_4^{3-}) variability to microbial metabolic shifts, this research provides a tool for monitoring ecosystem health and resilience under changing conditions. The findings also have implications for improving biogeochemical models and informing strategies to mitigate the impacts of urbanization and pollution on soil ecosystems.

Abstract ID: 3059

Microbially induced calcite precipitation: role of waste concrete fines properties and metabolic pathway of bacteria on the mechanical strength of bioconcrete

Presenting author: Assoc. Prof. Hana Stiborová, University Of Chemistry And Technology, Prague, Prague, Czech Republic

Co-authors: *Kristýna Kliková, Petr Holeček, Dana Koňáková, Václav Nežerka*

The construction and infrastructure repair sectors rely heavily on cement-based materials. However, the production of Portland cement (PC) poses significant energy and environmental challenges, as it involves the burning of limestone and clay at 1450°C, which accounts for approximately 7% of total anthropogenic CO_2 emissions. This underscores the urgent need for comprehensive research into improving circularity and sustainability through the partial replacement of PC.

Our investigation confirmed that microbiologically induced calcite precipitation (MICP) is a viable strategy to utilise waste concrete fines (WCF), improve their cohesion and facilitate the formation of robust conglomerates. We investigated the efficacy of *Sporosarcina pasteurii* DSM 33 and two *Bacillus* species that use different metabolic pathways (urea hydrolysis and oxidation of organic compounds), resulting in different $CaCO_3$ crystal formations. In addition, we tested three different WCFs that differed in age, particle size distribution and chemical composition.

Scanning electron microscopy provided a detailed insight into the morphology and quantity of crystals formed on WCF grain surfaces. Our results showed that sterile samples without MICP treatment showed only a minimal increase in calcite due to spontaneous crystallisation. Notably, the degree of biological carbonation correlated positively with the strength of MICP consolidated WCF material. *S. pasteurii* DSM 33 showed a 10-12% increase in calcium carbonate content, significantly improving the cohesion and strength of WCF over time (Holecek et al., 2024). Most notably, *Bacillus cohnii* DSM 6307 produced hexagonal gypsum crystals in one of the WCFs, identified by X-ray diffraction (XRD) as the AFt phases of ettringite and thaumasite, which significantly improved the integrity of the conglomerates. This resulted in an impressive 172% increase in stiffness compared to sterile samples (Kliková et al., 2025).

Our research has also focused on isolating bacteria capable of MICP from the limestone Koněprusy Cave in the Czech Republic. We collected samples from different karst

formations and dripping waters, enriching the MICP-capable bacteria utilizing different pathways for the formation of $CaCO_3$.

Our findings highlight the potential of MICP as a sustainable recycling method for WCF, promoting more efficient concrete waste management and supporting the principles of a circular economy.



Figure 1: Original WCF grains (left) and indentation of hardened samples using a conically-shaped steel indenter forced into a sample (middle) and the uniaxial compression test (right), taken and adapted from (Kliková et al., 2025)

Abstract ID: 3863 / Poster Number: 121.15F

Microbially Mediated Carbon and Nitrogen Cycling in the Deep Atlantic: Insights into Particulate Flux and Organic Matter Transformation from Metagenomic Analyses

Presenting author: Dr. Maria Papadatou, University of Vienna – Department of Functional and Evolutionary Ecology, Vienna, Austria

Co-authors: *Gerhard J. Herndl, Zihao Zhao*

Marine snow aggregates play a pivotal role in the biological carbon pump, facilitating the downward flux of particulate organic carbon (POC) and influencing oceanic carbon sequestration. However, microbial transformation of these aggregates modulates the balance between carbon export and remineralization. The MIMEPOC project investigates the phylogenetic and functional composition of particle-attached and free-living microbes in the North Atlantic Ocean using metagenomics. Water samples (475-3800m) were collected across 14 stations using CTD-Niskin bottles and fractionated into 0.2, 1, and 3µm size classes. Taxonomic analysis of 21 metagenomes revealed distinct microbial communities based on particle size and depth, with Pseudomonadota, Planctomycetes-Verrucomicrobia-Chlamydiae (PVC) group, and Terrabacteria dominating all samples. The phyla of the Thaumarchaeota-Aigarchaeota-Crenarchaeota-Korarchaeota (TACK) group, Myxococcota, Nitrospinae/Tectomicrobial group and Acidobacteriota were predominantly present in the large aggregate fraction (3µm). Functional annotations identified key metabolic pathways contributing to carbon, nitrogen, and methane metabolism. Genes linked to carbon fixation (*RuBisCO*, *PEPcase*) and degradation (*Enoyl-CoA Reductase*, *Thiolase*) were widespread, whilst nitrogen metabolism genes indicated nitrogen uptake (*ABC_tran*) and organic nitrogen degradation (*Pro_CA*, *Aldedh*). Methane metabolism genes (*Fer2*, *Metalloenzyme*) were enriched in particle-associated communities, suggesting microenvironments favorable for methanogenesis and methane oxidation. These findings underscore the role of microbial communities in regulating deep-sea biogeochemical cycles. The presence of functionally distinct taxa across particle sizes highlights microbial mediation in organic matter transformation, with implications for carbon sequestration and greenhouse gas dynamics in the ocean. Understanding these processes is crucial for predicting microbial responses to changing ocean conditions and their impact on global carbon and nitrogen budgets.

Abstract ID: 3441

Microbially mediated soil GHG fluxes in alpine ecosystems have seasonal and diurnal patterns

Presenting author: Prof. Ianina Altshuler, EPFL, Sion, Switzerland

Introduction: Anthropogenic climate warming disproportionately affects alpine and Arctic environments, including European permafrost-affected ecosystems. The European Alps are experiencing accelerated warming and snow cover loss, resulting in the replacement of boreal and alpine tundra environments with warmer temperate climates. Currently, research on soil greenhouse gas (GHG) fluxes and their relation to microbial dynamics in high-altitude ecosystems is limited. Additionally, the effects of seasonality and the diurnal cycle on GHG fluxes are poorly understood. This makes it difficult to quantify the current annual GHG fluxes in permafrost-affected ecosystems and predict how fluxes will alter with warming.

Objective(s): Determine *in-situ* CO_2 and CH_4 fluxes in high-altitude alpine systems across the four seasons and the diurnal cycle. Relate the direction (+ve vs. -ve flux) and magnitude of GHG soil fluxes to physicochemical properties and microbial diversity. Determine how future warming would interact with these parameters via experimental *in-situ* warming.

Method: Passive warming chambers were installed across high-altitude alpine ecosystem sites to determine the effects of warming (1.5-2.5°C) on GHG fluxes and the microbiome structure. *In-situ* GHG soil fluxes, physicochemical soil parameters, and microbiome composition were determined across the spring, summer, fall, and winter seasons. Additionally, we investigated the effects of the diurnal cycle by measuring gas fluxes and microbial diversity during the day and nighttime across three consecutive days.

Findings: Across all sites we report positive fluxes of CO_2 and negative fluxes of CH_4, suggesting that high altitude soils act as CH_4 sinks and CO_2 sources. Both seasonality and the diurnal cycle have an impact on the extent of GHG fluxes, with the magnitude of the CO_2 flux being primarily affected by temperature and artificial warming, while the CH_4 flux not responding to warming and instead being more tightly linked to soil moisture and abundance of methanotrophic microorganism.

Conclusion: Future warming will potentially exasperate microbially mediated soil emissions of CO_2, while CH_4 uptake will likely remain unaffected in alpine soils.

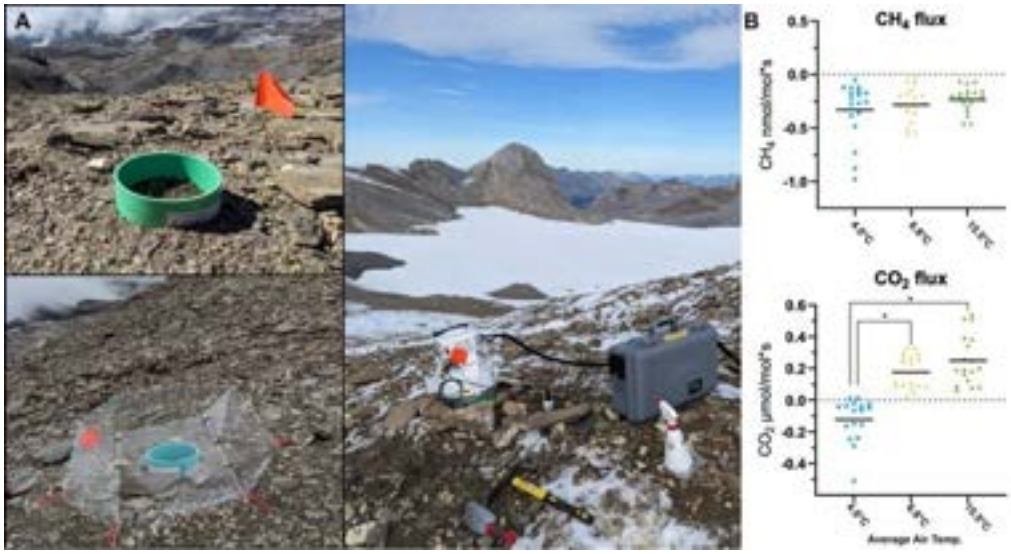


Figure 1: *A) Example of experimental warming plots and GHG flux measurements in high-altitude permafrost affected sites. B) CO_2 and CH_4 fluxes across three fall days.*

Abstract ID: 2363 / Poster Number: 423.17P

Microbiological and ecological state of Lake Chorne Kyiv region, Ukraine

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Co-authors: *Liudmila Khomenko, Serhii Skrotskyi, Nataliia Matviienko, Danguolė Montvydienė*

Background: Continuous hostilities on the territory of Ukraine, caused by Russia, lead not only to huge human losses, but also to serious polluted ecosystems, decrease of biodiversity and to a negative impact on aquaculture. The microbial communities in water bodies are an extremely sensitive bioindicator systems that can reflect the state and changes in microbial assemblages when anthropogenic toxic substances enter the aquatic environment. Moreover, the introduction of various toxic chemical compounds into the aquatic environment has a significant negative effect on aquaculture and its microbiota, potentially causing diseases and reducing the populations of valuable fish species.

Objectives: – conducting microbiological monitoring of Lake Chorne, studying microbiota of aquaculture and selecting potential microorganisms – biodestructors of explosives or their derivatives.

Materials and methods: For microbiological monitoring of samples of Lake Chorne (water, bottom sediments and fish) were used standardized methods (according to ISO 8199).

Findings: A microbiological study was conducted on the water and bottom sediments of Lake Chorne, Kyiv region (50.41293, 30.15799), where cruise missiles fell into the reservoir. The research identified the primary ecological-trophic groups of microorganisms in the water and calculated the trophic index. Microorganisms that absorb 2-dinitrotoluene were detected in water samples (1.6±0.01 CFU/g, lg). It was shown that the total number of saprophytic microorganisms in the water is 100 times greater than in the sediment, amounting to 5.18±0.06 CFU/g, lg and 3.58±0.07 CFU/g, lg respectively. The number of conditionally pathogenic microorganisms is significantly higher in the bottom sediments than in the water. The large number of these pathogenic microorganisms poses a significant threat to the normal functioning of the entire lake, since these microorganisms from bottom sediments can easily enter the water and cause infectious diseases of aquaculture and humans. In addition, the trophic index in

the water sample is 20% higher when compared with the sediment sample and is 1.22 and 1.0, respectively. The water in this lake, according to the ecological and trophic index, corresponds to water bodies polluted with organic matter.

The highest number of lactic acid bacteria and bacteria of the genus *Enterococcus sp.* was isolated from the Common Rudd fish, 4.86±0.09 CFU/g, lg and 2.0±0.5 CFU/g, lg, respectively. While in the upper reaches, the number of LAB was the lowest among the studied fish specimens, amounting to 3.67±0.2 CFU/g, lg, and the bacteria of the genus *Enterococcus sp.* were <10 CFU/g. It was established that the highest number of bacteria of the genus *Staphylococcus sp,* Coliform bacteria, and micrmycetes were found in *Leucaspius delineatus*.

The study of Lake Chorne revealed changes in the quantitative and qualitative composition of the main ecological-trophic groups of microorganisms. The presence of microorganisms capable of degrading 2-nitrotoluene indicates contamination of the lake with explosive substances. This contamination has caused a shift in the microbial composition, with an increase in the number of pathogenic microorganisms and a decrease in oligotrophic microorganisms. For further development of water purification technologies, isolates of microorganisms with biodegradative activity against nitroaromatic compounds, which are components of explosive substances or their derivatives, have been obtained.

Abstract ID: 2628 / Poster Number: 369.16P

Microbiological assessment of Enterobacteriaceae in pork meat: bacterial load and species diversity

Presenting author: Ms. Celia Arraiz-Fernández, University of La Rioja, Logrono, Spain

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Pork meat is a commonly consumed food, but its microbiological contamination can pose a risk to public health. Bacteria of the family Enterobacteriaceae, present in this type of product, include both non-pathogenic species and potential disease-causing agents. Assessing the bacterial load and microbial diversity in pork is crucial to identify risks and ensure food safety.

The objective of this study is to assess the bacterial load and species diversity of Enterobacteriaceae in pork samples, determining the bacteria present and their counts.

Thirty-nine pork samples were analysed microbiologically. Bacteria were isolated on MacConkey agar medium and Enterobacteriaceae species were counted and identified by MALDI-TOF mass spectrometry.

Of the 39 samples analysed, 25 had counts below 1 log CFU/g, and 5 above 4 log CFU/g. With regard to the strains isolated from the MacConkey agar medium of the pork samples, 10 species of Enterobacteriaceae were identified. The predominant bacterium was Serratia liquefaciens, although other bacteria such as Yersinia enterocolitica, Klebsiella oxytoca or other Serratia genera were also isolated. However, Escherichia coli was not detected in any of the samples.

In conclusion, this research indicates the variability of the bacterial load in pork samples,with some showing elevated bacterial counts. In total, ten species of the Enterobacteriaceae family were identified, with S. liquefaciens being the most common, and with potential pathogens such as Y. enterocolitica. The absence of E. coli is significant, but the presence of other pathogens underlines the importance of monitoring and controlling bacterial contamination in meat products to ensure food safety.

Abstract ID: 3053 / Poster Number: 374.16P

Microbiological evaluation of milk powdered formulae for infants sold on the Italian market

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Introduction: Infants are vulnerable to foodborne infections because their immune system is not completely developed.

Objective: Objective of this study was to evaluate the microbiological quality and safety of powdered formulae for infants sold on Italian market. A total of 83 samples including 23 infant formulae (PIF), 43 follow-on formulae (FOF), and 17 formulae for special medical purposes (SMP) were analysed.

Method: Total bacterial count (ISO 4833), anaerobic bacterial count, lactic acid bacteria (ISO 15214), *Pseudomonas* spp. (ISO 13720), *Enterobacteriaceae* (ISO 21528-2), *Escherichia coli* (ISO 16649-1), clostridia (ISO 15213-2), *Enterococcus* spp. (Slanetz-Bartley Agar), yeast and moulds (ISO 21527-1), *Bacillus cereus* (ISO 7932) and Coagulase-positive Staphylococci (ISO 6888-1) were enumerated. The presence of *Salmonella* spp. (ISO 6579-1), *Cronobacter sakazakii* (ISO 22964), *Listeria monocytogenes* (ISO 11290-1) and *Yersinia enterocolitica* (ISO 10273) was also investigated. Isolates were identified using MALDI-TOF MS.

Findings: The total bacterial count was $<5x10^2$ CFU/g in 17 SMP (100%), 20 PIF (87.0%), and 42 FOF (97.7%), indicating good quality according to the Code of Hygienic Practice for Powdered Formulas for Infants and Young Children. In most samples anaerobic bacteria count showed low levels. LAB counts were $<10^2$ CFU/g in 17 SMP (100%), 19 PIF (82.6%), and 37 FOF (86.0%), while high counts were rarely evidenced. Moulds were sporadically detected in almost all samples. Presumptive *B. cereus* was enumerated in low counts in two PIF and one SMP samples. Other parameters were always under the limit of detection (10 CFU/g). Other pathogens were never detected in the products.

Abstract ID: 439 / Poster Number: 437.16P

Microbiology Education for a new era: Integrating AI and Skills-Based Assessments to Enhance Student Learning and Employability

Presenting author: Dr. Katie Solomon, University of Exeter, Exeter, United Kingdom

Co-authors: *Sally Rogers, Alan Brown, Mark Clements*

Introduction: Ensuring microbiology graduates have the skills and attributes for workplace readiness is of utmost importance. Higher Education processes and policy have struggled to adapt quickly enough to meet the requirements of an AI-enabled workplace, creating a disconnect between how University students are prepared for employment and the world in which they will enter.

Objective: To address the evolving needs of our microbiology graduates by embedding a wider range of updated employability skills and attributes into our module teaching and assessments, ensuring students can clearly identify and evidence which skills they have attained, to thrive in an AI-enabled workplace.

Method: A novel 'Skills and Mindsets Framework' informed redesign of assessment practices, policies and attitudes across microbiology teaching to address the needs of an AI-enabled workplace. Skills such as AI/digital fluency, entrepreneurship and resilient self-advocacy are embedded through authentic coursework assessments i.e. debates, podcasts, consultancy reports and infographic data presentation rather than essay-based exams. Student experience was surveyed at three points across the academic year and declarations of AI use across all assessments collected.

Findings: Students reported greater satisfaction with teaching and assessments. The wider range of engagement and expression methods promoted greater inclusivity of diverse learners and balanced workload more evenly throughout the term. Students were also more confident that new assessment styles prepared them for employment.

Conclusion: This project underscores the potential of curricular innovations to enhance student experience whilst bridging the gap between academic preparation and real-world employment in an AI enabled world.

Abstract ID: 2213 / Poster Number: 012.17S

Microbiome alterations in peri-implantitis: comparing affected implants, healthy implants, and saliva

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Introduction: The increasing use of dental implants has been paralleled by the growing prevalence of peri-implant diseases, with peri-implantitis (PI) being particularly concerning. PI is a biofilm-driven inflammatory condition that affects the tissues surrounding dental implants, resulting in progressive bone loss and, ultimately, implant failure. Comprehensive studies are urgently needed to elucidate the microbial dysbiosis associated with PI. Such insights are critical for advancing our understanding of its pathogenesis and developing effective management strategies.

Objectives: To gain a deeper understanding of the microbiome composition, diversity, and functional potential of the subgingival biofilm in both healthy implants and those affected by PI, and to evaluate the extent to which the salivary microbiome reflects the peri-implant subgingival microbiome.

Methods: Following approval by the institutional Ethics Committee and adherence to predefined inclusion and exclusion criteria, patients were recruited for this study. Saliva and subgingival peri-implant biofilm samples were collected, resulting in 100 samples from 40 participants. These participants included individuals with either exclusively healthy implants or a combination of diagnosed PI-affected and healthy implants. The collected samples were subsequently analysed using shotgun metagenomic sequencing.

Results: By applying log ratio calculations and differential ranking analyses, we identified the key species that most effectively distinguished between the healthy and diseased study groups. *Mogibacterium timidum*, *Schaalia cardiffensis*, *Parvimonas micra*, *Filifactor alocis*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis* and *Olsenella uli* were associated with the subgingival peri-implant biofilm. In contrast, *Neisseria* sp oral taxon 014, *Haemophilus parainfluenzae*, *Actinomyces naeslundii*, *Rothia mucilaginosa* and *Rothia aeria* were more prevalent in healthy peri-implant biofilms. Functional pathways, such as arginine and polyamine biosynthesis, including putrescine and citrulline biosynthesis, showed stronger correlations with PI-affected implants. In

contrast, peri-implant health was characterized by the predominance of pathways involved in purine and pyrimidine deoxyribonucleotide de novo biosynthesis, glucose and glucose-1-phosphate degradation, and tetrapyrrole biosynthesis. Species such as *Veillonella atypica*, Veillonella *parvula*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Bifidobacterium dentium*, and *Neisseria sicca* changed the most between the saliva of patients with PI and those without PI, with these species being more prevalent in saliva associated with PI.

Conclusions: Our findings indicate that healthy implants in PI-free oral cavities exhibit significant differences in microbial composition and functional pathways compared with healthy implants coexisting with PI-affected implants, which closely resemble PI-associated profiles. A similar pattern was observed in salivary samples, where microbial and functional biomarkers followed comparable trends.

Abstract ID: 1836

Microbiome and antibiotic-resistant bacteria in the water-soil-plant continuum under unplanned water reuse practices

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The climate crisis is severely disrupting the water cycle leading to water scarcity and reduced access to clean water. In the Mediterranean region, wastewater treatment plant (WWTP) effluents account for a significant river flow downstream from urban areas and often contain traces of antibiotics, antibiotic resistance genes (ARGs), and antibiotic-resistant bacteria (ARBs) that are not fully removed. Many water sources impacted by WWTP effluents are used for crop irrigation, an unregulated practice known as unplanned (*de facto*) water reuse. This practice can facilitate the transfer of aforementioned contaminants to crops, posing potential food safety risks. Furthermore, the low antibiotic levels present in WWTP effluents can drive the development of resistance. At the same time, optimizing water use is crucial for coping with the effects of climate change and different irrigation methods may impact on the transfer of contaminants through the food web.

This study aims to evaluate the presence of ARB resulting from unplanned water reuse in the water-soil-plant system under different irrigation practices, as well as to assess the occurrence of multidrug-resistant bacteria (MDR phenotype).

We selected an agricultural plot cultivated with *Lactuca sativa* L. (lettuce) and located in the Jarama's river lower meadow, irrigated with its surface water, which is highly impacted by effluents from the main WWTPs of the Madrid region. Lettuce has a high contaminant bioaccumulation capacity, it is widely consumed in the Mediterranean area and is typically eaten raw. The plot was divided into two sectors, one irrigated by gravity-fed furrow irrigation and the other by sprinkler irrigation, over a period of 45 days. Water, plant tissues, and soil samples were collected at different times during this period. For the plant tissues, external and internal leaves were differentiated, and phyllosphere and endosphere were extracted. The different matrices were processed to perform colony counting of coliforms and total aerobes. Subsequently, a representative number of

isolates were used in a screening to detect ARB. Then, antibiotic susceptibility tests (disk diffusion) were conducted to confirm data and identify MDR phenotype.

The counts of coliform bacteria and total aerobes in the phyllosphere increase over time with furrow irrigation. Globally, most of the resistant coliforms and total aerobic bacteria isolated from the different types of samples were resistant to one or two antibiotics, with a small percentage exhibiting MDR phenotype. In the case of soil samples, there is an increase in resistant coliforms over time in the sprinkler irrigation system, whereas in the furrow irrigation system the number of resistant coliforms returns to an initial state. Regarding the plant samples, a higher number of resistant coliforms were found in the phyllosphere of inner leaves of the lettuces irrigated by sprinklers. Additionally, an increased prevalence of MDR coliforms was observed at the intermediate time point in both the phyllosphere and endosphere under sprinkler irrigation.

The results suggest that irrigation methods play a role in the distribution of ARB in the water-soil-plant continuum. The observed increase in ARB under sprinkler irrigation suggests that this practice may facilitate greater resistant bacterial dispersion.

Abstract ID: 1304 / Poster Number: 208.15P

Microbiome dynamics in early life stages of the precious Mediterranean red coral Corallium rubrum

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Microbial colonization during the early life stages of corals is of fundamental importance for coral fitness. However, for many octocorals, it is not yet known whether symbionts are acquired from parental colonies, the environment or both. In this study, we explored the acquisition of the bacterial microbiome by *Corallium rubrum*, the precious red coral, a species that contributes to Mediterranean coralligenous assemblages and associated diversity. We examined the bacterial community composition at various early life stages, including newly released larvae, 5- and 10-day-old larvae, 3-month-old settlers, 1-year-old recruits, and 3-year-old juveniles. By employing a direct PCR-based method for 16S rRNA gene metabarcoding, we were able to analyze the microbiome of individual larvae and settlers. Our findings reveal that the microbiome of *C. rubrum* undergoes significant maturation within the first year, with dominant symbionts such as *Spirochaetaceae* being acquired from the environment. These results deepen our understanding of the dynamic process of bacterial colonization and its crucial role in the functioning of the coral holobiont.

Abstract ID: 2296 / Poster Number: 232.15P

Microbiome dynamics in white truffle ecosystems: a comparative analysis of soil communities in primary and expanding forests

Presenting author: Dr. Mara Rondolini, University of Perugia, Perugia, Italy

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Truffles are highly valued underground fungi with considerable economic significance. While the cultivation of various truffle species has progressed, the harvesting of white truffles (*Tuber magnatum* Picco) continues to depend on natural production. Promoting the expansion of productive forests is crucial to safeguarding these natural resources. This study explores the microbiome dynamics in both established and newly expanding truffle forests through amplicon sequencing of the fungal ITS region and the prokaryotic 16S rRNA gene. The objective was to monitor and compare the development of soil biological communities in a primary productive forest and an adjacent expanding area over two years. Key findings revealed that certain fungi, particularly from the genus *Mortierella*, colonized newly available ecological niches. Additionally, interactions between saprotrophic fungi and ectomycorrhizal fungi (ECM) were examined. The bacterial community in both forest types was predominantly composed of Pseudomonadota, Planctomycetota, and Actinomycetota. Notably, the genus *Tuber* exhibited distinct behaviour compared to other ECM fungi, showing positive correlations with bacterial taxa such as *Ktedonobacter*, *Zavarzinella*, and *Sphingomonas*. The study offers an initial insight into the evolving habitats of white truffles, highlighting the complex interplay between fungal and bacterial communities. Further research is essential to unravel the potential relationships between specific taxa and their roles in truffle ecosystem dynamics.

Abstract ID: 1847 / Poster Number: 058.16P

Microbiome of pediatric patients with inflammatory bowel disease in the Czech Republic

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Introduction: Inflammatory bowel disease (IBD) is a chronic condition that usually begins in childhood, significantly impacting patients´ quality of life. It includes Crohn's disease and ulcerative colitis, leading to symptoms like abdominal pain, diarrhoea, and fatigue.

Objective: This study aimed to characterize the gut microbiome in children with IBD (Crohn's disease and ulcerative colitis), compare it to healthy controls, and to investigate potential associations of microbiome composition with IBD.

Method: This study included 62 children with pediatric inflammatory bowel disease (PIBD) and 26 healthy controls. Stool and inflammatory mucosal biopsies were collected. DNA was extracted from samples, followed by 16S rRNA gene sequencing targeting the V1-V2 hypervariable regions for microbiome analysis. Clinical data, including disease activity score and inflammatory markers, were collected for each participant.

Findings: Microbial diversity and bacterial taxa composition differed between IBD patients and healthy controls. PIBD patients exhibited a significant enrichment of taxa *Enterobacteriales* and *Hemophilus*, whereas the family *Oscillospiraceae* was significantly depleted compared to healthy controls. Moreover, Crohn's disease patients experiencing relapses exhibited significantly lower alpha diversity compared to those in remission, suggesting a potential link between reduced microbial diversity and disease activity.

Conclusion: Bacterial taxa significantly different between PIBD patients and controls may contribute to the pool of microbes associated with IBD, either enriched or depleted. Importantly, reduced microbial diversity in Crohn's disease patients experiencing relapses suggests a potential role of microbial dysbiosis in disease activity and progression.

Abstract ID: 3207 / Poster Number: 318.15P

Microbiome over the depth of the alpine Cenote Abyss ice cave

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Co-authors: *Daniele Ghezzi, Martina Cappelletti, Francesco Sauro, Alessio Romeo, Andrea Firrincieli*

Introduction: Microbial life at the cryosphere represents a unique archive of slow biogeochemical processes in ancient and chilly environmental conditions. The Cenote Abyss cave, located at 2940 m a.s.l. in the Regional Park of Fanes, Sennes and Braises, is an impressive carbonatic ice cave of 285 m deep and hosts one of the most voluminous cave glaciers of the Dolomites.

Objective: The enormous ice deposits identified within this cave are a remote, oligotrophic environment that represents an excellent accessible model system for understanding the fundamental microbial-mineral interactions that contribute to the subsurface biosphere and is of great interest for the study of the palaeoclimate and climatic evolution of the Dolomites over the last few thousand years, with insights into the recent melting of the cave glacier.

Methods: In this study, ice samples were taken at different depths within the Cenote Abyss, and the microbial communities present were characterised on a taxonomic andmicrobial functional potential by means of 16S NGS microbial sequencing and metagenomic analysis, respectively.

Findings: 16S sequencing analysis shows that the microbial communities of Cenote Abyss were dominated by members of Actinobacteria, Bacteroidetes and Proteobacteria well adapted to extreme temperatures and possibly involved in different steps of the carbon and nitrogen cycles in accordance with the cave depth.

Conclusion: This study revealed the presence of genes involved in the various metabolic processes of the nitrogen geochemical cycle including nitrogen fixation, as well as several genes of the primary metabolism of carbon such as the Rubisco gene.

Abstract ID: 2132

Microbiome-based solutions and soil improvers for healthy soils: a data-mining approach and application of tailored solutions in greenhouse and field

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Introduction: There is an urgent need to develop new approaches for improving soil health in EU agricultural soils and increasing agricultural productivity. Soil microbiome is crucial for sustaining the production of healthy and high-quality food. On the other hand, the use of food processing waste streams along with the multifunctional microbial consortia has the potential to improve soil health and enhance crop productivity, thereby contributing to independence to chemical inputs, in the context of a circular bioeconomic approach.

Objective: The main objective is to explore the impact of microbiome-based solutions and soil improvers derived from food processing side streams on soil health and crop productivity.

Method: Firstly, a data mining approach was employed to investigate the effect of soil improvers and biofertilizers on enhancing soil health and promoting soil biodiversity, with the target to explore their ecological role in the soil ecosystem. Subsequently, innovative and locally produced soil improvers (hydrothermal carbon and pelletized digestate) derived from the food industry (vegetables) were applied in greenhouse on spring wheat, in combination with tailored microbiome-based inoculants. A Synthetic Community (SynCom) was set up according to the genome analysis of each member and the high content of gene clusters encoding Plant Growth Promoting (PGP) traits. The inoculum was applied as seed coating and a refresh inoculum was applied as a liquid suspension 15 days after sowing. The concentration of the inoculum as well as its viability were measured using a culture-based approach and Flow Cytometry assay. During the experiments, plant growth parameters were measured, and soil samples were collected for biological and chemical analysis. In field, biochar derived from food processing

residues was applied on tomato, in combination with tailored microbiome-based inoculants and/or different types of biostimulants (*Rhizophagus intraradices*, Micosat F and Rhizovital). The effect of this application on soil health and plant parameters was investigated.

Findings: Preliminary results suggested that multifunctional microbiome-based solutions can be applied as green biofertilizers in crop production without altering the biodiversity or the resident microbiota, thus avoiding risks of long-term impacts. The Whole Genome Sequencing of PGP strains composing the microbial consortium permitted to exclude the presence of pathogenicity traits and revealed the presence of a large plethora of PGP traits boosting their use as microbial biofertilizers on a large scale. The collection and an in-depth analysis of available data from EU projects and long-term field experiments through a Python script, along with VOSviewer, permitted to define the main driver factors influencing soil health, thus providing the basis for the development of strategies and policies aimed at promoting sustainable agricultural practices. The ongoing microbiological investigation of rhizospheric soil samples and the evaluation of agronomic performance in greenhouse and field experiments will permit to identify new biomarkers of soil health and select the best combination of soil improvers/microbiome-based solutions for field application.

Conclusion: Our study can provide a first evaluation of how such soil improvers can affect soil health indicators, allowing for more precise recommendations on sustainable soil management practices. This study underscores the strategic importance of scientifically validated microbiological indicators of soil health and their key role in the entire food chain, supporting a solid and sustainable 'farm to fork' strategy.

Abstract ID: 3993

Microbiome-Driven Urban Design: Insights from the UniBiome Project on Biodiversity and Health

Presenting author: Antonia Bruno, PhD, University of Milano-Bicocca, Milan, Italy

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Introduction: Humans spend approximately 90% of their time indoors[1], predominantly interacting with the microbial communities of artificial environments. These microbial ecosystems, collectively referred to as the microbiome of the built environment (BE), are shaped by human activity, architectural design, and urban features[2,3]. The urbanization of human habitats over the past 10,000 years, and particularly the shift to modern urban lifestyles, has significantly altered the human exposome and microbiome[4]. This transition has led to a marked decrease in microbial diversity and a rise in specific microbial groups associated with modern living, contributing to civilization-related diseases[5]. Despite the vital roles microbes play in ecosystem equilibrium and health, the bidirectional relationship between BE microbiomes and host microbiomes remains largely unexplored. This knowledge gap poses a significant scientific challenge, compounded by its underappreciation in societal discourse. As part of the MUSA initiative (Multilayered Urban Sustainability Action), funded by the European Union's NextGenerationEU (NRRP), the UniBiome Project is a collaborative endeavor between scientists and students, recognizing the urgency for consensual urban biodiversity renovation plans.

Objectives: The UniBiome Project investigates the microbial characteristics of two university campuses in Milan, Italy, and their interaction with the student skin microbiome. Specific aims include a) characterizing the BE and human microbiome composition; b) assessing the impact of green spaces on microbial diversity; c) identifying potential links between BE microbiomes and human skin microbiomes.

Method: Over Spring and Autumn 2023, more than 160 volunteer students from the University of Milano-Bicocca and Politecnico di Milano donated skin microbiome samples, alongside the collection of over 500 environmental samples per season. By high-throughput DNA sequencing, we analyzed both host-associated and environmental microbiomes, estimating alpha and beta diversities and identifying shared core microbiomes. These results were correlated with metadata from participant questionnaires and environmental parameters.

Findings: Our analysis revealed a high environmental microbiome diversity, encompassing over 2000 identified genera. As expected, skin microbiome samples exhibited significantly lower alpha-diversity (Shannon diversity index, Kruskal-Wallis p-value <0.001), with about 800 genera identified. Notably, Bray–Curtis dissimilarity indicated significant differences between indoor and outdoor microbial compositions (PERMANOVA test, p-value = 0.001). Indoor microbiomes predominantly consisted of skin-associated bacteria, while indoor spaces with access to green areas exhibited significantly greater biodiversity (Kruskal-Wallis p-value <0.001), resembling outdoor microbiomes (core microbiome analysis). The analyses of student skin microbiomes further suggest shifts towards healthier microbiomes in such environments. **Conclusions**: These findings support the hypothesis that well-designed green interventions can enhance both environmental and human microbial diversity. The decisions we make about built environments have profound implications for microbial diversity and human health[6]. By integrating advanced research with participatory urban planning, UniBiome aims to transform urban ecosystems, fostering a sustainable and health-oriented future. Rooted in the One Health framework[7], this research represents a pivotal step toward microbiome-aided urban design and global health equity.

Abstract ID: 1818 / Poster Number: 221.15P

Microbiota from alluvial sediments and its metabolic response, an example from karst cave Postojnska jama, Slovenia

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Rivers deposit organic and inorganic sediments along their course, which change the environment and have a significant impact on the ecosystem. This is particularly important for oligotrophic environments such as karst caves. Microorganisms and their short-term metabolic responses were studied in recently deposited alluvial cave sediments, in a sediment deposited by a palaeo-river (20,000 – 780,000 years ago) and in a karst soil outside the cave. Chemical composition, community-level physiological profiling and 16S rRNA amplicon sequencing were used to characterise the sediments and their microbiota. The chemical composition varied, an old sediment showed elevated levels of some metals, in particular Be, Cd, Cr, Pb, V and Zn. Members of 26 bacterial phyla were found in the sediments. Pseudomonadota dominated in all cave sediment samples with an abundance between 40.9 % and 63.9 %. They were followed by Acidobacteriota (2.4 % – 20.7 %) and Nitrospirota (0.2 % – 17.0 %). Bacteroidota also represented a considerable proportion of the diversity (0.1 % – 9.8 %). Among the abundant genera, members of Acidobacterium, Denitratisoma, Gemmatimonas, Geobacter, Holophaga, Methylibium, Nitrospira and Pseudomonas were found in all samples, representing the core microbiome of the cave sediments. Metabolic fingerprints showed moderate effects of temperature (10°C, 20°C and 30°C) during aerobic cultivation on the microbial utilisation of 31 different substrates. The microbial ability to degrade substrates decreased significantly during anaerobiosis. Nutrients increase the level of microbial metabolism in alluvial sediments, especially when exposed to oxygenation and mixing, which also promote physical contact and drift of microbes between sites.

Abstract ID: 2361 / Poster Number: 034.17F

Microbiota Modulation and S1P2R Inhibition: Evaluating Ticagrelor as a Potential Therapeutic for Cholestatic Liver Diseases

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Cholestatic liver diseases (CLD) result from impaired bile acid (BA) secretion, leading to intrahepatic accumulation, inflammation, and fibrosis. Emerging evidence suggests a strong link between gut microbiota dysbiosis and CLD progression. Sphingosine-1-phosphate receptor 2 (S1PR2) plays a critical role in CLD pathogenesis by activating pro-inflammatory and fibrotic signaling pathways. This study investigates the therapeutic potential of ticagrelor, an FDA-approved antiplatelet drug, as an S1PR2 inhibitor in a bile duct ligation (BDL) murine model and its impact on gut microbiota composition and function.

Male Sprague Dawley rats (n=40) were divided into five groups: sham-operated, sham + ticagrelor, BDL, BDL + UDCA, and BDL + ticagrelor. Ticagrelor and UDCA were administered orally for 14 days post-BDL. Blood and hepatic tissue samples were collected for biochemical and molecular assessments, including liver function tests, BA levels, inflammatory cytokines (NLRP3, IL-1β, IL-18, NF-κB), and fibrosis markers (α-SMA, COL1) using ELISA and qRT-PCR. Western blotting assessed key molecular pathways, while histopathological analyses, including immunohistochemistry and transmission electron microscopy, evaluated liver tissue morphology and cellular integrity.

Microbiota analysis was conducted at three time points—day 1, day 8, and day 15 post-surgery—providing longitudinal insights into gut microbial alterations in response to ticagrelor treatment. Changes in microbial diversity and composition were assessed using 16S rRNA sequencing, revealing significant modulation of gut microbiota profiles associated with improved liver health. Molecular docking studies demonstrated high binding affinity of ticagrelor to S1PR2, suggesting its potential to modulate gut-liver interactions through key molecular pathways.

Ticagrelor treatment significantly attenuated liver fibrosis and inflammation, improved bile acid homeostasis, and promoted hepatic regeneration by modulating cellular proliferation and apoptosis. The observed gut microbiota shifts correlated with reduced

hepatic injury, indicating a potential gut-liver axis mechanism underlying ticagrelor's therapeutic effects.

These findings emphasize the role of gut microbiota modulation in CLD management and highlight ticagrelor's potential as a novel therapeutic agent targeting S1PR2 signaling and gut-liver interactions. Further investigations are warranted to explore its clinical relevance in cholestatic liver diseases.

Abstract ID: 1610 / Poster Number: 321.16F

Microencapsulation for enhanced in situ bioremediation of PAH-contaminated sites: improving microbial stability and degradation

Presenting author: Ms. Amelia Foley, Duke University, Durham, United States

Co-authors: *Claudia Gunsch*

Polycyclic aromatic hydrocarbon (PAH) contamination threatens environmental health due to the carcinogenic, mutagenic, and toxic properties of these persistent chemicals. *In situ* bioremediation via bioaugmentation is a technique of particular interest because it is cost-effective and minimally disruptive to the environment. However, repeated efforts have found that bioaugmentation schemes are inconsistently successful due to the transience of exogenous strains. Thus, additional approaches are required to ensure stable *in situ* populations. Here, we consider microencapsulation—a cell immobilization strategy that provides support and protection to microbes by surrounding them in a biodegradable carrier material which functions as a controlled release microbial delivery vehicle.

This project assesses the biodegradation capacity of encapsulated microbes compared to traditional planktonic microbes, leveraging known PAH-degrading bacterial strains previously isolated from contaminated site sediments (representative genera include *Novosphingobium*, *Stenotrophomonas*, *Hydrogenophaga*, and *Sphingobium*).

Growth and release of encapsulated microbes were characterized in defined media to compare differences in bacterial survival based on microcapsule formulation (polymer composition, capsule coating, cell concentration, and strain). We identified an optimal capsule design and prepared six encapsulated monocultures to compare the degradation of three PAHs (naphthalene, phenanthrene, and fluoranthene) in simple liquid media. Over 42 days, PAH concentrations and bacterial levels were monitored with GC-MS and plate counts, respectively. Follow up experiments with encapsulated consortia will feature field-contaminated media and microbial community analyses. Our findings demonstrate the impact of microencapsulation on bacterial survival and PAH degradation for several monocultures, informing strain selection and the design of future bioremediation schemes.

Abstract ID: 1896 / Poster Number: 276.16F

Micronet: identifying perturbation-associated subnetworks in microbial communities

Presenting author: Ms. Lusine Adunts, Armenian Bioinformatics Institute, Yerevan, Armenia

Microbiome communities are dynamic networks of microorganisms critical to host health. Despite the links between microbiome composition changes and diseases, current analytical methods often neglect microbial interactions and their temporal dynamics. To this end, we developed *Micronet*, an R package designed for constructing co-occurrence networks of microbial taxa and identifying subnetworks that are significantly altered between treatment groups.

Micronet employs robust calculation of correlations between taxa, generating co-occurrence networks that capture microbial interactions. By comparing changes in taxa abundance between treatment groups, it extracts subnetworks most impacted by external perturbations or differing conditions. Further, *Micronet* does functional analysis of these subnetworks based on biological pathways and known disease associations.

We applied *Micronet* to diverse datasets, including bee microbiome samples with different treatment statuses, oral microbiome samples from smokers and non-smokers, and saliva samples from healthy individuals and cancer patients pre- and post-treatment. The smoker-associated subnetwork showed significant enrichment in taxa linked to periodontitis, underscoring the connection between smoking and oral health. In cancer-associated saliva samples, infection-related taxa clusters were predominantly identified post-treatment, suggesting potential hospital-acquired infections.

Micronet's ability to integrate co-occurrence networks with differential abundance and functional analyses makes it a powerful tool for investigating microbial community dynamics. This package offers a comprehensive framework for researchers aiming to uncover microbial interactions, predict functional implications, and advance understanding of the microbiome's role in diverse environments.

Abstract ID: 3228

Microorganisms are active over the entire depths of two 5 and 20-million-year-old pristine oil reservoirs

Presenting author: Prof. Doc. Rainer Meckenstock, University of Duisburg-Essen, Essen, Germany

Co-authors: *Lisa Voskuhl, Sebastian Beilig, Erling Rykkelid, Joachim Rinna, Sadjad Mohammadian, Johannes Koch, Ann-Christine Severmann, John Köhne, Tony Guiterez*

Biodegradation in oil reservoirs was previously thought to occur only at the oil-water contact (OWC) zone. This study investigated fresh sediment cores from two pristine oil reservoirs located in the North Sea. Microcosm experiments and reverse stable isotope labeling measurements revealed oil mineralization rates between 1.95 and 16.80 µM $CO_2 \cdot g_{sediment}^{-1} \cdot year^{-1}$ in the oil zone indicating a large potential for biodegradation. This was supported by 16S rRNA gene amplicon sequencing indicating heterogenous microbial communities over the whole reservoir. CT-scans and fluorescence microscopy indicated that microorganisms live as microcolonies on sediment surfaces in small water-filled voids. Although the measured degradation rates were 3-5 times smaller than rates measured at the OWC, the greater volume of the oil-bearing zone shows a large potential for biodegradation of oil reservoirs.

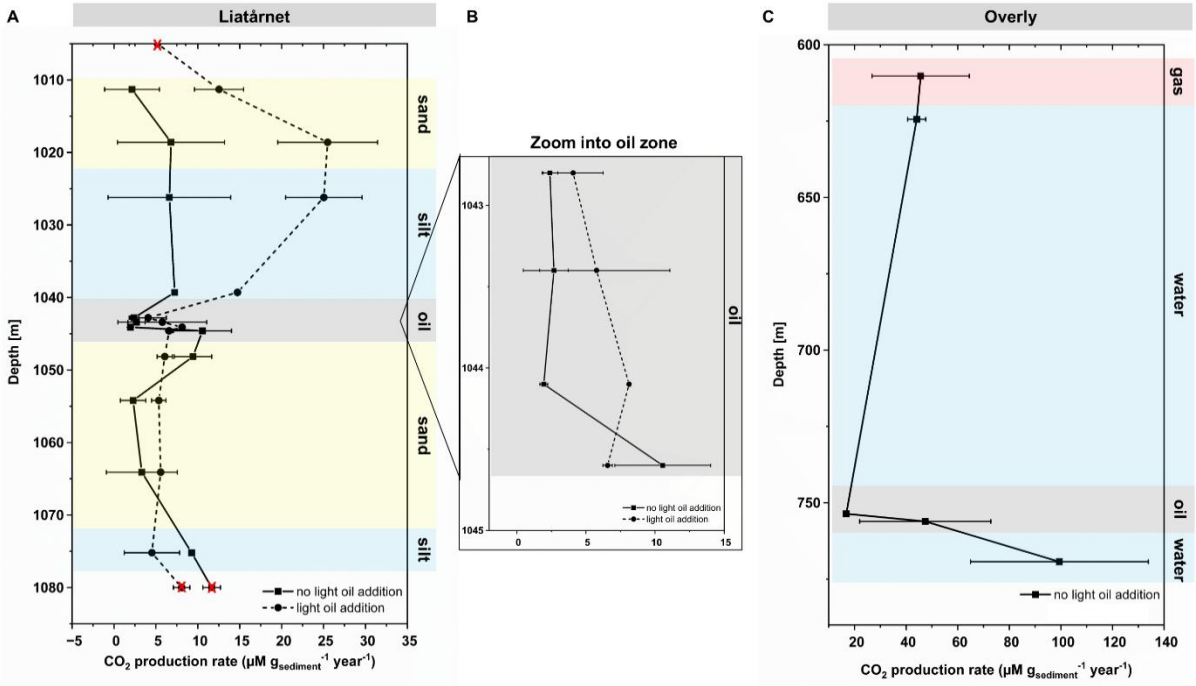


Figure 1: Microbial mineralization rates measured in microcosm experiments with sediment samples from the two oil reservoirs. Colors indicate different geological layers along the reservoir. Data points and error bars represent the means and mean deviations from two replicate microcosms, measured values from the negative controls were subtracted from the sample values. Note the scale differences between plots A, B, and A, C). Sediments from the Liatårnet reservoir incubated without extra carbon source addition for 1.5 years (solid line, squares) and with the addition of light oil to all microcosms incubated for one year (dashed line, circles). Datapoints, highlighted with red crosses, above and below the colored layers represent mineralization rates from drilling fluid (OBM) before (1005 m MD) and after (1080 m MD) the drilling. B) Magnified CO_2 production rates of four samples within the Liatårnet oil zone. C) Sediments from the Overly reservoir were incubated without extra carbon source addition for 0.7 years (solid line, squares).

Abstract ID: 1015 / Poster Number: 405.17P

Microparticle-enhanced cultivation (MPEC) of Streptomyces rimosus in various process conditions – analysis of the cellular morphology and secondary metabolism

Presenting author: Dr. Anna Ścigaczewska, Lodz University of Technology, Lodz, Poland

Co-authors: *Tomasz Boruta, Weronika Grzesiak, Marcin Bizukojć*

The secondary metabolism of actinomycetes *Streptomyces* is an inexhaustible source of biologically active compounds from which the biotechnology industry draws [1]. Oxytetracycline and rimocidine are examples of *Streptomyces* secondary metabolites widely used in human and animal treatment [2,3]. Cellular morphology is one of the key factors influencing the bioprocess when cultivating filamentous microorganisms. Therefore, a tailored method to control *Streptomyces* morphology is required. One of the developing morphological engineering techniques is microparticle-enhanced cultivation (MPEC), however, it used to be mainly tested on filamentous fungi like *Aspergillus*. *Streptomyces* species are still underrepresented in this field [4]. Hence, the main objective of the presented work was to analyse the effect of talc microparticle addition on cellular morphology and secondary metabolite production by *Streptomyces rimosus*. The experiments were conducted in two inoculum variants:

- the first one started from spores introduced into the bioreactor;
- the second one started from the preculture introduced into the bioreactor. The preculture had been earlier grown for 24 hours in shake flasks.

Each variant consisted of three bioreactor runs varying by the amount of talc microparticles added: 0 g L^{-1} (control), 5 g L^{-1} and 10 g L^{-1}.

S. rimosus morphological forms observed during the experiments (Fig. 1a) and the amount of the exemplary metabolite oxytetracycline (Fig. 1b) strictly depended on talc microparticle concentration and inoculum variant. The enhancement of oxytetracycline production was obtained in the variant inoculated by spores and conducted with the 5 g L^{-1} of microparticles. In this case enhancement factor (EF) exceeded 1, which meant that oxytetracycline concentration in the run with microparticles (MPEC) was higher than in the control. In the variant started from the preculture lower oxytetracycline production was observed.

In conclusion, the novelty application of MPEC technique has proven to be an effective tool for morphology tailoring in actinomycete cultivations. However, the outcome of the bioprocess strictly depends on the culture conditions, including the inoculation procedure and microparticle concentration.

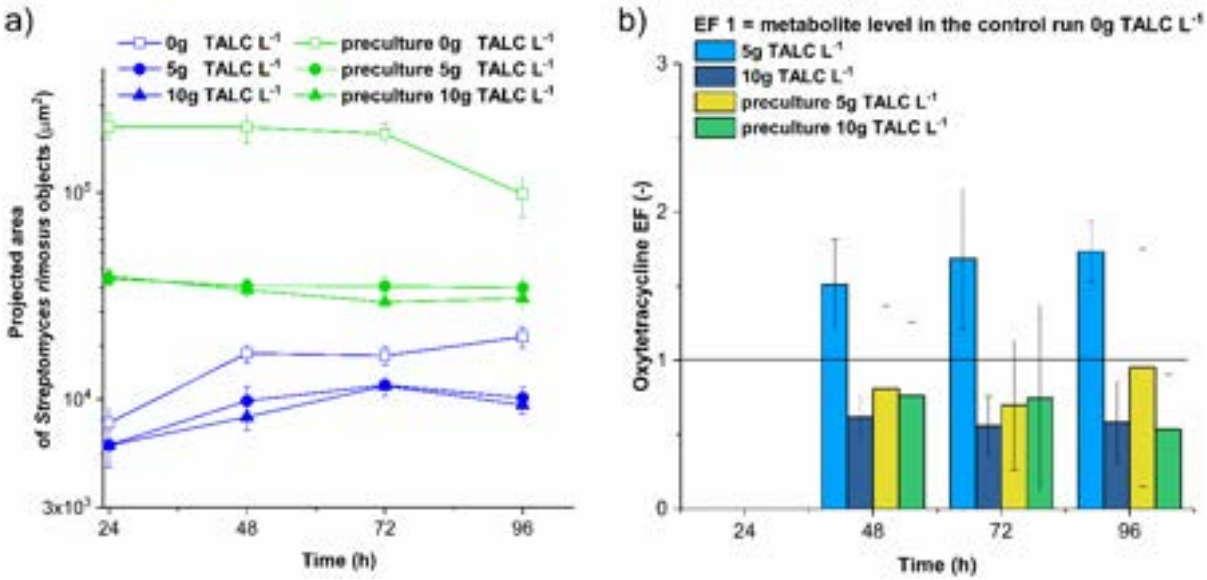


Figure 1: a) Projected area of *S. rimosus* objects and b) oxytetracycline Enhancement Factor EF in bioreactor experiments conducted with and without precultures

Abstract ID: 3570 / Poster Number: 183.17P

Microplastics as carriers of antibiotic resistant bacteria in the Tiber (Italy): sources, pathways and fate

Presenting author: Jakob Grosfeld, PhD, Deltares, Delft, Netherlands; Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Co-authors: *Matti Gralka, Rik Oldenkamp, Frans Buschman*

In rivers, microplastics (MP) can act as a transport medium for microbes. The microhabitat on plastic particles is called 'the plastisphere'. Microbes attach easily to MPs and can form a biofilm. There is increasing evidence the plastisphere forms a reservoir for antimicrobial resistance (AMR). Additionally, MPs can be transported over long distances as they have a similar density as water. The combination of elevated AMR in biofilms and the increased mobility of MPs suggests that MPs significantly enhance the spread of AMR in the environment. This study presents a conceptual framework on the sources, pathways and interactions between AMR and MPs in the Tiber river. The framework serves as a blueprint for hydrological transport- and water quality modelling for AMR harbouring MPs.

Important sources of AMR and/or antibiotics are effluents from waste water treatment plants (WWTP) and hospitals. MPs may originate from WWTP effluent, urban runoff and atmospheric fallout. In the river, interactions between bacteria, antibiotics and MPs can occur. MPs can adsorb antibiotics or other AMR selective compounds. This makes the MP surface selective towards colonisation by antibiotic resistance genes (ARGs) carrying bacteria. These bacteria can exchange ARGs with other species in the plastisphere through horizontal gene transfer . This leads to increased AMR on MPs downstream of pollution sources. Finally, hydrologic behaviour drives the transport of AMR harbouring MPs to downstream environments. This may increase societal exposure to potentially harmful resistant pathogens.

Abstract ID: 391

Microplastics as Vectors for Fecal Bacteria and ARGs in Urban Aquatic Environments

Presenting author: Prof. Elisenda Balleste, Universitat de Barcelona, Barcelona, Spain

Co-authors: *Hongxia Liang, Laura Migloriato, William De Haan, Gabriel Roscales Garcia, Cristina Garcia-Aljaro*

Introduction: Plastic debris enters natural ecosystems due to neglect, improper disposal, or mismanagement. Once in water, plastic surfaces rapidly generate a nutrient-rich layer facilitating bacterial adhesion. Due to their ability to act as substrates and their long persistence in the environment, plastics have been identified as vectors for bacterial dispersal. This study evaluated the presence and persistence of faecal bacteria and antibiotic resistance genes (ARGs) adhering to biofilms on plastics collected from sewage effluent, river, and coastal waters. Additionally, microcosm experiments assessed the ability of *E. coli* to adhere and persist under controlled conditions.

Method: We collected 36 microplastic samples from sewage effluent, urban-impacted rivers, and coastal water. We monitored the presence of faecal indicators (*E. coli* and Enterococci) and three ARGs using culture methods and qPCR. The bacterial community was assessed using 16S rRNA metabarcoding. Additionally, we performed microcosm experiments with 20L of water, adding 10^4CFU/mL of *E. coli* and environmental plastic pellets. Over one month, the abundance of *E. coli* and autochthonous bacteria attached to the plastics was evaluated using culture, qPCR, 16S rRNA sequencing, and SEM.

Findings: Environmental microplastics were covered by bacterial biofilms with densities ranging from 4.9×10^4 to 1.3×10^7 gc/mm^2. Faecal indicators were detected in 66%, 80% and 67% of plastic samples from sewage, river water and coastal water. On average, 37, 11, and 68 CFU of *E. coli* per plastic item were detected in sewage, river, and coastal floating plastics, respectively. ARGs were detected in 29% to 100% of plastic samples, with concentrations ranging from non-detectable to 9.9×10^5 gc/mm^2. Densities were higher than those observed in water. Microcosm studies showed that colonization began within 24 hours, developing an “early-biofilm” community (5 days), followed by a “later-biofilm” community. *E. coli* adhered to the biofilm during the first day, reaching a maximum concentration of 2.1×10^3 CFU/mm^2 and persisted for up to 7 days.

Conclusion: Our results demonstrate that faecal indicators, particularly *E. coli*, can attach to plastic biofilms in moderate numbers, sometimes reaching higher

concentrations on individual plastic items than in 100 mL of surrounding water. *E. coli* was detectable in plastic biofilms by culture for up to 7 days, indicating its potential for survival and dispersal with plastic debris. The colonization patterns observed in microcosm studies were consistent with those in environmental samples.

This study highlights the role of plastic debris as a vector for the dispersal of afecal bacteria and ARGs in aquatic environments. The ability of *E. coli* to adhere to and persist on plastic surfaces underscores the potential public health risks associated with plastic pollution. The findings of this study have significant implications for public health and environmental management.

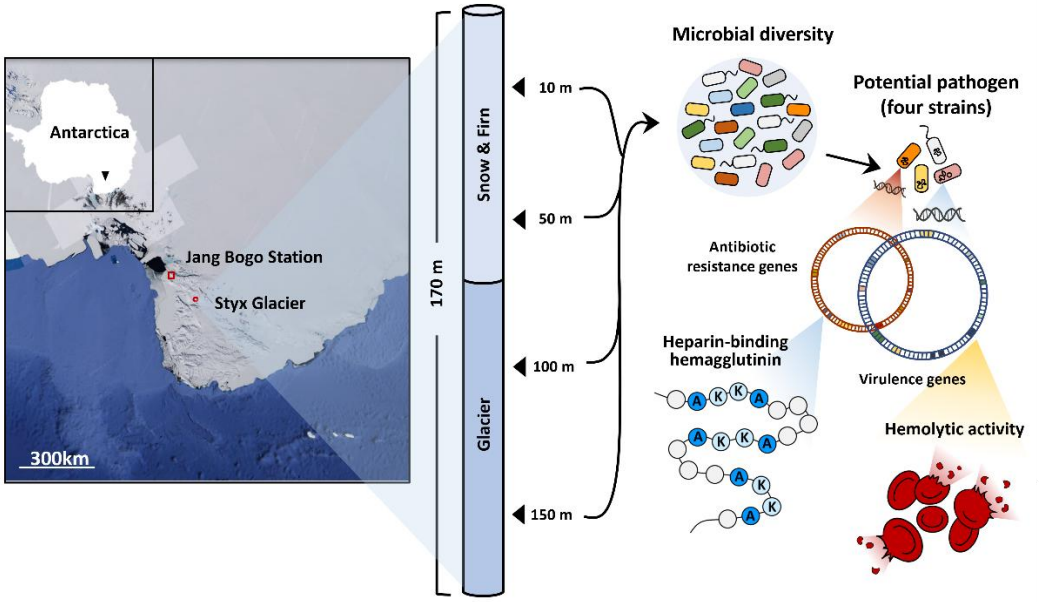
Abstract ID: 1817 / Poster Number: 087.15P

Millennial-scale depth-resolved ancient microbial diversity and pathogenic potential in Styx Glacier, Antarctica

Presenting author: Dr. Minkyung Kim, Korea Polar Research Institute, Incheon, South Korea

Co-authors: *Hanbyul Lee, Subin Lee, Yeongcheol Han, Ok-Sun Kim*

The cryosphere offers crucial insights into historical ecosystems and biomes on our planet, preserving ancient microorganisms and other biological materials. The release of pathogens from permafrost and Arctic regions has emphasized potential threats to human health. However, the ancient microbial diversity in Antarctic glaciers remains largely unexplored. To address this limitation, we investigated the microbial diversity and the presence of potential pathogens in the Styx ice core with millennial-scale dated between 530 and 1972 CE in Northern Victoria Land, Antarctica. A total of 656 isolates, representing 27 species, were obtained via incubation in four different media at 10°C and 25°C. Our cultivation data showed that the Alphaproteobacteria was the predominant class, with *Sphingomonas echinoides* (70% at 10°C) and *Bradyrhizobium elkanii* (76.2% at 25°C) being the most prevalent species across all depths. Genomic analysis of four isolates (*Roseomonas mucosa*, *Gordonia hongkongensis*, *Paenibacillus glucanolyticus*, and *Dermacoccus nishinomiyaensis*) among nine identified species with suspected to be potential pathogens revealed the presence of attachment-related genes in all analyzed species, along with the reference strain. Amino acid sequences of HbhA, a mycobacterial surface antigen, in *G. hongkongensis* strains exhibit AKKA repeat motifs, similar to those found in pathogenic *Mycobacterium tuberculosis* and *Nocardia cyriacigeorgica*. Furthermore, the hemolysin produced by *R. mucosa* and *P. glucanolyticus* showed no activity at 25°C but exhibited activity at 37°C. This study demonstrated that glacial ice contains core microbiomes and also highlighted the presence of potential opportunistic pathogens, resonating between ancient microbiology and contemporary environmental health concerns.


Antarctica
Jang Bogo Station
Styx Glacier
300km
170 m
Snow & Firm
Glacier
10 m
50 m
100 m
150 m
Microbial diversity
Potential pathogen
(four strains)
Antibiotic
resistance genes
Heparin-binding
hemagglutinin
Virulence genes
Hemolytic activity

Abstract ID: 3239 / Poster Number: 259.17P

Miniaturization of fermentations allows for a reliable, high throughput alternative to shake flask cultures for A. niger citric acid production

Presenting author: Mr. Johannes Theodoor Zwerus, Vienna University of Technology, Vienna, Austria

Introduction: Small-scale fermentations are usually performed using either microtiter plates or shake flasks which allows for relatively high throughput. However, it is complicated to analyze the product during the fermentation since most methods are invasive. Therefore, the product is often only analyzed after the fermentation is finished, resulting in little data about the process itself. In contrast, laboratory-scale fermentation tanks allow for online monitoring but are limited in throughput. Furthermore, this disparity in methods when trying to up-scale might cause false strain or medium selection leading to negative downstream effects.

Objective: The aim of this research is to address this translation problem and develop a robust cultivation protocol to enable reproducible A. niger fermentations.

Method: The BioLector XT allows for a high sample throughput while also enabling online sample measurement. In addition, it grants the ability to control oxygen levels of the experiment. Therefore, it allows for higher throughput compared to shake flasks and fermentation tanks, and higher reproducibility compared to microtiter plates.

Findings: Citric acid production was used as a measure of success to select for both high-performing strains and media. Both the biomass and the oxygen levels in each well could be monitored carefully throughout the fermentation giving valuable insights on the process.

Conclusion: Fermentations performed in the BioLector XT are suitable to obtain quantitative data about the growth of A. niger. In addition, it allows for more insights in fermentation processes and helps to either scale-up or scale-down fermentation experiments reliably

Abstract ID: 2620 / Poster Number: 014.16P

Minimal expression of antimicrobial resistance genes in frailty elders without active infection

Presenting author: Ms. Ana Barberá, Foundation for the Promotion of Health and Biomedical Research of the Valencian, Valencia, Spain; CIBER in Epidemiology and Public Health, Madrid, Spain

Co-authors: *Rosario Ortolá, Fernando Rodríguez-Artalejo, Andrés Moya, Susana Ruiz-Ruiz*

The gut microbiome serves as a hotspot for the selection of antibiotic resistance genes (ARGs) and their bacterial hosts due to continuous exposure to antibiotics from diverse sources. These genes accumulate and become more complex along life, potentially influencing health and contributing to aging-associated disorders such as frailty syndrome (FS). To elucidate the role of the gut resistome in FS, we profiled metagenomics and metatranscriptomics of 203 fecal samples from the Seniors-ENRICA-2 cohort, categorized into frailty (FT) and non-frailty (NFT) groups. The volunteers had not taken antibiotics and showed no active infection prior to sampling. Amino acid ORFs sequences were analyzed using the Reference Gene Catalog and CARD databases with set parameters for identity and coverage. Of the 10,424 functional ortholog genes identified in metagenomics data, 140 (1.34%) and 119 (1.14%) were associated with ARGs from each respective database. The K18220 (tetM/tetO) tetracycline resistance gene was the most prevalent, besides seven additional functions found in over 90% of individuals related to β-lactam, macrolides, and streptomycin, forming a potential core resistome in the elderly. Also, a significantly higher prevalence of ARGs was observed in the FT group (p-value < 0.005). In contrast, metatranscriptomic data revealed a low ARGs expression, with only 19 transcribed genes detected, showing no significant differences between FT and NFT groups. The distinct resistome profiles in elderly gut microbiota suggest bacteria may act as ARGs reservoirs, transcribing these genes only when needed, such as during antibiotic exposure, highlighting their role in maintaining resistance potential without constant expression.

Abstract ID: 4000 / Poster Number: 150.15P

Mining of genetic determinants for polyethylene biodegradation in Rhodococcus opacus R7 from a transcriptomic approach to enzymatic functions

Presenting author: Dr. Jessica Zampolli, University of Milano-Bicocca, Milano, Italy

Co-authors: *Marco Mangiagalli, Marina Lotti, Diletta Ami, Antonino Natalello, Luca Bertini, Federica Arrigoni, Patrizia Di Gennaro*

Introduction: Plastic waste management has become a global issue, with polyethylene (PE) being the most produced synthetic plastic worldwide and a major contributor to pollution. While various microorganisms have been investigated for biodegrading PE including *Rhodococcus* bacteria, the exact biodegradation process and molecular mechanisms remain unclear.

Objective: This study aims to identify the genetic determinants behind PE biodegradation in *Rhodococcus opacus* R7 to develop effective biodegradative technologies.

Method: RNA-seq of *R. opacus* R7 grown on PE, compared to a reference condition, was applied to determine the activated genes, supported by RT-qPCR. Novel extracellular laccase-like multicopper oxidases (LMCOs) were cloned, expressed, and purified as recombinant proteins (LMCO2 and LMCO3). They were biochemically and biophysically characterized and their oxidative activity was assessed on untreated PE using FTIR spectroscopy and GC-MSD analysis. Structural analysis and density functional theory calculations further supported their oxidative potential.

Findings: *R. opacus* R7 can grow on untreated PE as the sole carbon and energy source in a few days. The transcriptomic analysis highlighted the key genes involved in PE metabolism, including three laccases, an alkane monooxygenase, a cytochrome P450 hydroxylase, and a few transporters. Gene activation was recorded for up to 28 days, investigating potential inducers to enhance gene expression such as lignin-derived compounds and carboxylic acids. Selected laccases, LMCO2 and LMCO3, demonstrated PE oxidative activity within 24 hours, supported by predictive structural analysis and electronic structure calculation.

Conclusion: Understanding PE biodegradation mechanisms offers insights to develop biotechnological applications for plastic waste management.

Abstract ID: 4080 / Poster Number: 118.16P

Mitigating shiga toxin producing *Escherichia coli* contamination in raw milk cheese: the role of High Pressure Processing (HPP) and milk sub-lethal thermal treatment

Presenting author: Claudia Cortimiglia, PhD, Università Cattolica del Sacro Cuore, Piacenza, Italy

Co-authors: *Giovanni Milani, Marta Bisaschi, Pier Paolo Becchi, Leilei Zhang, Pier Sandro Cocconcelli*

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is one of the major sources of foodborne illnesses. In recent years, outbreaks linked to raw milk cheese consumption have become more frequent, increasing public concern about their safety. To minimize the risk associated with dairy product consumption, it is crucial to implement effective control and prevention strategies against STEC, also considering that it was shown that STEC strains can persist and proliferate throughout the cheese-making process.

Objectives: The objective of this research was to assess: (1) the effects of a sub-lethal heat treatment applied to milk on the inactivation kinetics of STEC; (2) the behavior of STEC throughout the cheesemaking and ripening processes, as well as the efficacy of HPP in their eradication post-ripening. Furthermore, the dynamics of the fermenting microbiota were monitored throughout all phases to analyze the microbial interactions in the presence of STEC during the ripening period.

Methods: A challenge test was conducted at a dairy processing facility to simulate two distinct experimental scenarios: (1) raw milk was inoculated with STEC and subsequently subjected to sub-lethal heat treatment; (2) raw milk was inoculated and then treated with HPP at the end of ripening. Additionally, a control cheese production was carried out without STEC contamination. Samples of milk, curd, and cheese were collected and analyzed at various ripening intervals. Cheese samples that underwent HPP were also evaluated one month following the treatment. Throughout the study, microbiological analyses were performed to quantify coliforms, lactic acid bacteria (LAB), and enterococci. The curds and cheeses were further examined through enrichment protocols in accordance with ISO/TS 13136:2012. Moreover, amplicon sequencing and non-targeted metabolomics analyses utilizing high-resolution mass spectrometry (HRMS) were employed to investigate the curd and cheese samples.

Results: The sub-lethal thermal treatment significantly reduced the STEC count in milk, rendering it undetectable. However, in the early stages of ripening, STEC was present up

to the midpoint of the ripening period; however, no viable cells were identified by the conclusion of this phase. The treatment also led to a decrease in indigenous LAB populations, although these populations exhibited recovery during the early stages of maturation. Ultimately, the final counts of LAB and enterococci were found to be comparable to those observed in untreated cheese samples. Metagenomic analysis revealed that the curd and cheese samples subjected to heat treatment exhibited greater microbial diversity at mid-ripening compared to the microbial diversity recorded in the negative control. This pronounced variability in microbial composition diminished by the end of the ripening period. The application of HPP at the conclusion of the ripening period led to a statistically significant reduction in the load of STEC, with detectable levels observed in only one replicate. During the assessment of microbial dynamics in samples contaminated with STEC, we noted a higher diversity compared to the untreated control, alongside a predominance of taxa that were absent during the fermentation of untreated cheese. In terms of metabolomics, our findings indicated that the ripening process is the primary factor influencing the separation of samples. Furthermore, within each stage of ripening, the untreated cheese controls exhibited distinct modulation trends when compared to the other two treatment groups, with amino acids and lipid-derived compounds identified as the most significantly affected chemical.

Conclusion: Heat treatment of the milk drastically reduced the STEC load, but did not guarantee their total elimination during ripening. HPP treatment has been shown to be effective in killing STEC, although not completely. Considering the highest possible natural contamination of STEC load, both treatments may be promising in reducing the risk of pathogen-related food-borne diseases. Further research is required to evaluate the effects of these treatments on the flavor and texture of the cheese.

Abstract ID: 2511 / Poster Number: 003.16S

Mitigating soil carbon emissions through symbiotic interaction arbuscular mycorrhizal fungi and phosphate solubilizing bacteria

Presenting author: Ms. So Hee Park, Yonsei University, Wonju-si, Kangwon-state, South Korea

Co-authors: *Bo Ram Kang, Min Sung Kim, Tae Kwon Lee*

Soil carbon serves as a major carbon reservoir in climate change mitigation, actively regulating atmospheric CO_2 levels through complex microbial processes. While arbuscular mycorrhizal fungi (AMF) are widely recognized for their substantial contributions to soil carbon processes, the effect of interactions between AMF and phosphate solubilizing bacteria (PSB) in the rhizosphere. This study investigates the impact of co-inoculating AMF and PSB on soil carbon on soil carbon emissions. Sorghum (*Sorghum bicolor*) was used as a host plant to co-culture AMF (*Rhizophagus irregularis*) and PSB (*Burkholderia cepacia*). We measured the glomalin-related soil protein (GRSP), CO_2 emission, soil physicochemical properties and organic carbon composition by Fourier transform ion cyclotron resonance (FT-ICR). Co-cultivation of AMF and PSB significantly reduced total and available phosphorus after 8 weeks, while soil organic carbon and glomalin remained unchanged. CO_2 emissions decreased by 14% compared to the control group and 21% relative to AMF single treatment. FT-ICR revealed increased proportions of recalcitrant compounds CHO and CHONS by 14.7% and 0.4%, suggesting enhanced soil carbon stability and potential carbon sequestration mechanisms. These findings indicate that microbial interactions between AMF and PSB can effectively mitigate CO_2 emissions through the accumulation of resistant organic matter.

Abstract ID: 3183

Mixed species urinary catheter biofilm derived from spinal cord patients show resistance to colonisation by new invasive bacteria

Presenting author: Ms. Kailey Hamann, University of Technology Sydney (UTS), Sydney, Australia

Co-authors: *MD Mozammel Hoque, Iain G. Duggin, Bonsan Bonne Lee, Scott A. Rice, Diane McDougald, Parisa Noorian*

Long-term use of urinary catheters in spinal cord injury patients (SCI) results in the consistent colonisation of complex multispecies biofilms on the urinary catheters. These patients often experience persistent asymptomatic bacteriuria and frequent catheter-associated urinary tract infections (CAUTIs). Simple mono-species models fail to capture the complexities of microbial community behaviours and artificially mixed communities from lab-based organisms cannot present virulence and resistance diversity found in real-world infections.

In order to produce a multispecies biofilm inside urinary catheters that would not have these limitations, two stable and reproducible *in vitro* multi species biofilm models derived from clinical flora associated with catheter-related infections from SCI patients were established using a macrofluidic system.

Metagenomic techniques using short-read Illumina and long-read Oxford Nanopore sequencing was used to assess the community composition, produce metagenome-assembled genomes (MAGs), analyse strain-level phylogeny diversity and single nucleotide polymorphisms (SNP) of isolates. *In silico* analysis of antibiotic resistance further revealed an alarmingly high number of varied resistance genes present in these communities which were further highlighted by *in vitro* antibiotic resistance tests showing the drastic differences between planktonic bacteria, single-species, and multispecies biofilms.

The newly created biofilm models were then evaluated for their ability to inhibit colonisation by a new invasive bacterium on urinary catheters. Colony-forming unit (CFU) counts were used to determine the biomass of each species within the polymicrobial biofilms and evaluate the bacterial colonisation. The results demonstrated that whilst the invading bacteria was able to establish itself on the urinary catheter in the presence of the biofilm models, there was a lower rate of growth than when grown as mono-species biofilm. A 24-hour preformed biofilm of the multispecies model in the urinary catheter

was also shown to be more effective at preventing colonisation of the invading bacteria in comparison to co-inoculation of both the biofilm model and the invading bacteria in the urinary catheter simultaneously. The invading bacteria could not take over the biofilms at both time points, and the total biomass of pre-existing bacteria within the biofilm models remained unaffected by their presence. Competition assays between single isolates from the biofilm models and the invading bacteria also highlight potential species-specific interactions during biofilm formation and establishment of the urinary catheter.

The research presented here, will help in devising more effective strategies to prevent and treat catheter-associated infections by showing the protective role of pre-existing asymptomatic biofilms, suggesting potential avenues for new policies in CAUTIs treatments as well as developing novel treatment strategies.

Abstract ID: 303 / Poster Number: 350.17P

Mixotrophic Methanotrophs- Evaluating the performance benefits of supplemental hydrogen, carbon dioxide and carbon monoxide

Presenting author: Ms. Nadeesha Athukorala, Monash University, Melbourne, Australia

Co-authors: *Chris Greening, Oliver Schmidt, Surbhi Jain, Pok Man Leung*

Methanotrophs were traditionally considered to strictly rely on methane as their energy and carbon sources. However, recent research reveals that some methanotrophs can adapt to a mixotrophic lifestyle, fixing carbon dioxide (CO2) and utilizing hydrogen as an energy source in addition to methane, which could have potential applications in the conversion of mixed waste gases into biomass. Therefore, we evaluated the performance of Methylococcus capsulatus, Methylosinus trichosporium, Methylacidiphilum IT5 and IT6, under high methane (10%), hydrogen (10%), and CO2 (5%) levels in batch culture experiments. The four methanotrophs grew on mixed gases but could not grow in hydrogen and CO2 treatment without methane. Gas chromatography measurements revealed that methane is completely oxidized concomitant with CO2 accumulation, with no significant consumption of hydrogen and CO2. Proteomics analysis revealed that the substrate presence didn't significantly upregulate expression of hydrogenase subunits in M. trichosporium and hydrogenases and RuBisCO subunits in M. capsulatus. These results collectively showed that the four methanotrophs predominantly rely on methane, maintaining obligatory behavior under tested conditions. Nevertheless, stable isotope fractionation analysis revealed that at least some of the cell carbon is derived from CO2 fixation in Methylacidiphilum sp. IT6, M. capsulatus, and M. trichosporium, a finding that was not obvious from the gas chromatography alone. In contrast to the previously tested four methanotrophs, preliminary results show that Methyloferula stellata consumes hydrogen and CO (1%) in addition to methane, with supplemental hydrogen potentially benefiting growth, indicating its capability to consume multiple gaseous energy sources present in industrial waste gas mixes.

Abstract ID: 545 / Poster Number: 067.17P

Modulating antibiotic efficacy: the role of Teucrium polium in disrupting bacterial resistance mechanisms

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s were quantified spectrophotometrically. To determine the impact of TPE on antibiotic efficacy, minimum inhibitory concentrations (MICs) of ampicillin and kanamycin were measured in the presence of the extract. Additionally, bacterial growth kinetics and proton flux were analyzed.

Findings: TPE exhibited strong antioxidant activity (IC50 = 73.89 µg/mL) and possessed significant amounts of phenolic (181.7 ± 2.1 mg GAE/g) and flavonoid (95.4 ± 3.1 mg QE/g) compounds. While the TPE showed no direct antibacterial activity, it significantly reduced the MICs of both antibiotics. TPE decreases growth rate of antibiotic resistant E. coli strains, increased the duration of lag phases and at the same time inhibited the proton flux on membrane.

Conclusion: The extract of *Teucrium polium* shows high antibiotic-modulatory activity, significantly enhancing the efficacy of kanamycin against kanamycin-resistant *E. coli* and ampicillin against ampicillin-resistant *E. coli*. TPE possesses antibiotic-resistance-modifying properties through its influence on membrane proton flux.

Abstract ID: 2194 / Poster Number: 207.16P

Modulating Gut Microbiota to Manage Hyperammonaemia: Potential Therapeutic Approaches

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Background: Ammonia is a byproduct mainly produced by gut microbiota by protein fermentation and urease activity. Excessive ammonia accumulation also known as hyperammonaemia, happens when the equilibrium between production and elimination is disrupted with the liver malfunctions like cirrhosis or chronic hepatitis. This excessive ammonia results in neurological complications linked to the gut-liver-brain axis such as hepatic encephalopathy (HE).

Objective: This study investigates microbiome-targeted therapeutics, such as natural adsorbents and novel antimicrobial strategies to reduce ammonia levels by modulating the gut microbiome.

Method: Natural minerals, such as zeolites and bentonites, are being evaluated for their capacity to adsorb ammonia and reduce its levels in the gut. Their antibacterial properties are being tested, along with medium-chain fatty acids (MCFAs), to evaluate their selective antimicrobial effects on ammonia-producing bacteria, including urease-positive and proteolytic species. Bacterial batch cultures, a simulated gut model (RoboGut), and cell culture models are utilised to investigate the effectiveness and cytotoxic effects of natural minerals and MCFAs in a controlled environment.

Findings and Conclusion: Both zeolites and bentonites demonstrated significant ammonia adsorption, however their absorbing effect varies based on the ammonia concentration. While maintaining the overall microbial balance and preventing the development of antibiotic resistance, MCFAs reduced the growth of ammonia-producing bacteria. These findings highlight the potential of microbiota-targeted therapies for managing high levels of ammonia in the gut. Further research is required to enhance the effectiveness of natural minerals in combating hyperammonaemia and expand their clinical applications.

Abstract ID: 3733 / Poster Number: 194.17P

Modulation of antibiotic resistance by chloramphenicol derivatives in multidrug-resistant clinical isolates

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The increasing prevalence of multidrug-resistant (MDR) bacteria places a significant pharmaceutical and socio-economic burden on the healthcare system. One antibiotic whose effectiveness has been significantly decreased by the spread of resistance is chloramphenicol, whose antibacterial effect can be eliminated by its transport out of the cell or by modification of its structure by chloramphenicol acetyltransferase (CAT). Here, we compared the antimicrobial activity of 59 chloramphenicol derivatives with the parental structure of chloramphenicol against chloramphenicol-resistant and sensitive strains using the microdilution method according to ISO standard 20776-1. Next, CAT was recombinantly expressed, and isolated, and its enzymatic activity in the presence of all derivatives was investigated. Finally, the efflux pump activity of the isolates was measured with an ethidium bromide assay. Eight derivatives demonstrated antimicrobial activity in chloramphenicol-resistant *S. aureus* isolates. Antimicrobial activity was also observed for *E. faecium* and *E. coli* isolates, but only in chloramphenicol-susceptible isolates. Regarding enzymatic activity, 15 derivatives demonstrated up to 20-fold lower affinity for CAT than chloramphenicol, while two also reduced the ability of CAT to acetylate chloramphenicol, thus showing adjuvant activity. Three derivatives that demonstrated antimicrobial activity even against chloramphenicol-resistant strains are of particular interest because they did not interact with the efflux pumps and they were not CAT substrates. Thus, some of the prepared chloramphenicol derivatives have considerable potential for use as antimicrobial agents.

Abstract ID: 1166 / Poster Number: 086.17P

Modulation of antibiotic resistance by quercetin and catechins in E. coli DH5α-pUC18 and E. coli pARG-25: a potential approach for overcoming antibiotic resistance

Presenting author: Mrs. Alvard Minasyan, Yerevan State University, Yerevan, Armenia

Co-authors: *Aram Aramyan, Silvard Tadevosyan, Anush Babayan, Naira Sahakyan*

Introduction: *Ribes nigrum* L. is known for its high antioxidant activity. It is rich in bioactive compounds, especially flavonoids and phenolic acids providing high biological activity. The major components of the *R. nigrum* extract (RNE) are (epi)gallocatechin, (epi)catechin, quercetin, etc. Our recent studies showed that although RNE expresses no antibacterial activity, it can modulate the susceptibility of drug-resistant bacteria to several antibiotics like kanamycin, ampicillin and more by reducing their Minimum Inhibitory Concentrations (MIC).

Objective: This study aims to reveal some mechanisms by which the main components of RNE (quercetin and catechins) may influence bacterial cell metabolism.

Method: The antibiotic-modulating effects of quercetin, and catechins were investigated by determining the MIC values of ampicillin and kanamycin against antibiotic-resistant bacterial strains in the presence of these components. The impact of these compounds on membrane-associated properties was studied by the investigation of changes in proton fluxes and ATPase activity in ampicillin-resistant *Escherichia coli* DH5a-pUC18 and kanamycin-resistant *E. coli* pARG-25.

Findings: It was revealed that 0.25 mg/mL of quercetin and catechin reduced the MICs of ampicillin by at least two-fold in both of the tested bacterial strains. Quercetin decreased also the MIC of kanamycin by four-fold in *E. coli* pARG-25 strain. These compounds also modulated bacterial proton fluxes and APTase activities.

Conclusion: Quercetin and catechin exhibit antibiotic-modulatory potential. This effect is likely due to their ability to influence on bacterial membrane properties. These findings suggest that RNE and its individual components have potential as a natural adjunct in the fight against antibiotic resistance.

Abstract ID: 2582 / Poster Number: 068.16P

Modulatory effects of calcium-alginate encapsulated Akkermansia muciniphila in healthy and pathogen-infected faecal models

Presenting author: Ms. Rita Vedor, Universidade Católica Portuguesa – Escola Superior de Biotecnologia, Porto, Portugal

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Modulatory effects of calcium-alginate encapsulated Akkermansia muciniphila in healthy and pathogen-infected faecal models

Escherichia coli O157:H7 is a food and waterborne pathogen that produces Shiga toxin, disrupting intestinal microbiota composition and metabolic functions. Probiotics may be a potential solution to restore intestinal homeostasis. *Akkermansia muciniphila* and its components have been demonstrated to improve host metabolic health and intestinal homeostasis. This study aims to evaluate the impact of calcium-alginate capsules containing *Akkermansia muciniphila* (cell concentration: 10^9 CFU/mL) on the composition and metabolic functions of both normal and pathogen-infected faecal fermentations. A multifaceted approach is used, including metagenomic analysis, to provide comprehensive insights. The study examines the implications on ammonia production during colonic fermentation, showing an increase in ammonia production until the 30-hour mark, followed by a decrease up to 48 hours in conditions incorporating encapsulated *A. muciniphila*. These observations explore the probiotic's ability to consume ammonia intracellularly, which may potentially benefit gut health. The colonic fermentation process was also analysed in terms of generated short-chain fatty acids. It was found that butyrate was absent after 24 hours in conditions involving encapsulated *A. muciniphila*, while acetate and propionate levels increased by four-times. The analysis of gut microbiota composition showed a higher microbial abundance and diversity in conditions containing *A. muciniphila*. Exposure to *A. muciniphila* resulted in a decrease in the abundance (around 0.4) of the Escherichia genus, indicating a treatment capacity, as microbiota homeostasis is achieved. The incorporation of *A. muciniphila*, encapsulated in a calcium-alginate matrix, resulted in the modulation of healthy and *E. coli* O157:H7-infected microbiota, both concerning composition and metabolic functions.

Abstract ID: 1096 / Poster Number: 047.16P

Modulatory Effects of Sauerkraut Juice on the Gut Microbiome and Potential Impact on the Gut-Brain Axis

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Fermented foods are integral components of the human diet, known for their health benefits. Among them, sauerkraut, a traditional fermented cabbage product, has been extensively studied for its health-promoting properties, while its liquid by-product, sauerkraut juice (SKJ), which is normally consumed as a beverage in South Tyrol, remains relatively underexplored.

Our study investigates SKJ's effects on the human gut microbiota and explores the potential metabolic responses linked to the gut-brain axis.

The dominant lactic acid bacteria (LAB) species in SKJ were genotypically assessed and evaluated for their survival during gastrointestinal transit. Results set up the daily dose (300 mL/day) for the dynamic in vitro Simulator of the Human Intestinal Microbial Ecosystem (SHIME), which included mucin-covered microcosms. The metabolic response of the gut microbiota was analysed by measuring the production of short-chain fatty acids (SCFAs). Potential release of key neurotransmitters and their precursors was explored using a triple-quadrupole mass spectrometer, while the overall microbiota composition was assessed via 16S rRNA sequencing. Quantitative PCR monitored the dynamics of LAB colonisation throughout the SHIME experiment.

Preliminary results indicate that dominant LAB species from SKJ demonstrate robust survival and have the potential to modulate SCFAs profiles, suggesting an enhanced metabolic activity of the gut microbiota. Initial findings also hint at the possibility that SKJ may affect the bioavailability of some neurotransmitters and/or their precursors.

This study contributes to the growing evidence that functional beverages like SKJ can modulate the gut microbiota and potentially interact with gut-brain communication pathways.

Abstract ID: 4074 / Poster Number: 458.15P

Molecular Adaptations in Pseudomonas aeruginosa: Genomic and Epigenetic Insights into Phage Resistance

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Co-authors: *Petras Radžiūnas, Lina Aitmanaitė, Giancarlo Russo*

Bacteriophages are one of the major driving forces of evolution in bacteria [1]. Currently, they are often discussed as tools against bacteria in the form of phage therapy [2]; however, bacteria, notably opportunistic pathogens such as Pseudomonas aeruginosa, employ a wide arsenal of defensive strategies against their phage threats. Therefore, a deeper understanding of bacterial anti-phage defence strategies is needed to utilize phage therapy against bacterial infections.

Our study focuses on the coevolution of *Pseudomonas aeruginosa* 14 and its lytic phage DMS3VIR, aiming to delve deeper into the complex relationships between CRISPR-Cas, surface modifications, and various auxiliary defence mechanisms [3]. Our objective is to determine whether CRISPR-Cas is necessary for the survival of the host and to identify any additional functions this system may have beyond defence. Whole-genome sequencing through Oxford Nanopore technology was utilized to reveal genetic changes and methylation patterns.

During this study, we observed two phage-resistant morphotypes: larger, irregularly shaped colonies that consistently correlated with spacer acquisition in CRISPR Locus 2, and smaller, smooth-edged colonies that most likely arise from surface modifications [4]. However, phage clearance and the irregular colony morphotype also occurred in the CRISPR-Cas–deficient strain, indicating multiple layers of bacterial resistance. Genome sequencing revealed genetic changes and differences in methylation patterns between wild-type and phage-surviving escape mutants, highlighting the molecular adaptations enabling bacterial survival.

Abstract ID: 1872 / Poster Number: 059.16P

Molecular and Immunohistochemical Detection of Cutibacterium acnes in Sarcoid Granulomas

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Introduction: Sarcoidosis is a multisystem inflammatory granulomatous disorder. Despite numerous studies, its etiology remains unclear. It is hypothesized that the disease is caused by unknown antigen(s) in individuals with an abnormal immune response and genetic predisposition. *Cutibacterium acnes* (C. acnes) is an opportunistic bacterium with increasing medical significance. Some studies suggest *C. acnes* as a potential etiological agent in sarcoidosis.

Materials and Methods: We analyzed clinical samples (blood, lung biopsy, and bronchoalveolar lavage) from 52 individuals suspected of having sarcoidosis, 22 of whom were histologically confirmed. Conventional PCR was used to detect *C. acnes* in the patient samples. The role of *C. acnes* in sarcoidosis etiology was further investigated through immunohistochemistry (IHC) using PAB antibody, which is specific for lipoteichoic acids in the cell membrane of *C. acnes*. IHC was performed on formalin-fixed, paraffin-embedded (FFPE) lymph node tissues from two sarcoidosis patients.

Results: Approximately 67% of the PCR-tested samples were positive for *C. acnes*. However, molecular analysis could not exclude contamination. IHC with the PAB antibody was performed on two surgically removed lymph nodes from sarcoidosis patients. *C. acnes* was identified within the non-caseating granulomas of both patients.

Conclusion and Future Directions: Our findings support the potential involvement of *C. acnes* in the pathogenesis of sarcoidosis. Future research will include performing IHC with the PAB antibody on all FFPE tissue samples from the tested group of sarcoidosis patients.

Abstract ID: 3645 / Poster Number: 218.17P

Molecular characterisation of Escherichia coli Extended Spectrum Beta-lactamases producing strains isolated from preoperative rectal swab screening of colonised patients that undergo elective pancreatic surgery

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Introduction: In our hospital's Pancreatic Surgery Unit, an antimicrobial stewardship program is implemented, which includes a preoperative screening of multi-drug resistant (MDR) microorganisms. The prevalent MDR isolate is the *E. coli* Extended-Spectrum-Beta-Lactamases (ESBLs)-producer.

Objective: The aim of the study was the molecular characterisation of *E. coli* ESBL-producing strains that were isolated from preoperative rectal swab screening from patients who underwent elective pancreatic surgery during the year 2023.

Methods: Screening of MDR strains

Preoperative rectal swabs were streaked out on ESBLs ChromoID agar (BioMerieux, France). Colonies growth on agar plates were confirmed to be ESBLs producers with the NDP-ESBL test.

DNA extraction and Data analysis

Total DNAs were extracted from 23 strains isolated from preoperative rectal swab screening of 23 patients with MagMAX Microbiome Ultra Kit following the manufacturer's instruction. After DNA quantifications, DNA libraries were prepared with the Illumina DNA Prep Kit, and samples were analysed by Next-Generation Sequencing on the MiSeq instrument in paired-end mode (2 x 300 bp). Sequencing data were analysed with DRAGEN FastQC and with MultiQC v3.6.3. Reads assembly was performed with Velvet v1.2.10.

Findings: In our sample group, we have identified 7 out of 23 of the ST131 Sequence Type (30,43%), 2 out of 23 of the ST69 (8,70%) and other sequence types that do not form a clonal complex.

Different $bla_{\text{CTX-M}}$ genes, coding for ESBLs, were detected in 19 out of 23 strains: 8 $bla_{\text{CTX-M-15}}$ and 4 $bla_{\text{CTX-M-27}}$. Other $bla_{\text{CTX-M}}$ genes detected were: -M1, -M14, -M36, and -M55. In

addition to the *bla*$_{CTX-M}$ genes, we have detected the *blaTEM-1B* on 8 samples and the *bla*$_{SHV-28}$ in a unique genome, *bla*$_{OXY2_27}$ and *bla*$_{OXY2_28}$ and *bla*$_{ACT-7}$.

The *bla*$_{CTX-M-15}$ gene is associated with the quinolone resistance genes (*qnr*) in three samples. The quinolone resistance gene is associated with the *bla*$_{CTX-M-1}$ beta-lactamase gene in one sample. Different point mutations were present in *gyrA* and *parC* genes.

IncFII, usually associated with antibiotic resistance, is the most prevalent plasmid in the analysed strains

Conclusion: We have shown the prevalence of *E. coli* ST 131, a very spread high-risk clone.

bla$_{CTX-M}$ genes, encoding for the most prevalent class of ESBLs, were present in 82,6% of the analysed strains and *bla*$_{CTX-M-15}$ was associated with 34,8 % of the strains.

This data could contribute to the prophylaxis and antimicrobial stewardship in the pancreatic surgery unit

Abstract ID: 1239 / Poster Number: 362.17P

Molecular characterization of exopolysaccharide from Periweissella beninensis LMG25373 and technological properties in plant-based food production

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Periweissella beninensis LMG 25373^{T}, belonging to the recently established *Periweissella* genus, exhibits unique motility and high adhesion capabilities, indicating significant probiotic potential, including resilience under simulated gastrointestinal conditions. However, its ability to *in situ* synthesize exopolysaccharides (EPS), nor its EPS chemical composition, was ever studied before.

In this study, the composition and structure of the EPS and the genomic determinants of its biosynthesis were revealed. The ability of the strain to synthesize EPS during chickpea and lentil fermentation, and its performance (growth, acidification, proteolytic activity) as a starter for a plant-based yogurt-type ("gurt") were investigated for the first time.

This study demonstrated that, due to the peculiar presence of two putative dextransucrases, *P. beninensis* produces a dextran-type EPS, with an atypical branching pattern compared to that of other *Weissella* species. Indeed, a distinctive high degree of branching (approximately 71% of α-(1→6)-linkages and 29% α-(1→3)-linkages), often associated with enhanced emulsifying and thickening properties, was observed. The strain displayed effective pro-technological properties in gelatinized and non-gelatinized legume-based substrates, achieving EPS synthesis levels up to 1.3 and 2.7 g/100g, respectively. When used as a starter for a gurt prototype, *P. beninensis* produced a substantial increase in viscosity which remained stable during refrigerated storage, confirming the role of its unique structure pattern as a hydrocolloid. Furthermore, the strain demonstrated high viability throughout storage, an essential trait for probiotic food applications. Recognizing this strain as QPS microorganism could accelerate industrial research and facilitate *in vivo* studies to evaluate its full potential.

Abstract ID: 109 / Poster Number: 140.16P

Molecular Detection and Genomic Profiling for Hypervirulent K. pneumoniae recovered in a South African tertiary hospital

Presenting author: Dr. Olusesan Adelabu, University of the Free State, Bloemfointen, South Africa

Co-authors: *Jolly Musoke*

Introduction: Hypervirulent K. pneumoniae (hvKp) is an evolving pathotype that is more virulent than classical K. pneumoniae (cKp) and has emerged as a concerning global pathogen. In South Africa, There is paucity of information on its prevalence and virulence. Therefore, this study aimed at determining the prevalence of hvKp and virulence-associated factors in K. pneumoniae isolates from one of the largest tertiary hospitals in a South African province.

Methods: A total of 74 K. pneumoniae isolates were collected from Pelonomi Tertiary Hospital National Health Laboratory Service (NHLS), Bloemfontein. Virulence-associated genes (rmpA, capsule serotype K1/K2, iroB and irp2) were screened for, using Polymerase Chain Reaction (PCR). The iutA (aerobactin transporter) gene was used as the primary biomarker of hvKp. The extracted DNAs were sequenced using the next-generation sequencing pipeline and the curated sequences were used for phylogeny analyses using appropriate bioinformatic tools. The virulence of hvKp vs. classical Klebsiella pneumoniae (cKp) was investigated using the Caenorhabditis elegans nematode model.

Results: Nine (12.2%) isolates were identified as hvKp. Moreover, hvKp was significantly ($p < 0.05$) more virulent in vivo in Caenorhabditis elegans relative to cKp. The virulence-associated genes [rmpA, iroB, hypermucoviscous phenotype (hmv) phenotype and capsule K1/K2] were significantly ($p < 0.05$) associated with hvKp. A homology search of the curated sequences revealed a high percentage of identity between 99.8 and 100% with other homologous iutA gene sequences of other hvKp in the GenBank.

Conclusion: Findings from this study confirm the presence of hvKp in a large tertiary hospital in central South Africa. However, the low prevalence and mild to moderate clinical presentation of infected patients suggest a marginal threat to public health. Further studies in different clinical settings are required to establish the true potential impact of hvKp in developing countries.

Keywords: hypervirulent, Klebsiella pneumoniae, virulence, Caenorhabditis elegans, aerobactin, capsule, serotype.

Abstract ID: 994 / Poster Number: 044.16P

Molecular detection of Ehrlichia pathogen in brown dog tick (Rhipicephalus sanguineus) infesting dogs in Taiwan

Presenting author: Prof. Chien-Ming Shih, Kaohsiung Medical University, Kaohsiung, Taiwan

Co-authors: *Li-Lian Chao,*

Introduction: The brown dog tick (Rhipicephalus sanguineus) infesting dogs have been recognized as the major vector for various tick-borne pathogens. Due to the increasing amount of dogs rearing as a companion animal with humans, the higher frequency of tick-bite for humans has been recorded through the dogs infested with ticks. It is deserved to investigate the pathogens carried by this potential vector tick in Taiwan.

Objective: We conduct a zoological survey to investigate the prevalence of Ehrlichia pathogens in Rhipicephalus sanguineus ticks infesting dogs in Taiwan.

Method: We employed a nested polymerase chain reaction assay targeting the 16S rRNA gene to detect Ehrlichia pathogens in brown dog ticks collected form dogs in Taiwan. Sequence and phylogenetic analysis were performed by Neighbour-Joining (NJ) compared with Maximum Likelihood (ML) methods to estimate the phylogeny of the entire alignment using MEGA X software package.

Findings: The general prevalence of Ehrlichia pathogens was detected with a prevalence rate of 1.42% in Rhipicephalus sanguineus ticks. The highest seasonal prevalence of Ehrlichia pathogens was observed on August with a prevalence rate of 5.91%. Sequence and phylogenetic analysis revealed a 100% sequence similarity and genetic identity was identified as the genospeices of Ehrlichia canis.

Conclusion: Our results provide the first molecular detection and genetic identification of Ehrlichia canis in Rhipicephalus sanguineus ticks infesting dogs in Taiwan, and this result may highlight the potential threat for animal and human infections in Taiwan.

Abstract ID: 3930 / Poster Number: 199.17P

Molecular epidemiological analysis of Acinetobacter baumannii isolates from hospitalized patients at Dokuz Eylul University hospital between 2000-2017 through different typing methods

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Co-authors: *İbrahim Mehmet Ali Öktem, Zeynep Gülay*

Introduction: Turkey is considered an endemic region for carbapenem resistance *Acinetobacter baumannii* isolates[1]. Following up on the hyperendemic clones both in hospital settings and on a global scale is important to understand the transmission dynamics and to prevent/control *A. baumanni* infections[2].

Objective: This study aimed to evaluate *A.baumannii* blood isolates by different epidemiological analysis techniques collected during a seventeen-year-long period at a tertiary hospital.

Method: A total of 316 non-duplicated, multi-drug resistant isolates, were investigated by pulsed-field gel electrophoresis (PFGE)[3]. Representative isolates of each pattern were included in multi-locus sequence typing (MLST)[4]. Single locus sequence typing (SLST)[5] was performed to find out if it might be used for epidemiological clustering. Carbapenemase and 16S rRNA methylase genes were investigated[6,7].

Findings: Twenty-nine different PFGE patterns were found of which representatives were selected for MLST. Fifteen of twenty-nine isolates(51.7%) were clustered in IC2, the most disseminated clone worldwide[8]. This was followed by six representative isolates(20.7%) clustered in IC4, a hyperendemic clone from different regions[9]. STs belonging to IC1, IC7, and IC8 were also determined. SLST had discriminated 75% of all clonal complexes.

Conclusion: Given its time frame and isolate numbers, the study represents comprehensive molecular epidemiological data from Turkey. PFGE patterns for outbreak comparisons and sequence data for global analysis have been provided. The predominance of IC2 members and their widening resistance profile over the years have been shown. Four new STs (ST1624, ST2490, ST2491, ST2492) were submitted to PubMLST database, and members of IC7 and IC8 were shown for the first time.

Abstract ID: 2694 / Poster Number: 138.17P

Molecular epidemiology of carbapenemase-producing Klebsiella pneumoniae circulating in Slovenija in 2024 – results of the SLOSEQ project

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Co-authors: *Helena Ribic, Tjasa Zohar Cretnik, Andrej Golle, Maja Rupnik, Sandra Janezic*

Introduction: The increasing incidence of carbapenemase-producing Klebsiella pneumoniae (Kp-CP) poses a significant threat to public health. In Slovenia, a six -fold increase in the proportion of Kp-CP cases was observed between 2017 and 2022. Given the rapid spread of Kp-CP, it is essential to routinely monitor resistance mechanisms and potential transmission clusters. To address this challenge, we implemented a genomic surveillance of Kp-CP as part of the project "SLOSEQ – Consolidation and Integration of Whole Genome Sequencing (WGS) into Routine Surveillance in Slovenia".

Objective: We present data from the molecular surveillance of Kp-CP isolates collected from diverse Slovenian regions in 2024 at the National Laboratory for Health, Environment and Food.

Methods: A total of 210 Kp-CP isolates were subjected to whole genome sequencing (NextSeq2000; Illumina). MLST and core genome multi-locus sequence (cgMLST) analysis were performed with RidomSeqSphere+ software. Resistome analysis was performed using AMRFinderPlus.

Results: The most common mechanisms conferring carbapenem resistance were blaNDM-5 (65%) and blaOXA-48 (86%), with 135 isolates having both genes. MLST typing revealed 25 STs, with ST147 being the most prevalent (76%). cgMLST analysis revealed seven clonal clusters. The largest cluster comprised 149 ST147 isolates (71%), most of which harbored both blaNDM-5 and blaOXA-48. Clonal spread of Kp-CP was observed within and between healthcare facilities.

Conclusions: This study provides the first comprehensive insights into the genetic relatedness, dissemination patterns, and resistance mechanisms of Kp-CP in Slovenia. The findings underscore the dominance of ST147 as a high-risk clone driving the spread of Kp-CP within and between healthcare facilities.

Abstract ID: 429 / Poster Number: 170.16P

Molecular Epidemiology, Virulence, and Antibiotic Resistance of Campylobacter in Foodborne Disease Patients in Beijing, 2016–2023

Presenting author: Dr. Penghang Zhang, Beijing Center for Disease Prevention and Control, Beijing, China

Co-authors: *Xiaochen Ma, Yanlin Niu*

Introduction: Campylobacter is the leading bacterial cause of diarrheal illnesses worldwide. Campylobacter jejuni and C. coli are the most common species accounting for campylobacteriosis.

Objectives: To investigate the molecular epidemiological characteristics of Campylobacter in patients with foodborne diseases.

Methods: We analyzed the prevalence, virulence, and antibiotic resistance features of Campylobacter isolated from 27,691 fecal samples collected in Beijing between 2016 and 2023.

Finding: A total of 1,204 Campylobacter strains were isolated, including 1,005 Campylobacter jejuni, 198 Campylobacter coli, and 1 Campylobacter fetus. The infection rate among children was 0.71%, being significantly lower than that in other age groups. A higher isolation rate of Campylobacter was observed in summer and autumn. C. coli infections are increasingly prevalent in Beijing. C. jejuni causes more severe infections than C. coli, with C. jejuni carrying significantly more virulence genes, indicating that C. jejuni has greater pathogenic potential. Screening identified high-risk C. jejuni serotypes associated with Guillain–Barré Syndrome, including 141 strains of HS:2, 61 strains of HS:19, and 1 strain of HS:41. HS:2 was associated with clonal complexes CC-464 and CC21, and HS:19 with CC-22. C. coli had higher resistance rates than C. jejuni, with a decreasing trend in chloramphenicol resistance observed in C. jejuni and an increasing trend in C. coli.

Conclusions: This study provides reference data and insights for preventing and controlling campylobacteriosis. It also offers support for future public health strategies and antibiotic use policies to effectively address the challenges posed by Campylobacter-related foodborne diseases.

Abstract ID: 27 / Poster Number: 160.16P

Molecular Identification and Antifungal Susceptibility Assessment of Trichophyton rubrum Clinical Strains Isolated from Diabetic Patients

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Trichophyton rubrum is the main causative agent of superficial mycoses in Brazil and worldwide. Recently, there has been an increase in dermatophytosis cases both in immunocompromised and diabetic patients. Under these conditions, the lesions tend to be deeper and more invasive, making treatment more difficult. Despite the availability of antifungal therapies, cases of drug-resistant strains are frequent. Curcumin analogs emerge as a promising alternative for treatment, due to their s antifungal and anti-inflammatory activities. This study aimed to perform molecular identification and evaluate the minimum inhibitory concentration (MIC) of fluconazole and curcumin analogs in fungal samples collected from diabetic patients (approved by the ethics committee: CAAE 75654223.2.0000.5498). A total of 29 patients with fungal lesions were selected for the collection of nail and skin fragments. The material was cultured on Sabouraud agar medium with chloramphenicol and incubated at 28ºC for 20 days. Of the collected samples, five were subjected to DNA extraction and molecular identification using TaqMan probes specific for T. rubrum via quantitative PCR. For fluconazole, the clinical isolates presented MIC values ranging from 15.6 to 62.5 µM, indicating resistance when compared to the reference strain CBS 118892, which had an MIC of 3.9 µM. Five curcumin analogs were tested, with MIC values ranging from 1.56 to 12.5 µM against the isolates, showing fungicidal effects for most of the samples. In conclusion, the molecular identification of dermatophyte T.rubrum was effective, and curcuminoid compounds may represent a more efficient alternative for the treatment of dermatophytosis.

Abstract ID: 252 / Poster Number: 060.17P

Molecular Insights into a Lipopeptide's Antifungal Mechanism and Resistance Dynamics

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Introduction: Fungal spoilage is a critical issue in the food industry, particularly for bakery products. Lipopeptide biosurfactants produced by Bacillus subtilis offer potent antifungal properties, presenting a promising alternative to synthetic preservatives. However, understanding its molecular mechanisms and resistance dynamics is essential for effective application.

Objective: This study aimed to elucidate the antifungal mechanism of a lipopeptide, evaluate its interaction with spoilage fungi (Aspergillus niger) and baker's yeast (Saccharomyces cerevisiae), and assess resistance potential under prolonged exposure.

Method: Transcriptomic analyses were performed to investigate the lipopeptide's impact on fungal and yeast molecular pathways. Antifungal activity was assessed via in vitro assays, while whole-genome sequencing (WGS) was conducted on resistant strains to identify genetic adaptations. The lipopeptide's compatibility with bread-making processes was evaluated to determine its feasibility in food systems.

Findings: The lipopeptide disrupts sterol-rich fungal membranes, significantly inhibiting A. niger while sparing critical yeast fermentation functions. Resistance studies revealed minimal adaptation in spoilage fungi, supporting long-term antifungal viability. In yeast, moderate tolerance emerged, requiring dosage optimization. The compound demonstrated stability during baking, confirming its suitability for industrial applications.

Conclusion: This study provides molecular insights into a lipopeptide's targeted antifungal mechanism and resistance dynamics. The findings highlight its potential as a sustainable, natural food preservative while emphasizing the need for careful formulation to maximize efficacy and compatibility in bakery systems.

Abstract ID: 1626

Moss symbionts as natural water purifier for inorganic contaminants in cold climate conditions

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Human activities are rapidly intensifying at high latitudes, driving the accumulation of inorganic contaminants like metals in aquatic ecosystems. As global human impact continues to rise, the demand for innovative, nature-based solutions becomes more urgent than ever, especially in these vulnerable, high-latitude environments. Mosses could offer such a solution: they are capable of surviving in severe and harsh environments and, with the help of microbial symbionts, possess the ability to mitigate various contaminants from the environment. Floating hook moss (*Warnstorfia fluitans*) has demonstrated remarkable efficiency in removing metals and nutrients from Arctic waters. To better understand this capability, we investigated its microbiome—including endophytes—using advanced techniques such as amplicon sequencing and transmission electron microscopy (TEM and HAADF-STEM) to identify and map microorganisms and metals within moss tissues. Additionally, we isolated fungal strains on potato dextrose agar, recognizing that cultivation is a crucial step toward unlocking microbial-driven applications. We identified endophytic fungal species such as *Phialocephala bamuru* and *Hyaloscypha hepaticola*, which thrived in metal-rich conditions and demonstrated remarkable tolerance to 500 ppm levels of Ni, Cu, and As, as well as low pH. Intriguingly, the moss exhibited a unique ability to store metals—particularly iron—intracellularly near the cell membrane, in close association with these endophytic microorganisms. As the global shift from carbon-based energy to renewables accelerates, fueling increased mining activities to meet the surging demand for battery production, the challenge of managing metal-rich, acidic waters from mining operations is intensifying, especially in high-latitude regions. Our findings highlight the potential of floating hook moss, in partnership with its symbionts, as an innovative, nature-based solution for effectively removing metals from mining-impacted waters in subarctic and Arctic environments.

Abstract ID: 1696 / Poster Number: 056.16P

Mouse intestinal microbiota alterations and inflammatory responses caused by two Salmonella enterica serovars with different invasive potential

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Non-typhoidal *Salmonella* serovars Enteritidis and Dublin are closely related phylogenetically, yet differ significantly in their invasiveness. Serovar Dublin causes more severe, extraintestinal infections, compared to Enteritidis. During *Salmonella* gut infection, the resident microbiota exerts a 'colonization resistance' effect, impacting *Salmonella's* ability to proliferate in that environment. However, *Salmonella* benefits from the inflammatory response it triggers in the gut, which negatively affects the microbiota.

In this study, we aimed to assess if serovars Dublin and Enteritidis differ in their capacity to trigger an intestinal inflammatory response and compete with the microbiota. Using shotgun metagenome sequencing, we examined the cecal microbiota composition of C57Bl/6 mice orally infected with two clinical isolates of each serovar, compared to mock-infected controls. We also analysed the inflammatory response of the cecum mucosa, using RTqPCR. *Salmonella* infection resulted in significant alterations in bacterial microbiota composition and the induction of proinflammatory cytokines, chemokines and antimicrobial proteins. Infected mice exhibited markedly reduced alpha-diversity compared to controls, with the lowest alpha-diversity observed in mice infected with serovar Dublin strains. Beta-diversity analyses further indicated differences in the relative abundance of taxa between the infected and control groups. Interestingly, three families depleted upon *Salmonella* infection (*Sutterellaceae*, *Eggerthellaceae* and *Erysipelotrichaceae*) encode several respiratory-like reductases per genome, that allow microorganisms to perform anaerobic respiration using molecules alternative to O_2 as electron acceptors. Serovar Dublin infection resulted in significantly higher changes in microbiota composition and induction of antimicrobial proteins than *S*. Enteritidis infection, suggesting that the former is better adapted to compete with the microbiota.

Abstract ID: 2989 / Poster Number: 439.16P

Movies in company for preventing diseases: an educational program engaged with societal needs

Presenting author: Mr. Mario Pulido-Vadillo, Complutense University of Madrid, Madrid, Spain

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"Movies in company for preventing diseases" program integrates service-learning principles to bridge the gap between science education and societal needs, employing games and commercial films as tools to facilitate understanding and engagement in preventive health measures.

In collaboration with seven NGOs and the Madrid City Council, groups of university students visit social centers where different disadvantaged collectives are attended (homeless, prisoners, drug-addicts, women at risk).

The project is structured into these phases: an initial visit to the center to identify specific needs of the individuals related to infectious diseases, followed by academic work to prepare materials for colloquia and to select an appropriate movie to be screened with the center's users. Finally, a closing celebration is held, where all participants come together to share their experiences and reflect on the impact of the experience.

Over five editions, this initiative has engaged more than 250 university students and 30 instructors who reached approximately 800 individuals from disadvantaged backgrounds or at risk of social exclusion.

By integrating students from mixed academic levels and encouraging the application of acquired knowledge to real societal needs, the program provides a novel approach to both learning and science communication. Participants gain deeper understanding of infectious and other diseases, enhance practical skills, and develop a strong sense of social responsibility. The service-learning program exemplifies how higher education institutions can act as agents of change, and effectively engages academia and underserved communities, creating meaningful societal impact while equipping students with essential skills for the future.

Abstract ID: 2270 / Poster Number: 321.15P

Multidrug-resistant Klebsiella pneumoniae release outer membrane vesicles (OMVs) that facilitate bacterial survival in human serum

Presenting author: Prof. Shiri Navon-Venezia, Ariel University, Ariel, Israel

Co-authors: *Polina Geva, Tehila Rubinstein,*

Introduction: *Klebsiella pneumoniae* (Kp) is a major Gram-negative bacterial pathogen causing life-threatening bloodstream infections (BSIs).[1-4] Like other Gram-negative pathogens, Kp releases OMVs that serve as bacterial cargo nanostructures.[5] Although Kpn OMVs were shown to induce inflammatory responses, their role in Kpn survival in human serum has not been previously investigated. We hypothesized that Kp excretes OMVs that facilitate its survival in human serum. To prove this hypothesis, we isolated OMVs from a serum-resistant and a serum-sensitive Kp isogenic strains, and investigated the enhancement effects of bacteria-free OMVs derived from the serum resistant Kp strain on the serum survival of serum sensitive Kp strains.

Objective: To decipher the role of extracellular Kp OMVs to bacterial survival in human serum.

Methods: OMVs were derived from overnight cultures of three Kp isolates - KpB199[6], a serum resistant isolate, and from KpB10 and Kp ATCC13383, two serum sensitive strains. Kp OMVs were isolated using Tangential Flow Filtration apparatus and were characterized using nanoparticle tracking analysis plots (NanoSight) and visualized using TEM imaging following negative staining. Kp OMVs functionality was evaluated and compared by assessing their effects on the survival of each Kp strain in human sera, compared to the survival of the tested serum-sensitive Kp strain without exogenous OMVs.

Findings: Kp strains release OMVs with a diameter of 25-40 and 250-260 nm and bear a typical OMVs morphology (Fig. 1). The macromolecules composition of the OMVs consisted of proteins (130 - 160 µg/mL) and nucleic acids - DNA (2.4 - 4.4 µg/mL) and RNA (3.5 - 3.9 µg/mL). Extracellular OMVs derived from the serum-resistant KpB199 strain enhanced significantly (15 and 40-fold) the average serum survival of the two serum-sensitive Kp strains - KpB10 and Kp ATCC13383, respectively ($p<0.001$). In contrast, Kp OMVs derived from the two serum-sensitive Kp strains did not affect the serum survival of either Kp strain, suggesting the unique properties of the serum-resistant derived OMVs. Pre-incubation of bacterial-free KpB199 OMVs with human serum for one hour prior to the introduction of the tested serum-sensitive Kp strain, further enhanced the serum survival due to OMVs-induced deactivation of serum complement activity.

Conclusion: We demonstrate a new role of human serum modulation for OMVs derived from *K. pneumoniae*, a clinically challenging-to-treat BSI causative pathogen. Comparative structure-function OMVs studies are currently in progress to elucidate the mode of actions.

These findings highlight the important role of extracellular OMVs as a strategy for Kp-host pathogenesis during BSIs and may contribute to potential future diagnosis and targeted Kp BSI therapy.

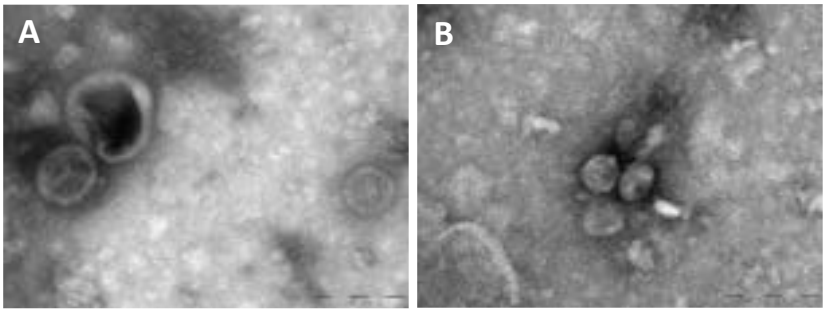


Figure 1: TEM images (negative staining) of extracellular OMVs of KpB199 (A) and KpB10 (B). (Scale bar- 100 nm; 300 X magnification).

Abstract ID: 3210 / Poster Number: 363.16P

Multifaceted and real-time biofilm characterisation of model and non-model yeast strains

Presenting author: Ms. Danelle Botha, Stellenbosch University, Cape Town, South Africa

Co-authors: *Gideon Wolfaardt, Elanna Bester-Klopper, Christoff Truter*

Biofilm characterisation has traditionally focused on bacteria, with a recent rising interest in fungal biofilms. Filamentous fungi, dimorphic fungi, and yeast biofilms frequently exhibit a layered architecture of yeast, hyphal and pseudohyphal cells. Encased in extracellular matrix, these phenotypes convey increased tolerance to fluctuating environmental and anthropogenic influences. Despite ongoing research on yeast biofilm establishment, maturation and the metabolic triggers thereof, knowledge on non-model organisms remains limited. This study addresses this gap through an integrated approach to fungal biofilm characterisation using static screening and non-invasive monitoring under continuous flow. The biofilm stress response capabilities of *Saccharomyces*, *Pichia*, *Cryptococcus* and *Rhodotorula* yeast strains were initially assessed through a high-throughput screen under conditions of decreasing nutrient concentrations, at a set temperature, and over different incubation periods. The biofilm growth phases of the top-performing strains were then monitored under continuous flow, using the previously validated carbon dioxide evolution measurement system (CEMS) combined with BioSpec. Metabolic activity through whole-biofilm CO_2 production was tracked in parallel with biomass accumulation and biofilm-derived planktonic cell production. The environmental strains *C. podzolicus*, *R. mucilaginosa*, and laboratory *S. cerevisiae* strains exhibited pronounced biofilm stress responses under varied static conditions. Under continuous flow, both *C. podzolicus* and *S. cerevisiae* established and matured as biofilms, extending the validation of this monitoring system from bacterial to fungal biofilms. The novel insight gained into yeast biofilm dynamics informs the potential future use of this growth mode for innovative, real-time environmental biomonitoring using engineered yeast.

Abstract ID: 2474 / Poster Number: 240.15P

Multiple lysine methylation of a cell surface nanofiber protein in a highly adhesive bacterium

Presenting author: Mr. Shori Inoue, Nagoya university, Nagoya, Japan

Co-authors: *Shogo Yoshimoto, Katsutoshi Hori*

Introduction: Protein lysine methylation is an important posttranslational modification in eukaryotes, playing a pivotal role in regulating diverse biological processes. Recent research has begun to reveal the potential role of methylation in modulating bacterial immune evasion and adherence to host cells. In this study, we analyzed the cell surface proteins of the toluene-degrading bacterium *Acinetobacter* sp. Tol 5 by label-free liquid chromatography–mass spectrometry (LC–MS) and coincidentally found multiple lysine methylation in its trimeric autotransporter adhesin (TAA), AtaA.

Objective: We aimed to identify and characterize multiple lysine methylation of AtaA in *Acinetobacter* sp. Tol 5.

Method and findings

We examined the cell surface proteins of Tol 5 by label-free LC-MS and found that the multiple lysine residues of AtaA were methylated by an outer membrane protein lysine methyltransferase (OM PKMT), which we named KmtA. KmtA was responsible for the methylation of over 130 lysine residues of AtaA, consisting of 3,630 amino acids and containing 232 lysine residues. Bioinformatic analysis showed that OM PKMT genes are widely distributed among gram-negative bacteria. Furthermore, the deletion of Tol 5 KmtA led to an increase in AtaA on the cell surface and enhanced bacterial adhesion, resulting in slower growth.

Conclusion: We discovered multiple lysine methylation on AtaA and identified the enzyme responsible for this modification. This study represents the first report of lysine methylation in TAA family proteins. Our findings contribute to a better understanding of posttranslational modifications in bacteria, which is important for both medical and engineering applications.

Abstract ID: 1688 / Poster Number: 158.15P

Multiple transporters protect Chromobacterium violaceum against manganese toxicity and oxidative stress

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Co-authors: *Beatriz Picinato, Tie Koide, José Da Silva Neto*

Manganese (Mn) is an important micronutrient in host-pathogen interactions, but it is less studied than other metals like iron and zinc. During infection, vertebrate hosts limit bacterial access to Mn by sequestering it in the protein calprotectin. Bacteria evade this mechanism through various strategies to supply Mn for their growth and survival. This study examines how *Chromobacterium violaceum*, a Gram-negative opportunistic bacterium, maintains Mn homeostasis with a focus on transporters. *In silico* analysis identified three potential NRAMP-type Mn importers (CV_0576, CV_3314, CV_3478) and five Mn exporters (CV_1005, CV_1359, CV_2442, CV_3108, CV_4389). The *yybP-ykoY* riboswitch, likely involved in Mn regulation, was found upstream of CV_1359 and CV_2442. RNA sequencing revealed 177 differentially expressed genes in response to Mn excess, with 91 upregulated (including CV_1359 and CV_2442) and 86 downregulated (including CV_3478). Many upregulated genes are linked to iron acquisition, suggesting a connection between Mn and iron homeostasis. RNA-seq data were validated by RT-qPCR. Mutants lacking putative Mn importers showed no growth defects in metal-chelated media but were more sensitive to oxidative stress. Mutants lacking the putative Mn efflux transporters CV_1005, CV_1359, and CV_2442 exhibited reduced survival in Mn-rich or oxidant conditions. Growth defects were rescued in the complemented strains. No cross-response to other metals was observed, but iron supplementation alleviated Mn toxicity. Virulence assays indicated that NRAMP-type Mn importers are not essential for pathogenicity. These results indicate that CV_1005, CV_1359, and CV_2442 play key roles in Mn detoxification and oxidative stress resistance, probably by acting as Mn efflux transporters.

Abstract ID: 3370 / Poster Number: 290.15P

Multi-stress effects on PGPM Social Behaviour

Presenting author: María García-Toledo, PhD, University of Granada, Granada, Spain

Co-authors: *Belén Juárez, Belén Rodelas, Clementina Pozo, Jessica Purswani*

The use of biofertilizers, composed of plant growth promoting microorganisms (PGPMs), are becoming increasingly widespread in agriculture. Unlike single species-strains, consortia provide greater PGP functionality and stability yet need to be resilient due to an ever-changing environment. Additionally, social behaviour can now be inferred to select a social consortia, however, environmental changes can lead metabolome disruption followed by the, production of new metabolites and thus possible changes in behavioural patterns. Since the BSocial tool (https://m4m.ugr.es/BSocial.html) deciphers individual social behaviour from complete combinatorial growth data, thus does the social behaviour change after physicochemical disturbance? and can we find a resilient social consortia? To answer these questions, social behaviour was assigned to 8 PGPMs (*Azospirillum, Bradyrhizobium, Ensifer, Mesorhizobium, Pseudomonas spp*.) after being exposed to 27 stresses, combined thermal (-20, 28, 50ºC), saline (0, 300, 450 mM NaCl) and water (0, 5, 35% PEG-6000) stresses. In addition, the resilience of the full combinations was calculated. Results demonstrated a highly resilient and social strain, *Mesorhizobium* all 27 disturbances, while *Pseudomonas* showed negative behaviour in all scenarios. *Azospirillum, Bradyrhizobium* and *Ensifer* showed social behaviour in half of the scenarios. Furthermore, increasing consortia species richness increased resilience, where social consortia were among the most resilient. Additionally, social behaviour remained constant in the recovery phase despite environmental changes, thus the BSocial tool allows for the selection of social and resilient consortia for biofertilizers.

Abstract ID: 2319 / Poster Number: 392.16P

Mushrooms at work: Lignocellulosic waste degradation by wood-rotting microfungi

Presenting author: Ms. Sheetal Joshi, North Eastern Hill University, Shillong, India

Co-authors: *Neha Chaurasia, Santa Ram Joshi*

Fungi are promising agents for the degradation of lignocellulosic waste, the main components of agricultural waste. This degradative capacity is due to their ability to produce an array of enzymes that break down cellulose, hemicellulose and lignin. The improper disposal of agricultural waste, particularly through burning during the winter months, contributes to rising particulate matter, exacerbating air pollution- a growing concern in Indian metropolises. To address this, the lignocellulosic degradation potential of wild wood-rotting macrofungi of Meghalaya was investigated.

Macrofungi were collected from various regions of Meghalaya, cultured, and identified using both morphological and molecular techniques. These isolates were qualitatively screened for the production of lignocellulolytic enzymes, including cellulase, laccase, and xylanase. Potential isolates were further evaluated for enzyme production using specific assays such as CMCase, xylanase and laccase assays.

The macrofungal isolates were confirmed as wood-rotting species through molecular identification using ITS markers and were found to belong to three families: Fomitopsidaceae, Polyporaceae and Pleurotaceae. The isolates were found to exhibit significant potential for the degradation of lignin, cellulose, and hemicellulose.

This study demonstrates the lignocellulosic degradation potential of wild wood-rotting macrofungi from Meghalaya, highlighting their enzymatic activity. These macrofungi could serve as bioremediation agents for sustainable agricultural waste management. Further research to optimise enzyme production could enhance their role in reducing the environmental impact of agricultural waste burning.

Abstract ID: 494 / Poster Number: 276.17P

Myco-fabricated silver nanoparticle by novel soil fungi from Saudi Arabian desert and antimicrobial mechanism

Presenting author: Assoc. Prof. Hana Sonbol, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia

Introduction: Biological agents such as fungal are getting a noticeable concern as efficient eco-friendly method for nanoparticle fabrication.

Objective: Applying fungi as sustainable approach to provide efficient AgNPs with antibacterial effect.

Method: Two fungal species (Embellisia spp. and Gymnoascus spp.) were isolated from the desert soil in Saudi Arabia and identified using 18S rRNA gene sequencing then used as bio-mediator for the fabrication of silver nanoparticles (AgNPs). Myco-synthesized AgNPs were characterized using UV–visible spectrometry, transmission electron microscopy, Fourier transform infrared spectroscopy and dynamic light scattering techniques. Their antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae were investigated. To detect their possible antibacterial mechanism, Sodium dodecyl sulfate and TEM analysis were performed for Klebsiella pneumoniae treated by the myco-synthesized AgNPs.

Findings: Detected properties of the fabricated materials indicated the ability of both tested fungal strains in successful fabrication of AgNPs having same range of mean size diameters and varied PDI. The efficiency of Embellisia spp. in providing AgNPs with higher antibacterial activity compared to Gymnoascus spp. was reported however, both indicated antibacterial efficacy. Variations in the protein profile of K. pneumoniae after treatments and ultrastructural changes were observed.

Conclusion: The study indicated the ability of two soil fungal for the biosynthesis of AgNPs and both myco-synthesized AgNPs could be recommended as antibacterial agents specially against K. pneumoniae where their mode of action was reported by TEM and SDS PAGE analysis.

Abstract ID: 1640 / Poster Number: 210.15P

Myo-Inositol metabolism in intestinal bacteria: Insights from proteomic-SIP experiments

Presenting author: Ms. Harshita Naithani, Universität Hohenheim, Stuttgart, Germany

Co-authors: *Amélia Camarinha Silva, Jana Seifert*

Introduction: Myo-inositol (MI) plays critical regulatory and metabolic roles in mammals and poultry. Despite its significance, the microbial utilisation and fermentation of MI in the intestine remains poorly understood.

Objective: The study focuses on identifying microorganisms in the chicken intestine, which are involved and show temporal and regional differences, in bacterial MI utilization using isotopically labelled MI (^{13}C) and proteomics-based stable isotope probing (SIP).

Method: Digesta samples from the ileum and caeca of two laying hens were either directly inoculated into isotopically labelled (^{13}C) MI medium (Treatment 1), or pre-incubated for 24 hours before inoculation (Treatment 2) under anaerobic conditions. A proteomic-SIP workflow was done with samples from 8- and 24-hours post-incubation to identify the active microbiome fraction using SIPROS 4 algorithm.

Findings: In the caeca, Treatment 1 showed higher ^{13}C-labelled protein abundance within 8 hours, with a decline by 24 hours. Ileum samples from Treatment 2 exhibited a gradual increase in ^{13}C-labelled protein abundance over time. The predominant bacterial class in almost all the samples was *Negativicutes*, followed by *Bacteroidia*, *Clostridia*, and *Bacilli*. At the genus level, *Megamonas* was abundant except in ileum samples from Treatment 1, where *Ligilactobacillus* and *Limosilactobacillus* dominated.

Conclusion: The findings suggest that MI metabolism occurs rapidly in the caeca, driven by well-adapted bacterial populations, while the ileum exhibits slower, sustained metabolism. The caeca appear to be the primary site of MI utilization, indicating further investigation into their microbial communities for insights into poultry MI metabolism.

Abstract ID: 1322 / Poster Number: 407.15S

NAD kinase as a new antibiotic target

Presenting author: Prof. Olivier Dussurget, Institut Pasteur, Paris, France

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Global burden of infectious diseases and antimicrobial resistance are major public health issues calling for innovative control measures. Development of new antibiotics and alternative therapies rely in particular on identification of new bacterial targets, as pointed out by the World Health Organization.

We have initiated characterization of such new target: the nicotinamide adenine dinucleotide (NAD) kinase (NADK). NAD+/NADH and their phosphorylated forms NADP+/NADPH are essential cofactors in all living organisms. NADK is the only enzyme that catalyzes synthesis of NADP(H) from NAD(H). Accordingly, it plays a critical role in controlling the NADH/NADPH ratio, which is necessary for proper balance of the redox state and optimal function of many metabolic pathways. Its activity has been shown to be essential for growth in clinically relevant pathogenic bacteria such as *Mycobacterium tuberculosis, Salmonella enterica* and *Pseudomonas aeruginosa*. Given its key role and its structural divergence from human NADK, bacterial NADK is a promising drug target for antibiotic discovery. The aim of this study was to develop NADK inhibitors and characterize further the role of NADK in pathogenic bacteria.

We initially solved the structure of *Listeria monocytogenes* NADK. Screening and *in situ* synthesis using NADK crystals led to the identification of a family of di-adenosine derivatives as NADK inhibitors. One compound, NKI1, exhibited inhibitory activity against *Staphylococcus aureus,* without any significant toxicity to human cells *in vitro*. Remarkably, NKI1 inhibited growth of both methicillin-sensitive and methicillin-resistant strains. CRISPR-interference knock-down of NADK gene expression confirmed its importance for growth of *S. aureus*. While NKI1 did not show any toxic effect in mice, it controlled the kidney bacterial burden in a murine model of acute hematogenous pyelonephritis induced by *S. aureus*.

In contrast to NADK enzymatic properties and metabolic functions, its role in bacterial pathogenesis remains to be fully characterized. We used CRISPR interference to knock down NADK gene expression in order to analyze the role of this enzyme in *S. aureus*

pathogenic potential. We found that NADK is a critical determinant of *S. aureus* infection in macrophages and in zebrafish, by protecting bacteria from antimicrobial defense mechanisms. Furthermore, we demonstrated that NADK is required for expression of major *S. aureus* virulence factors, including the quorum-sensing response regulator AgrA. Importantly, we also found that knock down of NADK by CRISPR interference sensitizes *S. aureus* to stresses induced by antibiotics targeting the envelop or inhibiting replication, transcription and translation.

Collectively, our findings indicate that NADK is a druggable target to develop innovative treatments against antibiotic-resistant pathogenic bacteria, which could be used in combination with current antibiotics.

Abstract ID: 2202

Nanobiotechnological design of M13 bacteriophage for egfr-targeted theranostic applications

Presenting author: Paolo Emidio Costantini, PhD, Università di Bologna, Bologna, Italy

Co-authors: *Luca Ulfo, Matteo Di Giosia, Alena Kaltenbrunner, Michela Nigro, Roberto Saporetti, Annapaola Petrosino, Manuela Sollazzo, Annamaria Porcelli, Eleonora Turrini, Vincenzo Cerullo, Matteo Calvaresi, Alberto Danielli*

Bacteriophages, especially the M13 filamentous phage, are gaining attention as nanomedicine platforms due to their low immunogenicity, efficient penetration of biological barriers, safety profile and targeting capabilities. Here, we present the development of a novel theranostic platform using the M13 phage. M13 was genetically engineered to display the 7D12 nanobody in fusion with the pIII protein on the phage tip, enabling for precise molecular recognition of the Epidermal Growth Factor Receptor (EGFR), which is overexpressed in several cancers with severe outcomes. The acquired tropism of $M13_{7D12}$ was demonstrated on both single and co-culture experiments performed with EGFR positive and negative cell line (fig 1B-E). In addition, we have successfully chemically conjugated photosensitizers (Rose Bengal – RB) or fluorescent dyes (CF594) onto pVIII proteins, resulting in a nanovector capable of delivering hundreds of molecules exclusively to cancer cells overexpressing EGFR. RB is widely used in photodynamic therapy (PDT), a promising minimally invasive cancer treatment that relies on photosensitizer-induced production of reactive oxygen species (ROS) upon light irradiation. After demonstrating that chemical conjugation did not alter the tropism of the engineered phage, the therapeutic performance in photodynamic therapy (PDT) of $M13_{7D12}$-RB was evaluated on both 2D (fig 1F) and 3D cell culture models. As a result, $M13_{7D12}$-RB exhibited potent and selective photodynamic killing activity at picomolar concentration of phage upon irradiation. Notably, in ex vivo tumor samples, the phage successfully penetrated the spheroid core and led to complete disaggregation of the spheroid cytoarchitecture upon light irradiation(1G). Furthermore, in vivo experiments in EGFR-positive tumor-bearing mice further validated its therapeutic potential, indeed laser irradiation after intratumoral injection of $M13_{7D12}$-RB led to marked tumor regression (fig 1H). This study demonstrates the potential of engineered M13 bacteriophages as precise and versatile platforms for targeted cancer therapy, addressing key limitations of PDT through enhanced tumor targeting. While the system was specifically developed for EGFR-overexpressing cancers, its modular design allows for adaptation to other tumor

markers, offering a powerful and customizable solution to overcome side effects and drug solubility challenges, enabling the targeted treatment of diverse cancer variants.

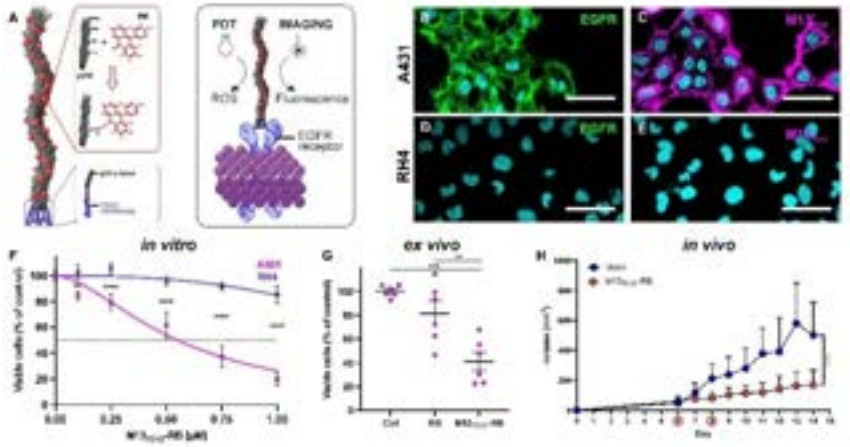


Figure 1: A) Scheme of genetic and chemical engineering of M13 phage. EGFR expression of B) A431 and D) RH4 cells and targeting of $M13_{7D12}$ to C) A431 and D) RH4 cells. F) Cytotoxic effects of $M13_{7D12}$-RB on A431 (magenta) and RH4 (purple) cells irradiated for 1 min with laser. Photooncolytic effect of $M13_{7D12}$-RB was evaluated also on G) ex-vivo tumoroid derived from patient with ovarian cancer and H) in vivo on xenograft.

Abstract ID: 1305 / Poster Number: 413.17P

Nano-Driven Biopesticide Encapsulation: Pioneering Sustainable Solutions for Managing Penicillium expansum and Rhizobium vitis in Agricultural and Environmental Systems

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Co-authors: *Kristina Ivanova, Garima Rathee, Paulina Tapia-Quirós, Leonardo Martín Pérez, Tzanko Tzanov*

Introduction: *Penicillium expansum* and *Rhizobium vitis* are common plant pathogens that cause significant postharvest losses in fruits like apples and grapes. These pathogens can also thrive after environmental disruptions from natural or man-made disasters. Traditional chemical fungicides raise concerns due to their environmental impact, toxicity, and resistance development. Biopesticides from natural sources offer a sustainable alternative, but challenges with stability, controlled release, and targeted delivery limit their effectiveness. Nanotechnology provides a solution by encapsulating biopesticides in nanoscale carriers, improving stability, bioavailability, and controlled release, thus enhancing their efficacy and offering a sustainable approach to addressing agricultural challenges.

Objective: The goal was to develop environmentally friendly, sustainable nanobiopesticides (NanoBP) by applying nanotechnology combined with eco-safe organic and non-organic carriers, such as zein, lignin, bacterial spores, layered double hydroxides (LDH), and mesoporous silica, for encapsulating bioactive compounds.

Methods: The antifungal and antibacterial activity of the nanoBPs was evaluated against two common phytopathogens, *Penicillium expansum* and *Rhizobium vitis*. Phenolic compounds (e.g., tannic acid, caffeic acid, trans-ferulic acid, rutin, and quercetin) and plant extracts (e.g., lavender oil, rosemary, ragweed, wormwood, nettle, and goldenrod) were used as antimicrobials for encapsulation. The preparation of NanoBPs involved the use of self-assembly, ultrasound- and template-assisted nanoparticle (NP) synthesis methods. Lignin-phenolated NanoBPs were produced through a combination of ultrasound and laccase treatment. The strain *Bacillus subtilis* subsp. *subtilis* CECT 39T (ATCC 6051) was used to obtain spores. The resulting NanoBPs were characterised for size, stability, and surface properties using dynamic light scattering (DLS) and Fourier-

transform infrared spectroscopy (FTIR). Antibacterial and antifungal activities were also assessed, confirming the grafting of phenolic compounds.

Findings: The encapsulation efficiency (EE) of NanoBPs ranged from 5% to 85%. Zein-based NanoBPs encapsulated with tannic acid (EE = 84.6% at 5 mg/mL) were the most stable, exhibiting high antifungal activity, reducing *P. expansum* growth by 63% at 1 mg/mL. The most effective NanoBP were formulated using spores of *B. subtilis*, where rosemary-extract-loaded spores eradicated the growth of *P. expansum* and *R. vitis* at all tested concentrations. Lignin-based NanoBPs phenolated with gallic and tannic acids inhibited *P. expansum* growth by 30-40%. Tannic acid-loaded LDH also showed strong antifungal activity, reducing *P. expansum* growth by up to 5 logs at 1 mg/mL and completely eradicating *R. vitis*. Pathogen-responsive multi-layered nanoformulations, such as mesoporous silica loaded with caffeic acid, were developed to target specific microorganisms. The pores containing caffeic acid were sealed with pectin, which targets *P. expansum* due to its pectinase production. Pectinase degrades pectin, releasing an antimicrobial compound that inhibits fungal growth without affecting non-pectinase-producing microorganisms. These MS_CA_NanoBPs reduced *P. expansum* growth by 30% and had no effect on *R. vitis*.

Conclusion: The study highlights the potential of nano-driven biopesticides as sustainable solutions for controlling specific agricultural pathogens, while minimizing ecological disruption. Future developments should focus on refining these formulations to enhance their specificity and efficacy, ensuring they target harmful pathogens without adversely affecting beneficial microorganisms in agricultural and environmental systems.

Abstract ID: 2710 / Poster Number: 377.17P

Nanoliposomal delivery of bacterial-derived β-cryptoxanthin for milk fortification: A novel approach to combat vitamin A deficiency

Presenting author: Ms. Debasmita Dutta, National Institute of Technology Durgapur, Durgapur, India

Co-authors: *Debasmita Dutta*

Vitamin A deficiency (VAD) is a significant global health challenge that requires innovative dietary interventions. *Kocuria marina* DAGII, isolated from the soil of NIT-Durgapur, has shown substantial potential for β-cryptoxanthin synthesis with a yield and purity of 96.67 mg/L and 93%, respectively. As a provitamin A carotenoid, β-cryptoxanthin showed efficacy in mitigating VAD but was limited by instability under adverse conditions. To overcome these challenges, β-cryptoxanthin was encapsulated in nanoliposomes and fortified in milk. The encapsulation efficiency of β-cryptoxanthin encapsulated nanoliposomes was 96.11±0.79%, with a particle size of 64.34±1.42 nm. Furthermore, 74.12±2.08% of β-cryptoxanthin was retained after 30 days of storage at 4 ºC with pH 7.4. A toxicity study showed >92% viability of human embryonic kidney cells against β-cryptoxanthin encapsulated nanoliposome concentrations up to 100 µg/mL. The genotoxicity study revealed no chromosomal aberration, and the haemolysis assay confirmed the absence of erythrocyte lysis. The fortified milk retained essential nutritional parameters, including fat, protein, dry sediment, specific gravity, and colour ($p>0.05$). Furthermore, >90% of β-cryptoxanthin was preserved after heat treatment (pasteurization and sterilization), demonstrating its thermal stability. Fortification significantly enhanced antioxidant properties, as evidenced by a two-fold increase of ferric-reducing antioxidant power, significantly reduced lipid peroxidation, and protein oxidation ($p<0.01$). Simulated gastrointestinal conditions revealed that fortified milk could supply 20%–47% of the recommended daily allowance for vitamin A. These findings demonstrate the significant potential of nanoliposome-encapsulated β-cryptoxanthin derived from *K. marina* DAGII, which could be a stable and nutritionally effective strategy for milk fortification to address VAD.

Abstract ID: 49 / Poster Number: 282.15P

Natural Microbial Pigment as a Sustainable Staining Agent for Nucleic Acids

Presenting author: Dr. Kalirajan Arunachalam, Mulungushi University, Kabwe, Zambia

Co-authors: *Dr.Mariselvan Ramaiah*

Introduction: Agarose gel electrophoresis is a technique used for the separation of DNA Sfragments by size, utilizing an intercalating dye like ethidium bromide for visualization. However, ethidium bromide poses risks to living organism.

Objective: The objective of this research was to explore the use natural pigments produced by *Serratia marcescens* as a safer alternative staining agent for DNA visualization.

Method: In this study,2 µl of microbial pigment was incorporated at a concentration of 0.2 µg into the agarose gel instead of ethidium bromide during DNA separation and FT-IR and GC-MS analysis was done to characterize the pigments. MTT assay was used to test the cytotoxic activity of bacterial pigments against MCF 7 Cancer celllines.

Findings: The results indicated that the bacterial pigment stained the Circulating tumor DNA, resulted in a reddish-orange coloration when observed under a UV transilluminator. Further, FTIR spectroscopy analysis revealed the functional groups (Peak at 3396 cm-1 which indicates amine group and–OH group where 2975, 2897 revealed the presence of aromatic C-H and –C-O-CH3 groups and peaks at 1649 cm-1 and 1599 cm-1 and the peak at 1649cm-1 showed the presences of –C=C- aromatic groups presented in the bacterial pigment closely resembled those found in prodigiosin, a known pigment produced by *Serratia marcescens*. The compound prodigiosin was confirmed by the mass spectral data. The results of the cytotoxic study revealed lesser toxicity of bacteria-based pigments.

Conclusion: The findings of this research will help develop microbial pigment based eco-friendly dyes to stain the DNA.

Keywords: Microbial pigments; Serratia marcescens; DNA staining, Breast cancer cell lines, cytotoxicity.

Abstract ID: 1995 / Poster Number: 289.16F

Natural Resistance-Associated Macrophage Proteins (NRAMPs) and Ferric Reductase Oxidase (FRO) crosstalk under abiotic and biotic (Verticillium wilt) stress in Gossypium spp

Presenting author: Dr. Surendra Pratap Singh, Dayanand Anglo-Vedic (PG) College, Kanpur, Kanpur, India

Cotton, a major fiber crop, often faces different stresses that greatly affect its growth and productivity. Natural resistance-associated macrophage proteins (NRAMPs) and ferric reductase oxidases (FROs) are crucial for metal transport in plants. This study presents a comprehensive genome-wide analysis of NRAMP and FRO genes across four Gossypium species, identifying 78 NRAMP (PF01566) and 65 FRO (PF01794) genes: G. hirsutum (21GhNRAMPs, 21GhFROs), G. barbadense (33GbNRAMPs, 21GbFROs), G. raimondii (12GrNRAMPs, 12GrFROs), and G. arboreum (12GaNRAMPs, 11GaFROs). Subcellular localization analysis revealed that NRAMP proteins localize to the plasma membrane, vacuole, and extracellular space, while FRO proteins are found in the plasma membrane. Phylogenetic analysis grouped NRAMP genes into 12 distinct clades and FRO genes into 15 clades. Gene structure analysis uncovered diverse exon-intron compositions, and chromosomal localization highlighted their distribution across the A and D sub-genomes, reflecting evolutionary dynamics. Cis-regulatory element analysis revealed their functional involvement in stress responses, hormone signaling, light responsiveness, and developmental regulation. Protein-protein interaction (PPI) network analysis demonstrated that FRO proteins interact with NRAMPs and Calcium-Dependent Protein Kinases (CDPKs). Expression analysis under various abiotic (heat, cold, salt, PEG) and biotic stress (treatment with the fungus *Verticillium dahliae*) further confirmed the regulatory significance of FRO and NRAMP genes. The selected GhNRAMP and GhFRO expression levels were checked by qRT-PCR under treatment with SA, MeJ, NaCl, PEG, and Cadmium (Cd) for different time intervals, suggesting their significant role. This study underscores the pivotal roles of NRAMP and FRO genes in stress tolerance, iron homeostasis, biotic stress, and development in cotton.

Abstract ID: 2289 / Poster Number: 403.16P

Nature-Based Solutions Using Natural Coagulants from Urban Parks as a Strategy for Intelligent Adaptation Systems to Address Sanitation and Water Scarcity in Catalonia

Presenting author: Dr. Nury Infante, Polytechnic University of Catalonia, Terrassa, Spain

Co-authors: *Beatriz Amante, Victor Lopez, Beatriz Escribano, Olga Sanchez, Jordi Morató*

The drought that Catalonia has suffered in the last 3 years has broken historical records, where the water level in the reservoirs of the internal basins of Catalonia has fallen below 15%, this being the lowest percentage of water stored in all of Spain. This critical situation directly impacts the availability of surface and groundwater resources, which are essential for all productive sectors, including ecosystem services. The resulting water scarcity increases the risk of cascading natural disasters, as climate related water risks propagate through food, energy, urban, and environmental systems. This study aims to address these challenges by evaluating the effectiveness of natural antibacterial agents and plant-based coagulants derived from species in Parc Sant Jordi, Terrassa, for potential biotechnological applications in sustainable water management. The research aligns with the European Union's "Water Smart Society" vision. Furthermore, evaluating the impact of Nature-based Solutions (NbS) is essential to understanding their role in mitigating or facilitating the dissemination of extended-spectrum beta-lactamase (ESBL)-producing bacteria in water and sediment samples from urban parks across Catalonia. The research unfolds in three phases: first, the physicochemical and microbiological characterization of water quality mainly a great spectral profile of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) from five representative urban parks monitored across four climatic seasons in Catalonia (Parc Vallparadis, Sant Cugat, Fluvial del Besòs, Ciutadella and Pegaso). These Urban parks contain canals, small streams and lagoons that act as wetlands. Second, the identification and evaluation of plant species for their coagulation-flocculation and antimicrobial properties, supported by a municipal database from Terrassa. Third, the investigation of plants-based coagulant and their integration into sustainable water management practices. 14 plant species was found with the highest efficiencies in the removal of contaminants in different types of synthetic prepared water, a database and a seedbed with potential plant-based solutions in biotechnological applications for water treatment was developed and tested with effectively inhibition growth targeting

waterborne pathogenic bacteria, including antibiotic-resistant strains between them *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These findings highlight the potential of urban park and their vegetation for biotechnological applications in water purification, offering sustainable and scalable solutions for regions facing water scarcity. This work underscores the critical role of urban biodiversity in addressing global water challenges while promoting ecosystem resilience and resource sustainability.

Abstract ID: 4142

Neurotransmitter-producing microbiota: Interaction with the host

Presenting author: Friedrich Götz, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany

Co-authors: *Dr. Samane Rahmdel*

Introduction: Monoamines are neurotransmitters (NTs) endogenously produced in mammals, but they are also synthesized by bacteria within the human microbiota. In many bacterial species, these NTs are produced by aromatic amino acid decarboxylases (AADCs). Once secreted, these NTs can interact with the host.

Objective: Do NTs produced by commensal bacteria impact human metabolism?

Results: Some bacterial AADCs, such as SadA, exhibit broad substrate specificity and can decarboxylate tryptophan, tyrosine, phenylalanine, dihydroxyphenylalanine (L-DOPA), and 5-hydroxytryptophan (5-HTP) to yield tryptamine (TRY), tyramine (TYM), phenethylamine (PEA), dopamine (DOP), and serotonin, respectively. Metagenomic analysis of the human skin microbiota reveals that SadA-type AADCs are widespread, particularly in the phyla Actinobacteria, Proteobacteria, Bacillota, and Bacteroidetes. Many of the genera harboring these AADCs belong to the core skin and gut microbiota.

The interaction between NT-producing bacteria and the host has been explored in various *Staphylococcus* species. Their secreted NTs promote internalization by human cells via activation of the α2-adrenergic receptor (α2-AR). Additionally, both purified NTs and NT-producing *Staphylococcus* strains accelerate wound healing by antagonizing the β2-adrenergic receptor (β2-AR) in keratinocytes.

Conclusion: A significant proportion of the human microbiota is capable of producing NTs. As these compounds are secreted by many bacterial species, they can cross epithelial barriers (e.g., in the intestine, skin, and nasal passages), enter the bloodstream, interact with neuroreceptors, and potentially affect host physiology and the central nervous system.

Abstract ID: 2497 / Poster Number: 241.15P

New Asgard Archaea enriched from hypersaline microbial mats from Shark Bay, Western Australia

Presenting author: Ms. Stephanie Nobs, University of New South Wales, Sydney, Australia

Co-authors: *Brendan Burns, Iain Duggin, Debnath Ghosal, Matthew Johnston, Julia Meltzer, Katharine Michie, Keiran Rowell, Xabi Vázquez Campos, Timothy Williams*

The microbial mats of Shark Bay, Western Australia harbour a substantial abundance of uncultured microorganisms including the Asgard Archaea – a difficult to culture clade with significant evolutionary implications as the closest prokaryotic relative to eukaryotes. Despite the abundance of metagenomes from environmental sequencing, phenotypic characterisation of the Asgard Archaea has been hampered by limited cultured representatives, with only three reported enrichments to date. As a result, little is understood about the physiology of Asgard Archaea in diverse environments.

Here, we present *Nerearchaeum marumarumayae* Loki-ASV2, a novel Asgard of the family *Promethearchaeaceae* that was cultured to a high enrichment (89%) to further expand our understanding of; 1) the underexplored physiology of the Asgard Archaea, and 2) un-cultured lineages from hypersaline microbial mats in Shark Bay. Loki-ASV2 was co-cultured with a sulfate-reducing bacterium, and, due to the low diversity of enrichment, we were able to assemble a complete 5.2 Mbp genome with a mix of long read and short read sequencing. High-resolution electron cryotomography combined with structure-based and sequence-based annotation methods allowed us to compare Loki-ASV2 with the three other cultured lineages of *Promethearchaeaceae*. Core features of *Promethearchaeaceae* include the 'pearls-on-a-string' morphology alongside key cytoskeletal and morphological proteins. In Loki-ASV2 we also have evidence of unique encapsulin nanocampartments, putative tubulin microtubules, and a large 5416 amino acid protein that may be involved in biofilms. This is the first phenotypic description of an Asgard Archaea from Shark Bay and our annotations suggest some niche adaptation of Loki-ASV2 to the hypersaline microbial mats.

Abstract ID: 1356 / Poster Number: 185.16P

New combinatorial therapies to treat intracellular Staphylococcus aureus infections in breast cancer

Presenting author: Ms. Helena Álvarez Ferrero, University of León, León, Spain

Co-authors: *Sergio Fernández Martínez, Blanca Lorente Torres, Pablo Castañera, Jesús Llano Verdeja, Farzaneh Javadi Marand, Álvaro Mourenza, Luis M. Mateos, Michal Letek*

Staphylococcus aureus is a versatile pathogen responsible for a wide spectrum of diseases, ranging from mild to life-threatening conditions, with a profound impact on global health. Its ability to invade and proliferate within host cells, coupled with its resistance to multiple antibiotics, presents significant treatment challenges. These characteristics not only complicate the management of intracellular bacterial infections but have also been implicated in the progression of certain cancers, including breast cancer.

To address this dual challenge, innovative therapeutic strategies are urgently needed. Drug repositioning, which identifies new applications for existing, approved compounds, represents a promising approach for developing effective treatments. This study screened 6,995 drugs with repurposing potential to identify compounds with potent antimicrobial activity against *S. aureus*. From this high-throughput screening, a range of candidates, including anticancer agents, were selected for further evaluation. Their effects were assessed both individually and in combination, using direct antibacterial assays and breast cancer cell models infected with intracellular *S. aureus*.

Our findings demonstrate that combinatorial therapies leveraging non-antibiotic compounds hold significant promise for addressing *S. aureus*-associated infections. These therapies not only mitigate the risk of antibiotic resistance but also provide a dual-action approach to target both breast cancer cells and the intracellular pathogen. These results highlight the potential of drug repositioning as a powerful strategy for tackling complex, multidimensional health challenges.

Abstract ID: 1722

New Genes Involved in Xylose and L-Arabinose Fermentation in the Thermotolerant Yeast Ogataea polymorpha

Presenting author: Prof. Andriy Sibirny, University of Rzeszów, Rzeszów, Poland; Institute of Cell Biology, NAS Ukraine, Lviv, Ukraine

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Introduction: Thermotolerant methylotrophic yeast *Ogataea polymorpha* grows at high temperatures up to 50-51°C and naturally utilizes and ferments major sugars of lignocellulose, glucose and xylose, still accumulates 100 times less ethanol from xylose than from glucose. Growth on L-arabinose is negligible. Feasible growth and fermentation at so high temperature makes *O. polymorpha* the promising organism for simultaneous saccharification and fermentation (SSF), however, xylose fermentation should be substantially enhanced. In our previous works, efficiency of xylose fermentations was increased 40 times due to combination of the methods of metabolic engineering and classical selection.

Objectives: In this work, we developed simple method of positive selection of the advanced ethanol producers from xylose by selection for mutants forming large colonies on plates with L-arabinose as sole carbon and energy source.

Methods: The standard methods of molecular genetics were used.

Findings: The best isolated mutant, A107, accumulated in xylose medium near 21 g/L of ethanol from xylose at 45°C which exceeds 50 times the levels accumulated by the wild-type strain (0.4 g/L). This is the highest known titer of ethanol from xylose at 45°C. The isolated strain A107 was also characterized by enhanced fermentation of L-arabinose and lignocellulosic hydrolysates relative to the parental strain. Whole genome sequencing of the mutant A107 revealed mutations in the *API1* and *IRA1* genes, which code for homolog of bacterial arabinose-5-phosphate isomerase and *Saccharomyces cerevisiae* Ras-GTPase activating protein involved in the Ras-cAMP pathway, respectively.

Conclusion: Overexpression of *API1* gene specifically activated L-arabinose fermentation whereas knock out of *IRA1* gene activated fermentation of both pentoses, xylose and L-arabinose. Future prospects of our strain implementation will be discussed.

Abstract ID: 1441

New insights on the phylogeny and function of the agricultural soil microbiome

Presenting author: Ioanna Chatzigiannidou, PhD, Technical University of Denmark, Kgs. Lyngby, Denmark

Co-authors: *Tanvi Taparia, Pi L. Johansen, Joseph Nesme, Søren J. Sørensen, Susanne Brix, Karsten Kristiansen*

Soil serves as a habitat for a rich and diverse microbial community. The soil microbiome is essential for ecosystem processes, including biogeochemical cycles, and is a primary source for discovering new compounds, such as antimicrobials. In agricultural settings, the soil microbiome influences soil fertility and plant health. Despite its importance, the soil microbiome is still not well understood, with only a few members cultured and a large proportion of uncharacterised phylogenetic and functional diversity. Nonetheless, comprehensive understanding of the agricultural soil microbiome composition and function is crucial for providing sustainable solutions for crop production.

In this study, we aimed to better characterize the agricultural soil microbiome through deep metagenomic sequencing and genome resolved metagenomics. We sequenced 455 soil samples collected in two depths of two agricultural fields in Denmark, with different physical properties. The above yielded ~70 TB of raw sequencing data from which de-novo genome assembly resulted in 58,831 medium to high quality metagenome-assembled genomes (MAGs). MAGs could be further clustered in 9,090 species-level genome bins (SGBs) spanning 46 different bacterial and 3 archaeal phyla. Our genome catalogue covers unexplored diversity as 96.2% of the SGBs have not been previously reported. Characterization of the functional potential of the SGBs revealed their role in biogeochemical cycling, for example carbon, sulphur, and nitrogen cycling. Focusing on the nitrogen metabolism, we identified different species – some of them novel – that were involved in nitrification, denitrification, and nitrogen fixation. Their presence and abundance differed significantly between soil-types and depths. Furthermore, we discovered 378 species (98.7% novel) that possess the nitrous oxide reductase (nosZ) gene enabling them to act as an environmental sink for N_2O, a major greenhouse gas.

In general, our catalogue greatly expands the known soil prokaryotic diversity and provides a valuable genomic resource for understanding the role of microbes in soil ecosystem processes. This information can be used to study the response of agricultural

soil in a changing environment and may be leveraged to design sustainable microbiome-based strategies to improve crop production and resilience.

Abstract ID: 837 / Poster Number: 152.16P

New synthesized two groups of heterocyclic hybrid derivatives of 2-quinolone with modified 2-thiohydantoin ring and rodanine ring. Study of their antimicrobial activity

Presenting author: Agnieszka Kania, PhD, University of the National Education Commission, Kraków, Poland

Co-authors: *Magdalena Greczek-Stachura, Anna Pawlak, Bartosz Różanowski, Waldemar Tejchman*

Based on literature, some quinolone, thiohydantoin and rodanine derivatives reveal antimicrobial activity. Expecting the synergistic effect, two series of heterocyclic hybrid compounds were synthesized: derivatives of 2-quinolone with modified 2-thiohydantoin ring and with modified rodanine ring. Taking into account that some of the obtained compounds are photosensitive and may generate reactive oxygen species, blue light irradiation was applied, expecting the enhancement of antimicrobial activity.

The aim of our study was to confirm the potential antimicrobial activity of new synthesized hybrid compounds.

The Knoevenagel synthesis was carried out, as a result of which new hybrid derivatives of 2-quinolone with 2-thiohydantoin as well as 2-quinolone with rodanine were obtained. Their chemical structures were confirmed by IR, NMR and ES MS. The compounds were characterized by UV-VIS and spectrofluorimetric methods. The measurements of singlet oxygen generation quantum yields were conducted. Blue light was applied to enhance the potential antibacterial effect. Antibacterial activity was tested on standard bacterial Gram-positive and Gram-negative strains using microdilution method or disk-diffusion method.

Moderate bacteriostatic activity is observed, the highest for derivatives containing a methyl group in the 2-quinolone system and simultaneously without an acetate group in the 2-thiohydantoin ring. Irradiation with blue light enhances the bacteriostatic effect. Gram-positive bacteria are more sensitive. The generation of reactive oxygen species causes an increase in bacteriostatic or bactericidal activity. These results were compared to hybrids of 2-quinolone ring joined with rodanine system.

Further chemical modifications are desired to improve chemical properties and enhance the biological activity of the examined compounds.

Abstract ID: 3606 / Poster Number: 360.15F

Niche breadth of microbial phage-host relationships along a salinity gradient from seawater to saturation

Presenting author: Jaime Alcorta, PhD, Universidad de Alicante, Alicante, Spain

Co-authors: *María Dolores Ramos-Barbero, Fernando Santos, Josefa Antón*

Background: Microbes are infected by viruses with interactions which can vary from specific to promiscuous. However, how these relationships change with environmental conditions remains elusive. Our aim was to characterize the distribution and composition of prokaryotic and viral contigs to determine variations in their predicted interactions along a salinity gradient from marine waters to salt saturation.

Methods: We sampled 6 sites across a salinity gradient in Bras del Port salterns (Santa Pola, Spain) from seawater (3.6 %) to saturation (39.0 %) and obtained one metagenome and virome per site. The assembled contigs were taxonomically assigned with refineM and reconstructed (metaWRAP) metagenome-assembled genomes with GTDB-tk. Viruses were characterized with geNomad and ANIcluster; and phage-host pairs were predicted with PHIST and WiSH.

Results and Discussion: A transition between the dominance of bacteria (mostly Pseudomonadota) in seawater (~45 % of the metagenomic reads) to the dominance of archaea in saturation (mostly Halobacteriales, ~60 % of the reads) was uncovered along the salinity gradient. A total of 170 dereplicated MAGs (95 % ANI) and ~50000 vOTUs (95 % ANI and 85 % aligned fraction) were obtained. Almost 4000 phage-host (vOTU-MAG) pairs were unveiled, and the number of samples in which the vOTUs were found was significantly higher than for their predicted hosts. The tendencies of the larger niche breadth of the viruses were mainly towards higher salinities than those in which their hosts were present. Altogether, this dataset revealed important trends in the strategies that are used by phages and hosts across environmental gradients

Abstract ID: 1587 / Poster Number: 351.15F

Non-flavonoid root exudates shape the plant 'cry-for-help' under polychlorinated biphenyl stress

Presenting author: Prof. Eleonora Rolli, Università degli Studi di Milano, Milano, Italy

Co-authors: *Elisa Ghitti, Lorenzo Vergani, Francesca Mapelli, Luigi Bazzana, Sara Borin*

Introduction: The holobiont dynamics in soils contaminated by recalcitrant xenobiotics are poorly understood and may rely on the plant "cry-for-help". Flavonoids are acknowledged as key factors under polychlorinated biphenyls (PCBs) stress, acting as inducers of the bacterial *bph* catabolic operon.

Objective: Decipher PCB-stress triggered root exudation pattern and role for degrading bacteria.

Method: Metataxonomic, metabolomic, qPCR analyses and in vitro assays were adopted.

Findings: A differential pattern of flavonoid exudation (WT, *tt4* null producer, *tt8* and *ttg* over-expressing lines) altered the structure of the rhizosphere microbiome in Arabidopsis plants grown in a PCB-historically contaminated soil, while the *bph* gene abundance was not affected. When exposed to flavonoid pure chemicals, the PCB-degrader *Paraburkholderia xenovorans* LB400 showed improved growth, proliferation, *bph* expression and rhizocompetence traits. Nevertheless, bacterial growth was promoted by the root exudates (REs) of mutant lines *tt4* and *tt8* under control conditions and reduced upon PCB-18 stress, showing no differences compared with the WT and *ttg*, indicating that unidentified plant metabolites could be involved. Moreover, strain LB400 promoted both plant growth and PCB resistance in all Arabidopsis lines, with similar bacterial colonization efficiency and root adhesion pattern, thus excluding flavonoid involvement in the process. A metabolomic approach revealed that under PCB stress plants over-exuded metabolites exploited as C and N sources for bacterial metabolism and reduced scopoletin exudation, due to its antimicrobial activity.

Conclusion: Under PCB stress, the plant 'cry-for-help' reshapes the root chemistry to provide more suitable growth conditions for degrading strains and flavonoids may play a minor role.

Abstract ID: 93 / Poster Number: 340.16P

Non-lactic fermented probiotic beverage enriched with microencapsulated Brazilian red propolis: microorganism viability, physicochemical characteristics, and sensory perception

Presenting author: Dirceu Melo, PhD, Federal University of Lavras, Lavras, Brazil

Co-authors: *Iara Ferreira, Disney Dias, Karina Magalhães-Guedes, Rosane Schwan*

This work aimed to develop a non-dairy functional beverage fermented with probiotic strains and fortified with Brazilian red propolis (microencapsulated and extracted). The non-dairy matrix consisted of oats (75 g), sunflower seeds (175 g), and almonds (75 g). It was fermented by a starter co-culture composed of Lactiplantibacillus plantarum CCMA 0743 and Debaryomyces hansenii CCMA 176. Scanning electron microscopy analysis was initially performed to verify the integrity of the microcapsules. The viability of the microorganisms after fermentation and storage, chemical composition (high performance liquid chromatography (HPLC) and gas chromatography coupled to mass spectrometry (GC-MS) analyses), rheology, antioxidant activity, and sensory profile of the beverages were determined. After fermentation and storage, the starter cultures were well adapted to the substrate, reducing the pH (6.50 to 4) and cell count above 7.0 log CFU/mL. Lactic acid was the main organic acid produced during fermentation and storage. In addition, 39 volatile compounds were detected by gas chromatography coupled to mass spectrometry (GC-MS), including acids, alcohols, aldehydes, alkanes, alkenes, esters, ethers, phenols, terpenes, and others. The addition of propolis extract increased the antioxidant and phenolic activity and the presence of volatile esters but reduced the beverage's acceptability. The addition of microencapsulated propolis was more associated with the presence of higher alcohols and had similar acceptance to the control beverage. The combination of a non-dairy substrate, a starter probiotic co-culture, and the addition of propolis culminated in a probiotic beverage with considerable functional attributes and potential for health benefits.

Abstract ID: 3424 / Poster Number: 339.17P

Non-Saccharomyces yeasts for ethanol production from sugarcane-based musts: stress resistance, growth and fermentation performances

Presenting author: Prof. Sandra Regina Ceccato-Antonini, Universidade Federal de São Carlos, Araras, Brazil

Co-authors: *Judieldo Morais Lima, José Machado Silva-Neto*

The main yeast used in the production of industrial bioethanol from sugarcane musts is the species *Saccharomyces cerevisiae*. However, non-*Saccharomyces* yeasts may present some advantages, such as greater adaptation to the fermentative environment and greater genetic diversity, resulting in better fermentation efficiency. Therefore, this work aims to evaluate the potential of non-*Saccharomyces* yeasts (*Wickerhamomyces anomalus*, *Meyerozyma guilliermondii* and *Scheffersomyces stipitis*) in sugarcane musts for ethanol production in comparison with industrial strains of *S. cerevisiae* (PE- 2 and CAT-1). Tolerance tests were carried out in microplates and spot assay, evaluating different stress situations regarding pH, ethanol, glucose and temperature. Growth tests were carried out in diluted sugar cane juice, molasses and mixture musts. Among *S. cerevisiae* yeasts, differences were identified in the tolerance profile to pH, temperature, glucose and ethanol stresses. Among the non-*Saccharomyces*, those that showed the greatest similarity with industrial yeasts in terms of stress tolerance were *W. anomalus* and *M. guilliermondii*, the first being more similar to PE-2 while the second more similar to CAT-1. The *M. guilliermondii* strain demonstrated potential for growth in high concentrations of ethanol and elevated temperatures, relevant characteristics industrial processes. The growth profiles in the sugarcane musts were very similar among the yeasts, showing adaptability to them for the yeast propagation stage in the production of first-generation ethanol. Fermentative tests with cell recycling and acid treatment are being conducted.

Abstract ID: 3130

Novel aerobic 1,2-DCA biodegrading consortia immobilized on biopolymeric scaffolds for enhanced bioremediation of contaminated groundwater

Presenting author: Laura Scirè Calabrisotto, PhD, University of Palermo, Palermo, Italy

Co-authors: *Elisa Maria Petta, Giovanna Carpani, Maria Clara Citarrella, Alberto Vassallo, Pietro Cusumano, Giorgio Schiera, Roberto Scaffaro, Marcello Tagliavia, Valentina Catania, Paola Quatrini*

Sustainable nature-based solutions are urgently needed to address environmental health challenges in line with the Sustainable Development Goals. 1,2-dichloroethane (1,2-DCA) is a recalcitrant, toxic and probably carcinogenic groundwater contaminant whose biodegradation is generally attributed to specialized anaerobic bacteria[1]. However aerobic hydrolytic dechlorination, mediated by the key enzyme DhlA, is also a potentially valid approach in bioremediation[1]. Biodegrading biofilms applied to bioremediation are a cost-effective strategy to enhance the clean-up of water contaminated by organic pollutants[2,3]. The aim of this work was to develop a bioremediation device based on novel aerobic 1,2-DCA-degrading consortia immobilized on biodegradable scaffolds to be exploited in bioaugmentation strategies.

The 3D biodegradable biopolymeric polylactic acid (PLA) scaffold, produced by electrospinning, was preliminarily assessed as the most suitable to host a microbial biofilm and absorb 1,2-DCA. Two novel 1,2-DCA degrading consortia (named A and B) were isolated from 1,2-DCA contaminated groundwater through enrichment cultures on mineral medium amended with 1,2-DCA as sole carbon source. The composition of the consortia was analyzed by Ion Torrent *16S rRNA* gene sequencing. The *dhlA* gene encoding for the hydrolytic dechlorination key enzyme was searched by PCR assay and its location was assessed by Whole Genome Sequencing. The 1,2-DCA degrading devices were obtained by immobilizing the consortia on the PLA scaffolds and the maintenance of their catabolic activity was tested after transferring them into a fresh 1,2-DCA amended medium. The scaffold colonization by the consortia was assessed by Scanning Electron Microscopy. 1,2-DCA removal by the devices before and after the transfer was monitored by Gas Chromatography-Mass Spectrometry (GC-MS).

The composition of the consortia was similar and included the known aerobic 1,2-DCA-degrading genus *Ancylobacter*, as well as other members with yet unclear role. *Ancylobacter* carried on its chromosome a *dhlA* gene identical to that of other known

aerobic 1,2-DCA-degraders. The consortium-scaffold systems (Fig. 1a, b) degraded up to 1000 ppm 1,2-DCA within seven days, and maintained the biodegrading ability after the transfer (Fig. 1c). In conclusion, these devices based on the novel highly efficient 1,2-DCA-degrading consortia immobilized on biopolymeric supports are suitable to be applied in enhanced bioremediation treatments, including permeable reactive barriers and bioreactors.

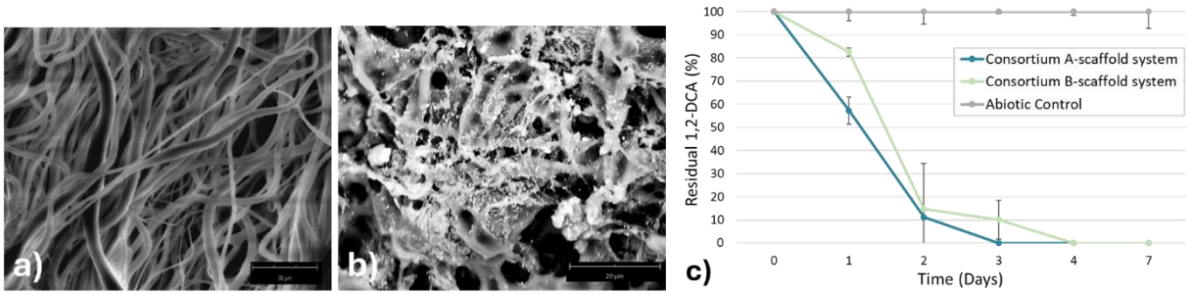


Figure 1: The PLA scaffold a) before and b) after colonization by the consortium A; c) 1,2-DCA (1000 ppm) degradation performed by the consortium-scaffold systems.

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Abstract ID: 2460 / Poster Number: 366.16P

Novel Biosurfactant produced by Bacillus rugosus HH2 Purification and Characterization

Presenting author: Prof. Young-Mog Kim, Pukyong national university, Busan, South Korea

Co-authors: *Min-Ung Kim, Geum-Jae Jeong, Kyung-Jin Cho*

Chemical-based surfactants have been used in various fields, such as coatings and cleaning industries. However, they pose environmental concerns due to their toxicity and resistance to degradation. Biodegradable biosurfactants offer a sustainable alternative, with various bacteria, including Bacillus species, known to naturally produce them. This study aimed to develop and characterize a biodegradable biosurfactant produced by Bacillus rugosus HH2, focusing on its structural properties, surface activity, and stability under varying environmental conditions. Biosurfactants from B. rugosus HH2 were purified using acid precipitation followed by solvent extraction. Structural characterization was performed using FTIR, UV-vis, and UPLC-Q-TOF/MS. Surface activity measurements, including surface tension and contact angle analysis, were used to determine the critical micelle concentration (CMC). Stability was evaluated through thermal degradation analysis (20-500°C) and emulsification index (EI) tests across various pH levels, NaCl concentrations, and temperatures. The purified biosurfactant exhibited structural characteristics consistent with surfactin homologs. The CMC was determined to be 0.1 mg/mL, demonstrating strong surface activity and wetting behavior. Desptie rapid thermal degradation occurred within the temperature range of 145°C to 330°C, the biosurfactant emulsion remained stable across a wide range of conditions, including temperatures (30-80°C), pH levels (4-10), and NaCl concentrations (3-18%). Based on these findings, the biosurfactant produced by B. rugosus HH2, a structural homolog of surfactin, exhibits structural stability and functional properties, making it advantageous for various industrial applications.

Abstract ID: 2788 / Poster Number: 146.17P

Novel findings on VOMG, a new drug candidate against Mycobacterium abscessus

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Introduction: *Mycobacterium abscessus* (*Mab*) is an emerging pathogen especially for cystic fibrosis (CF) individuals, the eradication of which represents a real challenge. VOMG was found to be a new drug candidate with bactericidal activity against *Mab* (MIC=0.25 µg/ml) and other pathogens.

VOMG inhibits cell division and has excellent *in vitro*, *in vivo* and ADME/Tox properties.

Objective: The aims of this work are: 1) to further investigate the mechanism of action of VOMG; to study its *ex vivo* and *in vivo* activity alone and in combination with amikacin (AMK).

Method: Selected cell division (CD) components are being mutated using a modified CRISPRi system to create conditional knock-down (KD) mutants whose characterization is in progress. We are testing the activity of VOMG alone and in combination with AMK in *Mab* infected mice, in Granuloma-LikeStructure (GLS) and macrophage monolayer assays.

Findings: Integrative vectors modified with the zeocin cassette were used to construct conditional KD mutants using the CRISPRi system. The expression of the target genes of KD mutants can be modulated by the addition of anhydrotetracycline (ATc). We have already obtained *ftsZ* KD mutants, while the other KD mutants of the CD genes are under development. The *in vivo* activity of VOMG alone or in combination with AMK is being tested in mouse models, in GLS and monolayer assays.

Conclusion: Our findings will pave the way for the development of potential new highly effective treatments against *Mab* infections, in particular for CF individuals.

Abstract ID: 3358 / Poster Number: 410.15S

Novel Insights into the Struggle Against Biofilm: The PsyOmp38 Protein from the Antarctic Marine Bacterium Psychrobacter sp. TAE2020

Presenting author: Ms. Diana Olimpo, Univerisity of Naples Federico II, Naples, Italy

Co-authors: *Caterina D'angelo, Marzia Calvanese, Maria Luisa Tutino, Ermenegilda Parrilli*

Background: Microbial biofilms pose widespread and persistent challenges across medical, environmental, and industrial settings. In healthcare, biofilms are major contributors to chronic, nosocomial, and medical device-associated infections, with *Staphylococcus epidermidis* being one of the most prevalent nosocomial pathogens. Moreover, biofilms' protective nature increases antibiotic resistance, contributing to the growing global crisis of antimicrobial resistance (AMR). An innovative strategy to address this challenge involves using antibiofilm molecules in combination with traditional antibiotics to enhance the efficacy against existing infections and prevent the biofilm formation of different pathogenic strains. Promising sources of antibiofilm molecules are Antarctic Marine Bacteria such as *Psychrobacter sp.* TAE2020, which produces an antibiofilm complex named Catasan. This polysaccharide-protein complex exhibits three distinct antibiofilm activities: it prevents adhesion, inhibits biofilm formation, and disperses mature biofilms of different *S. epidermidis* strains.

Objective: This study characterizes *Psy*Omp38, the Catasan protein, to evaluate its specific antibiofilm potential against *S. epidermidis* strains and to explore its capability to improve the effectiveness of traditional antibiotics on preformed biofilm.

Methods: The *Psy*Omp38 was recombinantly expressed in *Escherichia coli* BL21(DE3) and purified through anion exchange chromatography after recovery from inclusion bodies. The protein's anti-adhesive activity was assessed using a coating assay, while its antibiofilm activity was evaluated both during biofilm formation and on preformed biofilms using the crystal violet assay. Cell viability was analyzed through confocal laser scanning microscopy. Additionally, the synergistic potential of the protein with vancomycin was preliminarily assessed using a checkerboard assay.

Results and Conclusion: It has been demonstrated that the protein can interfere with all stages of *S. epidermidis* biofilm formation. It prevents adhesion and reduces biofilm formation at a concentration of 240nM, moreover, it disperses preformed biofilm at

2.4μM. Furthermore, confocal laser scanning microscopy confirmed that *Psy*Omp38 disrupts biofilm structures without affecting bacterial cell viability. Preliminary studies also revealed that *Psy*Omp38 increases the efficacy of vancomycin against *S. epidermidis* RP62A established biofilm, suggesting potential synergistic applications.

This work underscores the potential of *Psy*Omp38 as an innovative strategy to fight bacterial biofilms, opening new avenues for clinical and biotechnological applications.

Abstract ID: 2177 / Poster Number: 043.17P

Novel negative selectable marker loci for Trichoderma spp

Presenting author: Dr. Clara Baldin, University of Innsbruck, Innsbruck, Austria

Co-authors: *Anna Oberdanner, Julia Nagler, Laurenz Schark, Ulrike Schreiner, Susanne Zeilinger*

Introduction: The overuse of pesticides and antibiotics has contributed to the rise of antimicrobial resistance in various organisms. Beyond the implications for medically relevant pathogens, *Trichoderma* fungi highlight another aspect of this problem. Despite their importance in sustainable agriculture, *Trichoderma* species are challenging to genetically manipulate and characterize as, being isolates from heavily treated fields, they exhibit resistance to several antifungals commonly used in laboratories.

Objective: This study aims to develop new genetic tools to facilitate the manipulation of *Trichoderma* species by identifying and validating negative selectable markers. Focusing at first on *Trichoderma atroviride*, we proved the fungus susceptibility to 8-azaguanine and 5-fluoruracil, but not to 5-fluorocytosin.

Method: Using a CRISPR/Cas9 assisted protoplast transformation, we generated and characterized strains lacking the putative gene for the nucleobase analogues respective transporters. We also confirmed the absence of the gene homolog responsible for the internalization of 5-fluorocytosin in *T. atroviride*.

Findings: Deletion of the *azg1* gene resulted in strains resistant to 8-azaguanine but showing no other significant phenotypic differences from the wild type under various conditions. This locus was effectively used as a negative selectable marker with various insertions also in other *Trichoderma* species. Additionally, the insertion of *Penicillium chrysogenum's* **cytidine deaminase** gene into *T. atroviride* rendered it sensitive to 5-fluorocytosine.

Conclusion: The obtained findings pave the way for more efficient genetic engineering of *Trichoderma*, which could enhance its application in agriculture and biotechnology.

Abstract ID: 2605 / Poster Number: 443.15P

Novel Strategies for Monitoring the Expression of β-1,3-Glucan Production-Related Genes in S. cerevisiae Using AuNP-Based Biosensors

Presenting author: Mr. Rim Woo, Korea university, Seoul, South Korea

Co-authors: *Yoon-E Choi*

β-1,3-glucan, a physiologically active compound from sources such as yeast and microalgae, is widely acknowledged for its health benefits, including immune modulation and metabolic regulation. It is particularly notable for its potential in managing cancer and cardiovascular diseases, thereby offering clinical significance as a promising alternative therapeutic approach. The production of β-1,3-glucan is typically achieved through fermentation processes, which, although effective, labor-intensive, requiring continuous monitoring and detection throughout the entire process.

Therefore, this study aims to establish a novel monitoring strategy for β-1,3-glucan production, enabling the rapid identification of upregulated or downregulated gene expression in genetically engineered yeast strains.

Our focus was on *FKS1*, which encodes β-1,3-glucan synthase, as it is one of the key genes critical to β-1,3-glucan production. To this end, a gold nanoparticle (AuNP)-based biosensor was developed for the quantification of *FKS1* transcripts. This approach allows for the precise identification of optimal mutations in the *FKS1* transcript, thereby contributing to the enhancement of β-1,3-glucan production.

The AuNP-based biosensor developed in this study demonstrates significant potential for optimizing β-1,3-glucan production in the future by enabling real-time monitoring of the metabolites, facilitating genetic modifications, and streamlining efficient strain screening, which could lead to broad application across industrial biotechnology.

Abstract ID: 2892 / Poster Number: 387.17P

Novel Wet Lab Approach to Amplicon Concatenation to Enhance PacBio Sequencing Efficiency

Presenting author: Ms. Ece Silan, Quadram Institute, Norwich, United Kingdom; University of East Anglia, Norwich, United Kingdom

Co-authors: *Falk HILDEBRAND, Robert James, Darren Heavens*

Objective: Our protocol utilizes a one-pot polymer assembly reaction to concatenate full-length taxonomically relevant genes, such as 16S and ITS regions, into linear constructs that are barcode-ready for PacBio sequencing. The protocol is customizable, allowing the integration of any desired amplicons depending on study requirements. It also includes a clean-up step that removes background DNA and improperly assembled concatemers. The protocol was evaluated across multiple complex matrices, including mock microbial communities, faecal samples, and soil.

Methods: The novel method produced sequencing-ready libraries without requiring specialized library preparation kits. Key findings include: Superior taxonomic resolution compared to short-reads due to full-length amplicon sequencing, high reproducibility and robustness across diverse sample types, including stool and soil, significant cost reductions by eliminating the need for commercial library preparation kits and specialized equipment, flexibility in integrating user-defined amplicons to suit specific research objectives.

Findings: Golden Axe represents a cost-effective and versatile solution for microbial community profiling using long-read sequencing platforms. By overcoming limitations associated with short-read platforms and eliminating the need for expensive library preparation kits, our method enhances accessibility to high-resolution microbial diversity analyses across a wide range of studies and sample types.

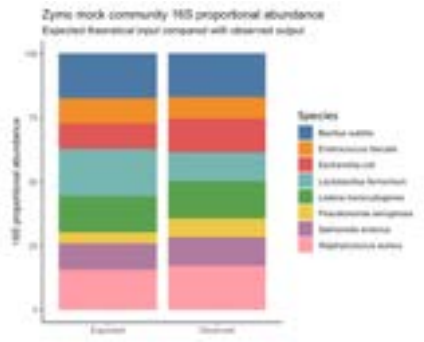


Figure 1: Relative abundance of Zymo Mock Community (Even) 16S observed results of Golden Axe protocol vs. Expected results.

Abstract ID: 2611 / Poster Number: 206.17P

Occurrence of multidrug-resistant Escherichia coli and ESBL-producing Klebsiella pneumoniae in an organic broiler farm depends on chick origin

Presenting author: Ms. Anna Maria Korves, German Federal Institute for Risk Assessment, Berlin, Germany

Co-authors: *Büşra Sardoğan, Kathrin Oelgeschläger, Frauke Umbach, Margret Krieger, Mirjam Grobbel, Bernd-Alois Tenhagen, Ulrike Binsker*

Multidrug-resistant (MDR) and ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* have been detected at different levels of the broiler production pyramid [1,2]. Recent studies suggest hatcheries as a reservoir for resistant strains and potential entry route into fattening flocks [3]. For organic broiler farms antibiotic use is strictly limited, but both the prevalence of resistant *E. coli* and *K. pneumoniae* as well as their source remain yet to be fully explored. This is of particular interest as the demand for sustainably and organically produced poultry meat is increasing.

The ProBioHuhn-project examines the prevalence, resistance determinants and relatedness of antimicrobial resistant bacteria in a longitudinal study on fourteen German organic meat chicken farms (including broilers, male layers and dual-purpose cockerels). Here we report on one of the broiler farms where MDR *E. coli* and ESBL-producing *K. pneumoniae* were frequently isolated.

Four consecutive flocks (Hubbard ISA-JA 757) were sampled at five time points each throughout the entire fattening period. Faecal samples were collected from the meconium of day-old-chicks on arrival on farm, in the rearing house at 10-14 days of life (DOL) and 26-34 DOL, in the outdoor house two weeks after first outdoor access and at the age of slaughter. Three *E. coli* and *K. pneumoniae* per sample were isolated on MacConkey agar with and without 1mg/l cefotaxime for selective screening of ESBL-producing strains. All isolates were tested for antimicrobial susceptibility via broth microdilution on EUVSEC3 microtiter plates. Whole Genome Sequencing was performed using both short-read sequencing (Illumina, NextSeq) and long-read sequencing (ONT, MinIon) for plasmid characterisation.

Resistant *E. coli* and ESBL-producing *K. pneumoniae* were frequently isolated from the first three flocks (66.7%, 30/45 resp. 78.6% 33/42) throughout the fattening period. All 33 *K. pneumoniae* belonged to a clonal ST307 strain with a conjugative InFII/IncFIB plasmid harbouring *blaCTX-M-15*, *blaTEM-1*, *aph(6)-Id*, *aph(3'')-Ib*, *sul2*, *dfrA14* and *aac(3)-IIa*

resistance genes. Compared to *K. pneumoniae*, the resistant *E. coli* population of flocks 1-3 showed an overall high genetic diversity with 15 different MLST. We found a distinct cluster of clonal MDR ST162 (15.6%, 7/45) with a conjugative IncFII/IncFIB plasmid harbouring *aadA1*, *aadA2*, *aac(3)-IVa*, *aph(4)-Ia*, *blaTEM-1B*, *cmlA1*, *sul3* and *tet(A)* resistance genes.

Flock 4 was purchased from a different organic hatchery than flocks 1-3. From this flock, only few resistances and no ESBL-producers were found. The majority of *E. coli* and *K. pneumoniae* isolates were fully susceptible (66.7%, 10/15 resp. 100%, 9/9).

The MDR strains therefore probably originated from the hatchery supplying chicks for flocks 1-3.

Our findings highlight the importance of chick origin for introducing antimicrobial resistant bacteria into organic broiler farms. Future studies targeting organic parent flocks and hatcheries are needed to evaluate the impact of possible transmission events and to develop targeted interventions if needed.

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Abstract ID: 4138

Oleaginous yeasts for biochemicals, food and a safe environment

Presenting author: Volkmar Passoth, Swedish University of Agricultural Sciences, Sweden

Yeast lipids produced from lignocellulose can serve as sustainable alternatives to vegetable oils (VO), which production is frequently accompanied with monocultures, land use changes, or rain forest clearings. We aim to understand the physiology of lipid production by oleaginous yeasts, optimise the production and establish novel applications of microbial lipid compounds (1). Those yeasts can even remove PFAS from water. Single cell oil (SCO) produced by oleaginous yeasts from lignocellulose could replace VO in aquaculture of arctic char (*Salvelinus alpinus*). Heavy metals and POP in yeast biomass cultivated on lignocellulose (wheat straw) hydrolysate were below the limits of the regulations defined by the EU. Moisture and ash contents, fatty acid profile and protein contents were similar between feed containing SCO and the control. No significant differences were found in the hepatic activity of 7-ethoxyresorufin-O-deethylase. Sensory analysis did not reveal any perceptible difference (3). System analysis showed that VO-replacement by SCO is sustainable: production of yeast oil required 10-38% less fossil energy input than the same amount of rapeseed oil (4). There was a high variability in formation of lipids and other interesting compounds such as carotenoids even between very closely related oleaginous yeast strains (1). When using advanced extraction methods, supercritical CO_2-extraction, torularhodin and torulene were demonstrated to be the major carotenoids in *Rhodotorula toruloides* (5). As a basis to understand the behaviour of the different strains and species, we sequenced a variety of genomes of *Rhodotorula* species. By a combination of long- and short read sequences, we could reconstruct most of the yeast chromosomes (6, 7). We identified 18-21 chromosomes and several circular structures. In general, a high diversity was found and some strains of the same species showed a phylogenetic distance to each other as to those of other species.

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Abstract ID: 3524 / Poster Number: 424.16F

Omics analyses of an isolated strain from an epoxy matrix as a sustainable method for thermoplastics recycling

Presenting author: Dr. Elias R. Olivera, Universidad de León, León, Spain

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Thermoset polymeric composites or epoxy composites are plastics frequently used in most industrial sectors (construction, aeronautic, automotive, renewable energy, sports). Their crosslinked microstructure of these polymers represents their main advantage, providing lightness and great mechanical strength; but also restrict their ability for reprocessing and recycling at end of life. Thus, landfilling or incineration are usually the only alternatives.

Biotechnological degradation or recycling of recalcitrant compounds is tackled by screening and/or adaptation of microorganisms capable of degrading the target compounds. Many factors influence the material degradation, such as: chemical composition, structure (pretreatment requirement), surface properties and appearance of the compound (historical microbial-material contact), microbial activity (need of Synthetic Biology approach), and degradative process (confined, microbial release). Development of solutions for microbial degradation of epoxy composites is the core of the EU-funded project ESTELLA (no. 101058371).

A *Pseudomonas putida* strain has been isolated from a commercial epoxy resin (RTM6) previously cured, grinded and metataxonomically analysed. An integral Omics approach has been developed around the strain, which includes genome sequencing, label free Proteomics and transcriptome validation, to evaluate its ability to survive and utilize the epoxy material as the carbon and energy sources. These studies are offering an intriguing view of the biodegradation of recalcitrant materials by a new microbial isolate of a well-known genus of material degraders.

Abstract ID: 1919 / Poster Number: 395.15P

Only Human: Uncovering Differential Human Core Taxa in the Primate Gut Microbiome

Presenting author: Mr. Samuel Piquer-Esteban, Universitat de València (UV), Valencia, Spain; FISABIO, Valencia, Spain

Co-authors: *Laia Domingo-Costa, Enrique Roig-Tormo, Vicente Arnau, Wladimiro Diaz-Villanueva, Andrés Moya*

Studying non-human primates (NHPs) offers a great opportunity to explore the evolution of symbiotic microbial relationships in humans and other primates [1,2]. Compared to previous primate studies using traditional metataxonomy [3,4], whole genome sequencing (WGS) data would allow for a more detailed examination of their core features providing better taxonomic resolution [5,6]. The present study aims to: investigate the prevalence of distinct core taxa across primates, assessing their potential as differentiating markers; identify distinctive core taxa between humans and other great apes; and search for differential core taxa among humans based on westernization. Using a novel curation workflow [7], we have assembled a large catalogue of public WGS primate gut metagenomes, consisting of 57 human adults and 18 NHP datasets, comprising more than six thousand primate individuals. After a thorough quality control process, taxonomic profiles were obtained using MetaPhlAn4.0.6. The aggregation of presence-absence data by host specie, country and lifestyle resulted in a total of 1200 local core species that allowed the separation of these groups based on prevalence data, showing clear effects in terms of phylogeny and lifestyle. We then prioritized taxa that were highly frequent core in humans, western humans, non-western humans and great apes. This resulted in a set of 120 core taxa whose differential coreness was evaluated by logistic regression, finding westernization and human-linked markers. Taken together, these results underscore the importance of phylogeny and westernization in the study of the primate gut microbiome, shedding light on what makes our microbiota human.

Abstract ID: 1375 / Poster Number: 443.16P

Open Industry Retraining Program for Infectious Disease Preparedness: Enhancing R&D Capabilities and Practical Competence

Presenting author: Prof. In Seop Kim, Department of Biological Sciences and Biotechnology, Hannam Univeristy, Daejeon, South Korea

Co-authors: *Yong Cheol Yoo*

Our research team is part of the "Specialized Workforce Training Program for Infectious Disease Research," funded by the Ministry of Science and ICT, Republic of Korea. This initiative aims to strengthen industrial competitiveness by enhancing the research and development (R&D) capabilities of professionals in diagnostics, vaccines, and therapeutics for emerging and re-emerging infectious diseases.

The program employs an open retraining model tailored to industry needs, allowing companies to propose necessary training topics and select courses freely. Based on extensive demand and satisfaction surveys, we developed a specialized curriculum in in vitro diagnostics, vaccines, and therapeutics. Designed for working professionals, the program is delivered online over four months, offering participants flexibility in accessing educational content.

To ensure the program's continuous improvement, we conduct satisfaction surveys to evaluate the training experience, identify strengths and weaknesses, and gather input for future curriculum development. Preliminary results show positive feedback on the program’s relevance and flexibility, with participants reporting improved practical competencies and readiness to address infectious disease challenges.

This poster will present the design, implementation, and outcomes of our training program, demonstrating its effectiveness in bridging industry needs with specialized education and its contribution to infectious disease preparedness.

Abstract ID: 3073 / Poster Number: 166.17P

Optimised methods for the targeted surveillance of extended-spectrum beta-lactamase producing Escherichia coli in human stool incorporating limited-diversity metagenomics

Presenting author: Dr. Sarah Gallichan, University of St Andrews, St Andrews, United Kingdom; Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Co-authors: *Sally Forrest, Esther Picton-Barlow, Claudia McKeown, Maria Moore, Eva Heinz, Nicholas Feasey, Joseph Lewis, Fabrice Graf*

Understanding transmission pathways of extended-spectrum beta-lactamase producing *Escherichia coli* (ESBL-EC) is essential for targeted prevention. To detect putative transmission using genomics, all ESBL-EC strains present in each niche need to be characterised at single nucleotide variant (SNV) level. Plate-sweep metagenomics provides a possible cost-effective method to achieve this at scale.

We tested available culture, DNA extraction, and sequence analysis methods to optimise plate sweep-metagenomic SNV characterisation (using the mSWEEP/mGEMS pipeline) of ESBL-EC in human stool samples (n = 6) and compared SNV calls to multiple colony picks (4-7 colonies) strategy.

Using 4-hour pre-enrichment in Buffered Peptone Water, plating on cefotaxime supplemented MacConkey agar and extracting DNA with the Lucigen MasterPure DNA Purification kit, we improved the recovery of ESBL-EC from 3 x 10^{-2} CFU/ mL to 1 x 10^{-10} CFU/mL from human stool and produced high-quality DNA for whole genome sequencing. Stool samples contained one to two ESBL-EC sequence types, with perfect agreement between metagenomic and multiple colony-pick methods. SNV calling of plate sweep metagenomics was directly comparable to the single colony picks, identifying on average 16 (IQR 0 - 88) extra SNVs compared to a reference genome.

The plate sweep approach offers a novel, cost-effective, albeit more computationally intensive, alternative to multiple single colony picks for describing within-host ESBL-EC diversity, facilitating improved inference of transmission events.

Abstract ID: 2027 / Poster Number: 119.16P

Optimization of Actinomycin D Structure for Antifungal Activity: Strategies to Improve Combat Against Fungal Infections

Presenting author: Dr. Sanja Škaro Bogojević, INSTITUTE OF MOLECULAR GENETICS AND GENETIC ENGINEERING, University of Belgrade, Belgrade, Serbia

Co-authors: *Veselin Maslak, Miloš Petković, Jelena Milovanović, Jasmina Nikodinović-Runić*

Actinomycin D (ActD), initially discovered as an antibiotic with anticancer potential, was discontinued as a chemotherapeutic agent due to severe side effects, including bone marrow and liver toxicity. However, ActD demonstrates significant antimicrobial activity, with minimum inhibitory concentrations (MICs) of 0.15 µg/mL against *Staphylococcus* and *Bacillus* species, as well as mild antifungal effects. These properties are especially important in the context of rising invasive *Candida* infections, which affect over 200,000 patients annually and face growing concerns over drug resistance and limited treatment options.

This study explores strategies to optimize the structure of ActD for enhanced antifungal activity against *Candida* species while minimizing its cytotoxicity. ActD's peptide component and chromophore were synthesized separately and biological screening, including MIC determination and cytotoxicity tests, was performed. Additionally, organic fragments with antimicrobial properties—curcumin, carvacrol, ferulic acid, and cinnamic acid—were tested for their ability to improve ActD's antifungal efficacy. We also analyzed the peptide length and amino acid composition of ActD to identify structural modifications that could enhance its activity.

Our findings show that the peptide component alone is biologically inactive, while the chromophore exhibits antifungal activity against *Candida parapsilosis* at 250 µg/mL. Notably, all fragments tested showed reduced cytotoxic effects on MRC-5 fibroblasts. These preliminary results highlight the potential of the ActD chromophore as an optimized antifungal agent, offering a promising strategy for co*mbating Candida infections and mixed Candida-Staphylococcus* infections, addressing a critical unmet medical need.

Abstract ID: 2680 / Poster Number: 315.16F

Optimization of enzymatic saccharification for steam exploded softwood by an enzyme cocktail

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Co-authors: *IKSU HA, JEONGHEE YUN*

Lignocellulosic biomass, such as *Pinus* sp., is a valuable renewable resource for biofuel production due to its abundance and sustainability. *Pinus* sp. offers significant potential for sustainable energy applications. However, its recalcitrant structure poses challenges for efficient conversion into fermentable sugars. This study aimed to optimize efficient enzymatic saccharification conditions for steam-exploded *Pinus* sp., with and without bark, to maximize saccharification rate. Initial experiments evaluated enzyme loadings (10, 20, 30, 40, 50 FPU) and substrate concentrations (9, 11, 13, 15%) individually, selecting three levels that showed significant differences in saccharification efficiency. Using Box-Behnken Design (BBD) statistical analysis, the effects of these parameters and the blending ratios of the enzyme derived from the *Trichoderma* sp. KMF006 cellulase and a commercial enzyme (Cellic CTec3) were evaluated within the mixing ratios ranging from 10:90 to 50:50 (*Trichoderma* sp. KMF006 cellulase:commercial enzyme). The enzymatic hydrolysis rate was observed to range from approximately 50% to 90%, with the highest rate of around 90% achieved under optimized conditions. Additionally, the saccharification yield of *Pinus* sp. with bark was higher than that of bark-free *Pinus* sp., highlighting its greater potential as a resource-efficient feedstock. The results further indicated that the maximum saccharification rate was not achieved simply by increasing enzyme loading or substrate concentration, but rather through a balanced combination of these parameters, including an optimized enzyme blending ratio. By establishing optimized conditions, this study provides a foundation for scaling up saccharification processes, serving as potential starting point for the production of biofuels or bioplastics.

Abstract ID: 2538 / Poster Number: 316.17P

Optimization of Tuna Meat Discoloration Prevention Using Response Surface Methodology with a Combination of Lactic Acid Bacteria Supernatant and Eisenia bicyclis Extract

Presenting author: Mr. Kyung-Jin Cho, PuKyong National University, Busan, South Korea

Co-authors: *Geum-Jae Jeong, Min-Ung Kim, Young-Mog Kim*

Tuna is widely consumed in many countries for its health benefits, such as its high protein content, polyunsaturated fatty acids, and a balanced composition of essential amino acids. The bright red color of raw tuna meat greatly affects consumer preference. However, due to oxidation during distribution, the red color of tuna meat turns into brown, leading to economic loss. Therefore, this study aimed to optimize the treatment conditions using lactic acid bacterial supernatant (LABS) and Eisenia bicyclis extract (EE), which have high antioxidant activity to prevent tuna meat discoloration. The Box-Behnken Design (BBD) of response surface methodology (RSM) was used to optimize experimental conditions and analyze the effects of the variables. The optimized model exhibited R2 values exceeding 0.950 for all dependent variables. The optimal conditions were an EE concentration of 0.02775 mg/mL, a ratio of LABS to EE of 1.12:1, and a tuna meat immersion time of 15.11 s. Consequently, the difference between the actual and predicted values was statistically significant ($p < 0.05$), and the optimized model using RSM was statistically valid. This study suggests that the combinational treatment with LABS and EE effectively prevents the oxidation and discoloration of red tuna meat, preserving its quality. Additionally, this study provides a promising approach to reduce economic losses in the tuna industry and enhance consumer preference.

Abstract ID: 863 / Poster Number: 278.17P

Optimizing Fungal Microencapsulation for Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs)

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Co-authors: *Claudia K. Gunsch*

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals with two or more fused benzene rings. Their aromatic structure makes them highly persistent, mutagenic, and carcinogenic. Mycoremediation, using fungi to degrade PAHs, offers a cost-effective and minimally invasive solution. However, bioaugmentation of PAH-degrading fungi from the lab to the field is challenging, as these strains often struggle to establish in new environments. To address this challenge, fungal monocultures were encapsulated in sodium alginate to enhance the viability and establishment of exogenous strains in situ. The growth of both *Trichoderma* (filamentous) and *Aureobasidium* (yeast-like) strains were compared in encapsulated versus free-living form to assess whether encapsulation promotes growth. Preliminary results under high-nutrient conditions show that encapsulation does not hinder fungal growth. Ongoing studies focus on how nutrient levels, initial fungal concentrations, and alginate density affect growth between treatments. In a separate set of experiments, fungal cultures have been grown in the presence of three model PAHs—naphthalene, phenanthrene, and fluoranthene—to determine if encapsulated cultures are more effective at metabolizing PAHs. Gas chromatography-mass spectrometry (GC-MS) is being used to measure PAH concentrations after 8 weeks and evaluate degradation. These PAHs, spanning low to high molecular weights, will provide insights into the effectiveness of encapsulated fungi in degrading PAHs. Overall, these findings suggest that encapsulation enhances fungal growth and this work serves as a baseline for future studies investigating the use of capsules for PAHs mycoremediation. Ultimately, this work aims to determine the viability of encapsulation for in-situ bioremediation.

Abstract ID: 1264 / Poster Number: 153.15P

Optimizing Osmoprotectant Production in Antarctic Psychro-Halotolerant Bacteria for Salinity Stress Mitigation

Presenting author: Ms. Aleksandra Goszcz, University of Warsaw, Warsaw, Poland

Co-authors: *Marcin Musiałowski, Robert Stasiuk, Aleksandra Junkier, Karolina Furtak, Joanna Wójtowicz, Klaudia Dębiec-Andrzejewska*

Introduction: Soil salinization is a critical threat to global agriculture, reducing productivity and yields. Osmoprotectants protect bacteria from osmotic stress, ensuring survival and functionality in saline environments. Antarctic psychro-halotolerant bacteria (PHTB), naturally adapted to extreme conditions, efficiently produce these compounds. Optimizing their production and application could enhance soil microbial activity, nutrient availability, and plant resilience, providing a sustainable strategy for combating salinity.

Objective: To establish an efficient methodology for optimizing osmoprotectants production in PHTB by refining culture conditions, enhancing stress tolerance through adaptive evolution, and assessing their application in mitigating salinity-induced plant stress.

Methods: Osmoprotectants production was optimized using Response Surface Methodology (RSM) to identify ideal culture conditions, while BIOLOG profiling revealed key substrates for biosynthesis. Adaptive Laboratory Evolution (ALE) improved bacterial stress tolerance and osmoprotectants yields. Plant experiments evaluated the effects of these compounds on growth and resilience under salinity stress.

Findings: RSM optimization emphasized the significance of medium composition and environmental conditions in maximizing osmoprotectants production. BIOLOG analysis identified sugars and carboxylic acids as critical substrates, while ALE enhanced osmoprotectants yields, particularly betaine, in both Gram-positive and Gram-negative strains. Plant trials demonstrated that osmoprotectants effectively mitigated salinity stress, with elevated cell division rates in salt-stressed plants, improving growth and resilience.

Conclusion: This study outlines a robust framework for optimizing osmoprotectants production in PHTB and applying them to combat salinity stress. These advancements provide a scalable, sustainable solution to improve soil quality and support agricultural productivity under saline conditions.

Abstract ID: 1671 / Poster Number: 130.16P

Optimizing Respiratory Microbiota Analysis from clinical samples: primer, DNA extraction,

and sequencing choices

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Co-authors: *Patricia Fernández de Córdoba Ansón, Silvia Serrano, Cruz Soriano, Raúl De Pablo, Teresa Coque,*

Introduction: Respiratory tract microbiota is vital for respiratory health, immune regulation, and defense. However, analyzing this microbiota is challenging due to its high human DNA content and small sample volumes. Optimized extraction, amplification, and sequencing methods are crucial for accurate microbiota characterization and for improving taxonomic resolution.

Objectives: (1) To select the optimal kit-based method for extracting bacterial DNA from respiratory clinical samples, **(2)** to determine the best primer option for analyzing the hypervariable region of the 16S rRNA gene, and **(3)** to compare the resolution of Ilumina and Nanopore sequencing technologies for the analysis of respiratory microbiota minimizing human DNA interference while reaching the maximum diversity resolution **(Figure 1).**

Material and Methods: We evaluated **(1)** two DNA extraction kits (Molysis and Qiagen), **(2)** two primers targeting 16S rRNA hypervariable regions (V1-V2 and V3-V4), **(3)** two sequencing technologies (Illumina and nanopore MinION) from broncho-aspirates (BAS) from critical patients. Diversity metrics Shannon index, Chao 1, Faith, and Bray-Curtis dissimilarity were used to assess alpha and beta diversity. Additionally, we statistically compared the relative abundance of human DNA and the most abundant taxa between conditions. Most analyses were based on R software.

Results: (1) Both DNA extraction methods did not significantly differ in diversity and composition, although the Molysis kit identified more exclusive taxa and significantly reduced human DNA compared to Qiagen. **(2)** Primers showed no differences in alpha and diversity but differed in taxa composition. V1-V2 primers detected more exclusive taxa, achieved species-level resolution, compared to V3-V4 and amplified less human DNA. **(3)** Nanopore sequencing identified more exclusive taxa compared to Illumina, with significantly less amplification of human DNA.

Conclusion: The Molysis kit and V1-V2 primers are the best choice for 16S rRNA Illumina analysis, improving detection of minority taxa and offering species-level resolution. Nanopore sequencing is more effective at detecting a broader range of bacteria while minimizing interference from human DNA.

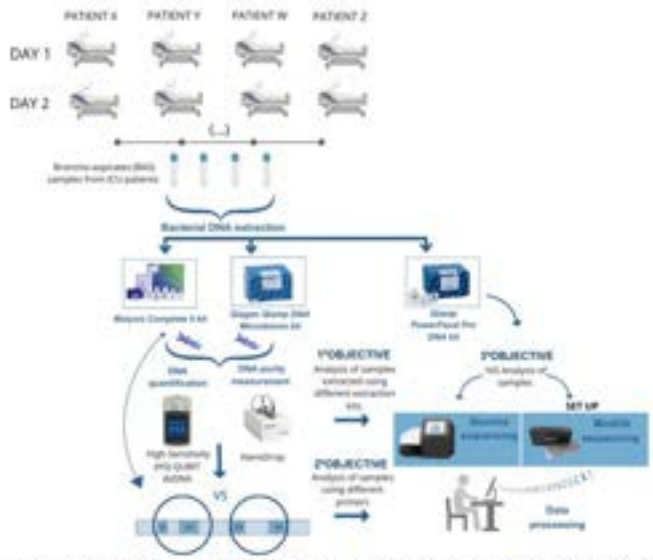


Figure 1: The study workflow details, molecular methods from sample collection to data analysis.

Abstract ID: 352 / Poster Number: 224.17P

Optimizing Spray Drying Parameters for Microencapsulation of Non-Saccharomyces Yeasts: Viability, Yield, and Characterization

Presenting author: Pâmela Mynsen Machado Martins, PhD, Federal University of Lavras, Lavras, Brazil

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Yeasts have many biotechnological applications. However, liquid storage is unfeasible due to the loss of cell viability, genetic stability, and contamination. Thus, this study aimed to optimize the spray drying conditions for the microencapsulation of three yeast strains: *Pichia kluyveri* CCMA0237, *Wickerhamomyces anomalus* CCMA1640, and *Hanseniaspora uvarum* CCMA0236. Whey powder (WP) was used as wall material. The wall material concentration (%) and the drying air inlet temperature (°C) were investigated using the Central Composite Rotational Design (CCRD). The microcapsules cell viability, drying yield, and water activity (aw) were selected as response variables. Microcapsules characterization included analysis of water solubility, hygroscopicity, moisture content, morphology, and particle diameter. The operating region with the most promising result for *W. anomalus* was 85 °C and 24.8%. For *P. kluyveri* was 88 °C and 18.1% and for *H. uvarum* it was 90 °C and 30%. Model validation was performed using these parameters, and the experimental results were similar to the model's. The cell viability of *W. anomalus, P. kluyveri*, and *H. uvarum* reached 94.08, 75.68, and 90.85%, respectively. The drying yield was 71.17, 71.63, and 65.36%, and the a_w was 0.29, 0.29, and 0.30, respectively. The moisture content ranged from 5.60 to 7.78%, hygroscopicity from 17.03 to 23.76 g/100g, water solubility from 87.33 to 89.33%, and particle size from 6.44 to 8.60 µm. The micrographs showed smooth and spherical microcapsules of different sizes. Given these observations, it can be concluded that *Pichia kluyveri* showed more sensitivity to the spray drying process.

Keywords: Whey powder, Pichia kluyveri, Wickerhamomyces anomalus, Hanseniaspora uvarum

Abstract ID: 955

Oral Microbiota as a Non-Invasive Diagnostic Tool for Early Detection of Lung Cancer: A Machine Learning Approach

Presenting author: Prof. Aycan Gundogdu, Erciyes University, Kayseri, Turkey; Enbiosis Biotechnology, London, United Kingdom

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Lung cancer remains a leading cause of cancer-related mortality globally, primarily due to its late-stage diagnosis. Early and non-invasive diagnostic approaches are critical for improving patient outcomes. Recent advancements suggest that the oral microbiome holds significant promise as a biomarker for the early detection of lung cancer, offering the additional capability of distinguishing lung cancer from other lung diseases and oral cancers. The use of easily accessible oral samples combined with advanced sequencing technologies presents a practical, non-invasive diagnostic tool that enhances accuracy and facilitates timely interventions.

This study aimed to evaluate the potential of oral microbiota-based diagnostics for lung cancer and its ability to differentiate lung cancer from other lung diseases. This prospective study included 55 patients diagnosed with lung cancer, 55 patients diagnosed with other lung or respiratory diseases, and 55 age- and gender-matched healthy controls who were recruited at a Chest Diseases Department. Oral swab samples were collected at the time of admission and processed using 16S rRNA amplicon sequencing.

Taxonomic profiles of the samples were generated using the QIIME2 pipeline, and the profiles were classified using the XGBoost machine learning algorithm. The alpha diversity of lung cancer patients was found to be significantly different ($p=0.003$), while the beta diversities for cancer and other groups also showed significant differences ($p<0.001$). Cross-validated experiments using a 10-fold validation approach demonstrated that lung cancer cases could be classified from the remaining samples with high accuracy (ROC AUC: 0.87 ± 0.03; Cliff's Delta effect size: 0.74). Furthermore, the classification algorithm effectively distinguished lung cancer from other lung diseases at admission with an ROC AUC of 0.84 ± 0.04 and a Cliff's Delta effect size of 0.66.

Taxa such as *Bacteroides*, *Faecalibacterium*, *Ruminococcus*, and *Parabacteroides* were significantly more abundant in lung cancer cases, highlighting their potential as diagnostic biomarkers for lung cancer.

This study underscores the potential of oral microbiota-based diagnostics as a non-invasive, early detection tool for lung cancer. The analysis achieved high sensitivity while maintaining low false positives, demonstrating strong pre-diagnosis utility and the ability to distinguish lung cancer from other lung diseases, paving the way for enhanced diagnostic accuracy and timely interventions.

Abstract ID: 172

Order and frequency shape multiple stressor effects on freshwater benthic microbial community function

Presenting author: Ms. Rose Fuggle, The University of Sydney, Sydney, Australia

Co-authors: *Mariana Mayer-Pinto, Miguel Matias, Ezequiel Marzinelli*

Introduction: In freshwater ecosystems benthic microbial communities underpin critical functions (e.g., decomposition and primary productivity) threatened by stressors such as salinisation and nutrient enrichment, both of which are intensifying due to climate change (Moyano Salcedo et al., 2024). These stressors often occur together, sometimes simultaneously or in alternating sequences (e.g. in extreme weather events) (Reid et al., 2019). However, the influence of temporal dynamics, particularly order, on stressor interactions remains poorly understood (Jackson et al., 2021).

Objectives: We aimed test the hypotheses (i) repeated exposures would have stronger effects than single exposures, (ii) stressor interactions would differ depending on the order of application, and (iii) treatment effects would weaken over time, with salinity effects (a press stressor) persisting longer than nutrient effects (a pulse stressor).

Methods: A manipulative experiment was conducted in 1000-L semi-natural freshwater ponds established over a decade ago. Biofilms were grown on ceramic tiles for 30 days before being transplanted into treatment ponds and subjected to elevated salinity and/or nutrient enrichment individually, repeatedly, or in combination in different sequences. Sampling at 1, 21, and 81 days post-treatment assessed physiological profiling of metabolic rates across 31 carbon sources, respiration rates, primary productivity, photosynthetic efficiency, chlorophyll a concentrations, and microbial community structure via 16S rRNA sequencing.

Findings: Different order of stressor exposure resulted in functional effects in opposing directions. When nutrient enrichment preceded salinity, gross primary productivity was halved, and carbon metabolic rates increased by 50% compared to treatments applied in reverse order.

Conclusion: Stressor order can be critical to the outcomes of multiple stressor interactions. Key freshwater microbial functions, including productivity and carbon cycling, may fluctuate more dramatically as stressors increasingly interact under global change.

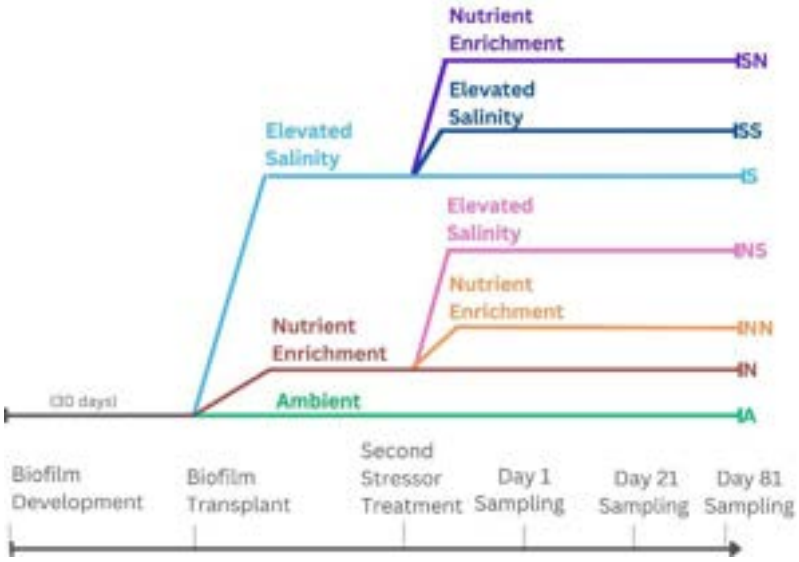


Figure 1: Experimental Design.

Abstract ID: 2868 / Poster Number: 149.17P

Outbreak of blaVIM Harboring Pathogens in an Italian Tertiary Hospital: Genetic Insights into Resistance and Transmission

Presenting author: Dr. Francesca Saluzzo, IRCCS San Raffaele Scientific Institute, Milan, Italy

Co-authors: *Federico Di Marco, Virginia Batignani, Kiarash Moghaddasi, Franca Averara, Silvia Bracco, Gabriele Del Castillo, Claudio Farina, Daniela Maria Cirillo*

Introduction: Between December 2021 and December 2022, 29 *bla$_{VIM}$* harbouring bacteria belonging to different species were consecutively isolated from 26 inpatients. This represented the first isolation of *bla$_{VIM}$* harbouring bacteria within the affected hospital, raising concerns about a potential outbreak.

Objective: To analyze the outbreak and investigate the genetic mechanisms facilitating resistance and transmission of *bla$_{VIM}$* harbouring bacteria.

Methods: Classical epidemiological investigation, including environmental sampling, was combined with molecular analyses. Short and long reads sequencing were performed with the Illumina MiniSeq platform and the MinION Mk1B platform respectively. Resistome analysis was performed with AMRfinderPlus v3.11.11. Virulence and plasmid were analysed using the VFDB and the PlasmidFinder database respectively. The integron analysis was conducted using Bakta v1.4.0 and Integron Finder v2.0.2.

Findings: Forty four *bla$_{VIM}$* harbouring isolates, including Enterobacterales and *Pseudomonadaceae*, were identified from patients (29) and environmental samples (15). The strains exhibited diverse resistomes and virulomes, with high mortality rates among infected patients. Plasmid analysis showed a correlation between replicon types and bacterial species for *Escherichia coli* and *Enterobacter hormaechei,* regardless of sample type. Whereas assembly analysis did not support an interspecies plasmid transmission, the genomic comparison revealed the presence of a shared class 1 integron, in both environmental and clinical strains, carrying *bla*$_{VIM-1}$, *IntI1* and *qacEΔ/sul1*. Only minor differences were observed in the composition of the related resistance gene cassette.

Conclusions: These findings emphasize the need for comprehensive infection control measures, including genomic-based surveillance, to investigate the sources of resistance spreading and persistence in the healthcare setting.

Abstract ID: 3304

Outreach with Tardigrades and 3D Printing for Primary, Secondary, and High School Students and Future Educators Aspiring to Teach

Presenting author: Prof. Masahiro Ito, Toyo University, Asaka-city, Japan

Co-authors: *Issei Suzuki, Ken-ichi Goto*

This outreach initiative aimed to spark scientific curiosity and engagement among elementary to university students by using innovative tools such as smartphone microscopes and 3D printers in hands-on science activities. Workshops included observing tardigrades, creating 3D-printed models, and monitoring tardigrade revival from desiccation. Students collected moss samples, extracted tardigrades, and utilized smartphone microscopes for detailed observations.

Surveys conducted before and after the activities assessed behavioural changes, particularly among elementary school students in grades 3 to 6. Over 90% of participants expressed satisfaction with the activities, with positive responses increasing by 10 percentage points compared to pre-event surveys. Feedback highlighted differences in comprehension and experimental skills between younger and older students, emphasizing the need for tailored support for younger participants. Across all groups, increased interest and motivation in science were evident, with schools adopting the tools introduced and university students showing greater interest in science education careers.

Ongoing analysis focuses on behavioural changes among middle to university students who participated in similar experiments over the past three years. A new activity featuring hands-on use of smartphone microscopes and tablets for an instant tardigrade observation event is planned.

The initiative demonstrated the power of integrating innovative tools and performance-based activities into science outreach, fostering curiosity, engagement, and positive behavioural changes. Tailored approaches for different age groups and continuous evaluation ensure inclusiveness and effectiveness, showcasing this model's potential to enhance science education and create lasting impacts.



Figure 1:(a) Three tardigrades observed under a smartphone microscope (indicated by arrows).

(b) Actual observation video captured using a smartphone microscope. https://www.youtube.com/watch?v=NO8jM6M-snU

(c) A photo showcasing miniature tardigrade models created using a 3D printer arranged to form text. After the tardigrade observation event, students are given their choice of tardigrade models in their favourite colours as a memorable keepsake from the event.

Abstract ID: 3100 / Poster Number: 335.16P

Overcoming Antagonistic Microbial Dynamics for the Growth Promotion of Biotechnologically Relevant Fungi

Presenting author: Mrs. Marie Bonduelle, Institut National de la Recherche Scientifique, Laval, Canada

Co-authors: *Audrey-Anne Durand, Simon Barnabé, Philippe Constant*

Background: Bacteria and fungi coexist in many ecosystems. Their interactions are essential for ecological processes and play a key role in environmental biotechnology industry, particularly organic waste recycling biotechnologies. One promising development is the use of mycelium-based composite materials, which harness the growth of Basidiomycota fungi to convert lignocellulosic biomass into biodegradable products. While current methodologies are suboptimal for achieving sufficient yields, the potential to optimize and stabilize bioprocess performance through the integration of microbial consortia has often been overlooked. This study addresses that knowledge gap by exploring how interactions between the biotechnologically relevant basidiomycete *Pleurotus ostreatus* and microbial communities influence fungal growth in lignocellulosic substrates.

Objectives: This study aims to investigate whether enrichment cultures displaying contrasting redundant functions can enhance fungal growth of *P. ostreatus* in lignocellulosic substrate.

Methods: *P. ostreatus* was cultivated on a lignocellulose substrate, both alone and in co-inoculation with microbial enrichment cultures exhibiting a gradient of functional redundancies for lignocellulose degradation, with or without complementary autotrophic metabolism. The microbial diversity of enrichment cultures and substrates colonized by *P. ostreatus* was analyzed through PCR amplicon sequencing, while the growth of P. ostreatus was assessed by quantitative image analysis.

Results: Overall, co-inoculation of lignocellulosic substrate with microbial enrichment cultures reduced the growth of *P. ostreatus*. Substrate degradation functions were not primary factors driving beneficial microbial communities for *P. ostreatus*, while additional functions beyond carbon metabolism are likely to promote beneficial interactions. Microbial community analyses and *in vitro* assays led to the identification of a single phylotype affiliated to *Brevundimonas* spp. which displayed neutral interaction with *P. ostreatus*.

Conclusion: These results suggest that specific bacterial species compatible with *P. ostreatus* must be selected to elaborate a compatible synthetic community. This bottom-up approach drives our current research, which aims to assemble compatible bacterial and fungal communities while identifying ecological traits (phenotypes, phylogeny, genetics) that promote fungal growth. Ultimately, this knowledge is expected to optimize fungal biomass production and lignocellulosic feedstock pretreatments for bioproducts and bioenergy production.

Abstract ID: 1586 / Poster Number: 233.15P

Overexpression of LexA in the filamentous cyanobacterium Anabaena sp. PCC 7120 explicates its regulatory role in optimizing photosynthetic responses under abiotic stress

Presenting author: Dr. Akanksha Srivastava, Banaras Hindu University, Varanasi, India

Co-authors: *Arvind Kumar, Subhankar Biswas, Vaibhav Srivastava, Hema Rajaram, Yogesh Mishra*

Abiotic stresses are known to disrupt the redox balance of photosynthetic electron transport chain in all photosynthetic organisms. Cyanobacteria have developed several photosynthetic responses to sustain their photosynthesis under stressful conditions, but the regulatory network underlying these responses is not well understood. Here, we attempted to answer this question by using cadmium (Cd) as an abiotic stressor, since it disrupts photosynthetic redox balance and a LexA-overexpressing recombinant *Anabaena* sp. PCC 7120 strain (An*lexA*$^{+}$), since LexA has been identified as a global regulator of stress responses in *Anabaena* 7120. Measurement of the photosynthetic responses of An*lexA*$^{+}$ and vector control AnpAM, under unstressed and Cd-stressed conditions using transmission electron microscopy, chlorophyll *a* fluorescence, and blue native (BN)-PAGE, indicated that several photosynthetic responses, including PSII photoinhibition, heat dissipation, PSI protection, and NDH-mediated cyclic electron transport were reduced in An*lexA*$^{+}$ under unstressed conditions. However when exposed to Cd stress, An*lexA*$^{+}$ elicited these responses similar to AnpAM, albeit at a slower rate, indicting a regulatory role for LexA in optimizing the photosynthetic responses of *Anabaena* 7120 under stressful conditions. This hypothesis was further supported by the fact that transcript, electromobility shift assay (EMSA), and bioinformatics investigations showed that LexA regulates more than 70 of the 90 photosynthetic component genes. In a nutshell, this work identifies LexA as one of the regulators involved in the optimization of photosynthetic responses of the model filamentous cyanobacterium *Anabaena* 7120 under normal growth and during abiotic stress through transcriptional regulation of some of the photosynthetic genes.

Abstract ID: 1783 / Poster Number: 237.15P

Oversexed but not out of control: transcriptome modulation and silencing of a rhizobial conjugative megaplasmid is dependent on the genomic background

Presenting author: Ms. Cristina De la Cruz Hernandez, Universidad Nacional Autónoma de México, Centro de Ciencias Genómicas, Cuernavaca, Mexico

Co-authors: *Laura Cervantes de la Luz, David Rene Romero Camarena*

Nitrogen-fixing symbioses between rhizobial bacteria and leguminous plants are of key importance for ecology and agriculture. Genes required for nodulation and nitrogen fixation are located on the bacterial symbiont, usually on mobile elements such as conjugative megaplasmids. In Sinorhizobium fredii GR64 (one of the symbionts of the common bean) the megaplasmid pSfr64a displays a high conjugative transfer rate (1x10-1) to a variety of rhizobial hosts. Interestingly, when pSfr64a is transferred to Rhizobium etli (another bean symbiont) conjugative transfer from this host became virtually undetectable; in contrast, transfer of pSfr64a from Agrobacterium tumefaciens is maintained at high rates. To address the causes of this host-specific reduction in pSfr64a conjugative ability, we undertook a transcriptional analysis of this plasmid in different hosts, involving RT-qPCR, RNAseq and analyses of transcriptional fusions. Our results reveal that in the R. etli background there is a marked reduction in the mRNA levels of certain genes related to conjugation in the foreign megaplasmid. Genetic regulation of mRNA levels can be modulated through xenogeneic silencers, DNA-binding proteins that act as global repressors. Inactivation of one of these silencers (mucR) on the R. etli background leads to a significant recovery in gene expression for some genes, but not for the genes involved in conjugation, which remain tightly silenced. We will present data of transcriptomic studies of pSfr64a in different hosts, as well as of the ongoing effort to identify the xenogeneic silencer responsible for quelling the conjugative ability of pSfr64a on R. etli.

Abstract ID: 505

Oxadiazolones and Staphylococcus aureus: multi-target approach against biofilms and antimicrobial resistance

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Co-authors: *Jordan LEHOUX, Thierry DURAND, Céline CRAUSTE, Stéphane AUDEBERT, Luc CAMOIN, Jean-Michel BRUNEL, Stéphane CANAAN, Jean-Michel BOLLA, Jean-Francois CAVALIER*

Staphylococcus aureus is a Gram-positive bacterium known for causing a wide range of infections, from skin abscesses to deadly bacteremia. Due to its virulence, biofilm formation, and antimicrobial resistance, *S. aureus* remains a high-priority pathogen as reported by WHO (1).

Enzymes with catalytic serine or cysteine residue (Ser/Cys-based enzymes) play a key role in bacterial growth, pathogenicity, and antibiotic resistance, making them attractive therapeutic targets. Oxadiazolone (OX) molecules, which are non-toxic to mammalian cells, represent a novel class of selective and efficient covalent multitarget inhibitors of such (Ser/Cys)-based enzymes. Recently, some OXs have already been described to efficiently impair the growth of *S. aureus* by targeting some virulence factors (2, 3).

In this context, the aim of our study was to evaluate a series of new OX compounds against planktonic and biofilm-associated *S. aureus*.

The best growth inhibitor, MpPPOX, displayed a strong antibacterial activity (MIC ~ 7.0 µg/mL), similar to vancomycin or linezolid (MIC ~ 2.0 µg/mL), against reference strains and clinical methicillin-resistant isolates (MRSA). Time-kill assays further confirmed the bactericidal properties of this inhibitor. Remarkably, the OXs antibiofilm activity was uncorrelated with their respective MICs. Indeed, the HPOX derivative, inactive against planktonic *S. aureus* (MIC > 128 µg/mL), exhibited a very strong anti-adhesive effect at sub-MIC values up to MIC/32.

Finally, click-chemistry activity-based protein profiling (ABPP) approach allowed us to determine the target enzymes of MpPPOX and HPOX, thereby confirming their respective mode of action, planktonic vs. biofilm, respectively. Of interest, FabH, an essential enzyme involved in lipid metabolism, was captured with all tested OXs inhibitors.

Overall, our results confirm the use of the OXs as potential therapeutic alternatives in the fight against *S. aureus* infections. Ongoing studies are validating FabH, at the molecular and structural level as an effective target, and are exploring the OXs efficacy against infected macrophages, a relevant reservoir for this Gram-positive bacterium.

(1) WHO bacterial priority pathogens list, 2024 (2) Lentz et al., Nat. Chem. Biol. 2018 (3) Jo et al., J. Am. Chem. Soc. 2024

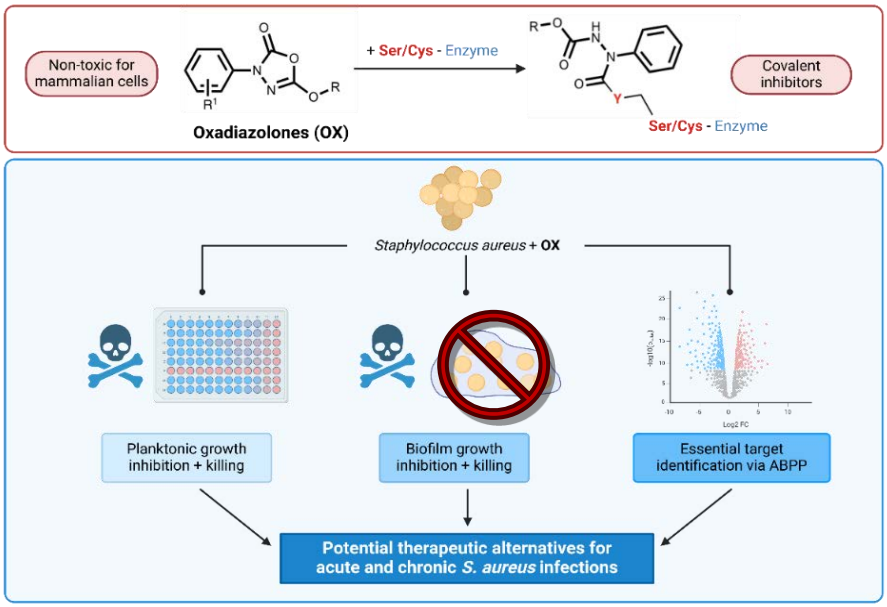


Figure 1: Oxadiazolone-core compounds inhibit and kill planktonic and biofilm-associated S. aureus through covalent inhibition of (Ser/Cys)-based enzymes, making them promising therapeutic antibacterial agents.

Abstract ID: 2037 / Poster Number: 108.15F

Oxidative stress drives vertical migration in diatoms microphytobenthic biofilms: An adaptative synergy between photoprotective motility and the xanthophyll cycle

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Co-authors: *Cédric Hubas*

Intertidal microphytobenthic biofilms (MPB) experience rapid and dynamic changes in irradiance throughout the day. These qualitative and quantitative variations in the electromagnetic spectrum can lead to cellular oxidative stress, characterized by the production and accumulation of reactive oxygen species (ROS) within the photosynthetic chains. This process can lead to harmful effects, such as cell death. To prevent such outcomes, autotrophic cells have evolved several photo-protective mechanisms.

This study investigates the behavioural and photo-physiological responses of diatoms under three distinct ROS conditions, highlighting the synergy between vertical migration and the xanthophyll cycle in unfavourable environmental conditions.

The experiment involved exposing cells to high-intensity white light (30 minutes at 1500 µmol photons m^{-2} s^{-1}), a condition known to stimulate intracellular ROS production. Additionally, extracellular ROS were applied either through atmospheric cold plasma (10 minutes), or by treating with a hydrogen peroxide solution (10 minutes at 200 µM). These treatments were applied to two types of MPB communities: one capable of vertical migration and the other non-migratory. Pulse-amplitude modulation fluorescence measurements were conducted to study photo-physiological parameters, and metabolic responses were assessed through the analysis of lipophilic pigment.

Pigment compositions vary depending on the stress, with contrasting effects on the activation of the xanthophyll cycle. Most interestingly, the results reveal that mobile diatoms exhibit vertical migration under all three oxidative conditions, indicating that ROS, especially hydrogen peroxide, play a key role in triggering this behavior.

The combined use of specific pigments and migration represents a rapid and efficient strategy for cell photoprotection.

Abstract ID: 3269 / Poster Number: 277.15P

Oxygen tolerant mechanism of commensal gut anaerobes from long term evolution: using Faecalibacterium as a showcase

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Co-authors: *Eleni Tsompanidou, Muhammad Tanweer Khan, Jan Maarten Van Dijl, Hermie Harmsen*

Although anaerobes are traditionally considered oxygen-intolerant, many possess defenses similar to those of aerobes, enabling survival under substantial oxygen exposure. However, the molecular mechanisms underlying oxygen tolerance, particularly in gut anaerobes colonizing the host colon after birth, remain unclear. In this study, we used *Faecalibacterium*, a strictly anaerobic, butyrate-producing gut microbe, to explore the genetic basis of oxygen tolerance in gut anaerobes.

We identified seven *Faecalibacterium* strains with varying oxygen tolerance levels using oxygen survival assays and subsequently performed comparative genomic analysis of the core genes in these strains. The results revealed a unique functional gene cluster present only in oxygen-tolerant strains. This cluster comprises two regulons: one encoding flavin reductases and the other involved in iron homeostasis.

A genome-wide association study further identified two SNPs within the cluster that significantly enhanced oxygen tolerance, despite structural variations in the cluster. These findings suggest the presence of additional transcriptional regulation mechanisms. To investigate this, we performed qPCR analysis, which showed a positive correlation between oxygen concentration and cluster transcription, with co-transcription observed among functional genes.

Additionally, a gene distribution analysis revealed that most of the genes within the cluster are widely observed in commensal gut microorganisms, including archaea. This indicates that the cluster likely resulted from long-term evolutionary processes.

In summary, we identified a widely distributed gene cluster responsible for strain-specific oxygen tolerance in the *Faecalibacterium* genus. The cluster's co-transcription pattern strongly influences bacterial oxygen tolerance.

Abstract ID: 3025 / Poster Number: 235.16P

Pasteurella Multocida Core Genome Multilocus Sequence Typing, And Taxonomic Life Identification Number Barcoding Systems

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Pasteurella multocida is a globally distributed bacteria that infects diverse hosts, causing diseases such as fowl cholera in poultry, haemorrhagic septicaemia, and pneumonia in livestock, with significant economic and public health implications. Despite its importance, limited genotypic characterisation of *P. multocida* has hindered effective surveillance and response. Recent advancements in whole genome sequencing have enabled the development of genome typing schemes to elucidate its population structure.

This study introduces a core genome multilocus sequence typing (cgMLST) scheme for *P. multocida*, along with a Life Identification Number (cgLIN) barcoding system. The cgMLST was developed using 990 genomes and validated with 208 additional genomes from diverse hosts and locations spanning 1922 to 2023. Potential core genes were identified from the development dataset and refined using the validation dataset to ensure the scheme's reliability. A core genome sequence type (cgST) was assigned to 98.38% of the development isolates and 95.67% of the validation isolates, identifying over 968 unique cgSTs, significantly surpassing the 219 STs identified by traditional seven-gene MLST.

The cgLIN nomenclature system further assigns unique numerical codes to genomes, stratifying them into hierarchical clusters based on cgMLST profile similarities, aligning with phylogenetic clustering. Together, the cgMLST-cgLIN code provides a standardized, high-resolution approach for analysing *P. multocida* population structure, addressing critical gaps in epidemiological surveillance. This study represents a significant advance in genomic taxonomy, offering a practical tool for global collaborative research, enabling effective monitoring and control on the emergence and evolution of *P. multocida*, which will be integrated into the PubMLST platform.

Abstract ID: 1306 / Poster Number: 198.16P

Pathobiome characterization and role in disease severity in children under 5-years with respiratory infection symptoms

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Introduction: The role of respiratory pathobiome in disease and progression remains poorly understood.

Objective(s): To improve knowledge of respiratory pathobiome and its impact on the onset, severity, and progression of respiratory diseases in children under 5-years.

Method: Nasopharyngeal swabs were consecutively collected from children under 5-years with respiratory symptoms admitted to Fondazione IRCCS Policlinico. Pathobiome was extracted using QIAamp-MinElute Kit and sequenced by QIAseq-xHYB Kit (Qiagen, Germany). Raw sequences were preprocessed using a standalone pipeline (1). Taxonomy was assigned using Kraken 2 (v2.1.2) and visualized by Pavian (2). Cytokines (IL-3,IL-6,IL10,IL-33,Rantes,PDGF,MIP1-alpha) were quantified by Luminex Assay. Shannon and Simpson indexes and PERMANOVA test were used to define correlations between species abundances and disease severity, resolution of symptoms within three days by hospital admittance, and cytokines profile.

Findings: Among the 54 hospital-admitted children (median age, IQR: 0.9 [0.4-1.8] years), 37 (68.5%) showed lower airway respiratory symptoms (LTRS), and 23 (42.6%) failed to resolve symptoms within three days by hospital admittance (FRS-day3). Metagenomics on nasopharyngeal swabs revealed that 48 (88.9%) samples contained at least one viral species, while the remaining 6 contained exclusively bacterial strains. The predominant viral species included RSV (relative-abundance:58.9%), HCoV-HKU1 (relative-abundance: 20.1%), Bocaparvovirus (relative-abundance:8.4%), Gammainfluenzavirus (relative-abundance:4.7%), and HAdV-C (relative-abundance:4.2%). Among bacteria, the most abundant species were Moraxella nonliquefaciens (relative-abundance: 44.5%), Haemophilus spp. (relative-abundance:33.1%), Moraxella catarrhalis (relative-abundance: 14.9%), and Streptococcus pneumoniae (relative-abundance: 6.1%). Lower

Shannon and higher Simpson indexes characterized the respiratory pathobiome of children with LRTS and FRS-day3 (p-value <0.05). By PERMANOVA, while a higher abundance of bacterial species was correlated with upper-respiratory-tract symptoms (P=0.01), a higher abundance of viral species was correlated with LRTS (P=0.003) and FRS-day3 (P=0.04). In line with that, a lower abundance of Moraxella catarrhalis and a higher abundance of RSV were related to LRTS (P=0.03 and 0.002) and FRS-day3 (P=0.04 and <0.001). A trend between higher abundance of HAdV-C(+/-AAV2) and FRS-day3 was also observed (P=0.097). No significant associations were found considering the cytokines levels.

Conclusion: Pathobiome in the respiratory tract of children under 5-years is characterized by significant diversity, that can significantly impact clinical onset and disease progression. Its knowledge can help in patients' management and care.

Abstract ID: 1789 / Poster Number: 159.15P

Pathogenic antibiotic-resistant bacteria transmitted by venomous snakes bites

Presenting author: Ms. Noelia Rechnitzer, University of Costa Rica, San José, Costa Rica

Introduction and Objective

Snake venoms have traditionally been regarded as sterile, but recent research suggests they may harbor microorganisms adapted to this unique environment, potentially contributing to severe infections. This study aims to confirm the presence of bacteria in the venom of *Bothrops asper* snakes, which are responsible for over 50% of snakebites in Latin America. Additionally, the study evaluates whether these bacteria are pathogenic to humans and resistant to commonly used antibiotics.

Methods

Venom samples were collected from 12 *B. asper* snakes at the Clodomiro Picado Institute in Costa Rica, using venom gland stimulation (8 samples) and a surgical method in sterile conditions (4 samples). The bacterial composition was analyzed via 16S rRNA gene metabarcoding, and isolates were cultured on blood agar medium and tryptic soy agar. Antibiotic susceptibility testing was performed using the VITEK 2 system, and the genomes of resistant bacteria were sequenced. Additionally, shotgun metagenomics analysis was conducted to identify antibiotic resistance genes in snake venoms.

Findings

16S rRNA metabarcoding showed microbial communities dominated by Gammaproteobacteria, primarily Enterobacterales. The most abundant genera were *Morganella* and *Stenotrophomonas*. Bacterial isolation confirmed the presence of these genera, along with other known human pathogens such as *Enterobacter*, *Providencia*, *Proteus*, *Serratia*, and *Aeromonas*.

Conclusions

The study concludes that *B. asper* venom is non-sterile and contains bacteria associated with human pathogenicity. Understanding the diversity and antibiotic resistance of these microorganisms could improve targeted antibiotic treatments in snakebite cases.

Abstract ID: 1301 / Poster Number: 153.16P

Pathogenic metamorphosis of Escherichia coli belonging to serotype O3:H2 associated with human infections

Presenting author: Ms. Daiany R. P. De Lira, Universidade Estadual Paulista (UNESP) – Instituto de Biociências, Botucatu, Brazil

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Introduction: Pathogenic isolates of *Escherichia coli* cause multiple types of infections in the human host. *E. coli* associated with gastrointestinal tract infection are termed diarrheagenic *E. coli* (DEC), while *E. coli* associated with urinary tract infection, sepsis, or meningitis are termed extraintestinal pathogenic *E. coli* (ExPEC). The high plasticity of the genome of this pathogen has allowed the emergence of isolates with unexpected genetic combinations, and these isolates are termed hybrids.

Objective: Our objective was to genetically characterize a collection of 18 *E. coli* isolates from the serotype O3:H2, obtained from stool or urine samples.

Method: We performed Illumina whole genome sequencing. Further, one representative isolate (UPEC34) was selected to perform MinION long-read sequencing.

Findings: All *E. coli* isolates belonged to phylogroup A and were divided into two sequence types (ST): ST10 (55.6%) and ST8087 (44.4%). Isolates from ST8087 were classified as enteropathogenic (EPEC), enteroaggregative (EAEC), or hybrid EPEC/EAEC. In contrast, isolates from ST10 harbored markers from the EAEC and ExPEC pathogroups, and thus, were classified as ExPEC/EAEC hybrid isolates. Using the UPEC34 as a model, we observed that all virulence factor-encoding genes related to the ExPEC pathogroup were co-located in a single chromosomal pathogenicity island with 159.2 kb in length and organized in 156 coding sequences.

Conclusion: Our data suggest that *E. coli* from ST10 have acquired a larger palette of virulence-encoding genes, including both DEC and ExPEC markers, thus emerging as hybrid pathogens able to cause intestinal and extraintestinal infections.

Abstract ID: 2823 / Poster Number: 283.15P

Pathways and Efficiency of Organic Nitrogen Utilization in Cyanobacterial Blooms

Presenting author: Ms. Huiting Chen, University of Bristol, Bristol, United Kingdom

Co-authors: *Charlotte Lloyd, Liz Bagshaw, Patricia Sanchez-Baracaldo*

Cyanobacteria, as ancient oxygenic autotrophs, are critical players in aquatic nitrogen cycling and bloom formation. Cyanobacterial blooms are major ecological challenges, closely linked to eutrophication and climate change driven by human activities. Nitrogen is a key driver of cyanobacterial blooms, yet research has predominantly focused on its inorganic forms, leaving the role of organic nitrogen underexplored. Although cyanobacteria can metabolize simple organic compounds like urea and amino acids, the utilization and pathways for more complex forms remain poorly understood.

This study aims to explore the uptake and utilization of organic nitrogen by five representative bloom-forming cyanobacteria: *Aphanizomenon*, *Dolichospermum*, *Lyngbya*, *Oscillatoria*, and *Planktothrix*. Eight nitrogen sources, including six organic forms representative of fertilizers, herbicides, pharmaceuticals, amino sugars, amino acids, and methylamines, as well as two inorganic forms (nitrate and ammonium), were selected to reflect key nitrogen inputs in natural waters. The effects of these nitrogen sources on cyanobacterial growth, photosynthetic performance, toxin production, and the generation of taste and odour compounds were evaluated. Preliminary results indicate that *Aphanizomenon klebahnii* CCAP1401/3 exhibited distinct growth rates and chlorophyll-a content under different nitrogen sources. Glutamate supported the highest growth, while atrazine showed no measurable utilization. Urea, caffeine, and methylamine were identified as effective organic nitrogen sources, supporting significant growth and chlorophyll-a production. To explore the genetic basis of nitrogen metabolism, comparative genomic analysis will examine genes involved in organic nitrogen utilization, linking genomic traits to phenotypic responses. This study provides new insights into cyanobacterial nitrogen metabolism, offering a foundation for bloom management strategies.

Abstract ID: 667 / Poster Number: 387.16P

Patterns of vaginal microbiome variations in distinct areas of the Republic of Armenia

Presenting author: Ms. Ruzanna Harutyunyan, Yerevan State University, Yerevan, Armenia

Co-authors: *Tigran Aleksanyan, Edita Gharibyan, Inga Bazukyan*

The vaginal microbiome plays a crucial role in women's health and reproduction. It is a dynamic ecosystem that undergoes hormonal changes throughout the various stages of a woman's life. The metagenome analysis has never been previously applied for the study of vaginal microbiome in the Republic of Armenia, thus, the investigation of the effects of various conditions with this method is the goal of the present study.

The samples were collected from 64 women of reproductive age residing in three regions with distinct ethnic ancestries. They were also categorized into three subgroups: healthy, pregnant, and those with vaginitis infections. The vaginal microbiome was analyzed both with traditional microbiological methods and V3-V4 16S rRNA amplicon-based metagenomic analysis.

The results from traditional microbiological methods aligned with those of the metagenomic analysis. *Lactobacillus iners* dominated in all the samples. The women of Armenian ancestry from the capital exhibited the greatest bacterial diversity at both species and phylum levels, while Molokans from Lori had diverse lactobacilli genera. Candidiasis resulted in the reduction in lactobacilli, accompanied by the presence of pathogenic species. Pregnant women typically have a healthy microbiome; however, pathogens may occasionally be detected due to a weakened immune system, especially among Yezidis from Aragatsotn. Regional variations are primarily observed at the phylum level, particularly among minority groups.

This study highlights the dominant presence of *Lactobacillus iners* and the diversity of the microbiome across different regions, it may lead to the development of bacterial associations for improving women's health and managing vaginal dysbiosis.

Abstract ID: 1338 / Poster Number: 166.16P

Performance Evaluation of Lodestar DX: A Rapid Point-of-Care Platform for Identifying Uropathogens – A Breakthrough in Urinary Tract Infection Diagnostics?

Presenting author: Dr. James Moore, Norfolk and Norwich University Hospital, Norwich, United Kingdom; Eastern Pathology Alliance, Norwich, United Kingdom

Co-authors: *Stuart Drazich-Taylor, Brian McCann, John Hayward*

Introduction

The Lodestar DX is a point-of-care test (POCT) utilising loop-mediated isothermal amplification (LAMP) to detect common uropathogens. Molecular POCTs offer potential as adjuncts in diagnosing urinary tract infections (UTIs) while awaiting urine culture results.

Objectives

To evaluate the diagnostic performance of Lodestar DX in detecting uropathogens in non-pregnant adults.

Methods

Between August and November 2024, the Lodestar DX was compared to traditional microbiological culture at the Eastern Pathology Alliance. Urine samples from non-pregnant adults were analysed. Discrepancies between the Lodestar DX and culture reports prompted a review of culture agar plates.

Findings

Of 380 urine samples tested, 345 (90%) passed quality controls. Samples included mid-stream urine (83%, n=287) and catheter urine (17%, n=57). Aggregated performance showed sensitivity 84%, specificity 89%, positive predictive value 54%, negative predictive value 96%, and accuracy 89%. Sensitivity for specific organisms was: *Escherichia coli* 87% (n = 67), *Enterococcus spp.* 80% (n= 20), *Staphylococcus aureus (S. aureus)* 27%(n= 8), *Staphylococcus* saprophyticus 92% (n=13), *Proteus mirabilis* 94% (n=16), *Pseudomonas aeruginosa* 84% (n= 19), and *Klebsiella pneumoniae* 100% (n = 2).

Conclusion

The Lodestar DX demonstrated strong performance in detecting most uropathogens, except *S. aureus*. It holds promise as a diagnostic tool for UTIs, though further comparison with other POCTs is warranted.

Abstract ID: 1168 / Poster Number: 159.16P

Phage Display Screening of BBB-Permeable Peptides for Improving Drug Delivery in Neurodegenerative Disease Treatment

Presenting author: Mr. Seungwoo Chung, Jeonbuk National University, Jeonju, South Korea

Co-authors: *Jiho Min, Yang-Hoon Kim*

Introduction: The incidence of neurodegenerative diseases such as Alzheimer's, Parkinson's, and stroke is rising due to an aging population. Effective treatment requires delivering drugs to brain lesions, but the blood-brain barrier (BBB) restricts external substances from entering the brain, posing challenges in drug development. As a result, overcoming the BBB has become a key focus of research. This study hypothesized that permeable peptides, screened via phage display in an enhanced BBB model, could improve drug delivery to the brain.

Objective: This study aimed to screen permeable peptides using phage display in an enhanced BBB model and analyze the permeability of selected peptide candidates.

Methods: To mimic the BBB, human brain endothelial cells (hCMEC/d3) and astrocytes (HA) were co-cultured on a membrane, and barrier function was enhanced using neural growth factors and cyclic adenosine monophosphate (cAMP). BBB integrity was confirmed through Transendothelial Electrical Resistance (TEER) measurements and FITC-dextran assays. A phage library was passed through the enhanced BBB model, followed by three rounds of biopanning. The permeability of selected peptides was evaluated by measuring their titers after passage through the BBB model.

Findings: The enhanced BBB model demonstrated TEER values exceeding 200 $\Omega \cdot cm^2$, and FITC-dextran assays confirmed improved barrier function. Phages screened via biopanning showed significantly higher permeability compared to controls.

Conclusion: This study successfully enhanced the BBB model and identified BBB-permeable peptides using phage display. These peptides exhibited high permeability, offering potential for effective drug delivery to the brain and overcoming barriers in neurodegenerative disease treatment.

Abstract ID: 1358 / Poster Number: 248.16P

Phage Dynamics in Chronic Diseases: Shotgun Metagenomic and Machine Learning Approaches in Colorectal Cancer and Inflammatory Bowel Disease

Presenting author: Prof. Aycan Gundogdu, Erciyes University, Kayseri, Turkey; Enbiosis Biotechnology, London, United Kingdom

Co-authors: *Mehmet Hora, Özkan Ufuk Nalbantoğlu,*

The gut microbiome, comprising bacteria, archaea, fungi, and viruses, is increasingly linked to chronic diseases. Bacteriophages (phages) within the microbiome regulate microbial communities and influence health. Despite their importance, phages remain underexplored. This study investigates the gut phage ecosystem in colorectal cancer (CRC) and inflammatory bowel disease (IBD) to identify disease-specific biomarkers using machine learning.

Shotgun metagenomic sequencing data from six datasets were analyzed: four datasets for CRC (383 patients and 446 healthy controls) and two datasets for IBD (468 patients and 267 healthy controls). Quality control and metagenomic assembly were performed using IDBA_UD, with bacterial taxonomic assignments conducted via MetaPhlAn2. Phage detection was carried out using the PhaBOX pipeline, excluding temperate phages. Three machine learning algorithms—Random Forest, Gradient Boosted Decision Trees (XGBoost), and Multi-Layer Perceptron (MLP)—were applied to classify disease states.

In total, metagenomic data from 1,564 individuals (approx 7.0 terabytes) were analysed. CRC metagenomes revealed that *Bacteroides fragilis*, *Bacteroides faecis*, and *Achromobacter xylosoxidans*' phages were significantly enriched in the patient group ($p<0.05$) and were consistent across other CRC datasets. Conversely, phages linked to *Roseburia intestinalis*, *Gordonia terrae*, and *Parabacteroides merdae* were significantly more abundant ($p<0.05$) in the healthy group, aligning with findings from other CRC datasets. In IBD metagenomes, phages associated with *Ruminococcaceae bacterium* and *Coprobacillus sp. AF13-15* were significantly enriched ($p<0.05$) in the patient group and consistent across other IBD datasets. Phage profiles demonstrated moderate predictive power (Table 1).

While these results indicate a significant differentiation between health and disease states, the observed performance may be influenced by the non-targeted nature of the datasets, which were not specifically designed for phage isolation and sequencing. The limited representation of phage diversity highlights the need for tailored wet-laboratory

protocols specifically designed for phage enrichment and sequencing. These findings underscore the potential of bacteriophages as non-invasive biomarkers and suggest directions for future research to optimize their diagnostic utility. Future studies should focus on elucidating the mechanistic roles of phages in disease pathogenesis and progression.

Table 1. Classification Performance of CRC and IBD Datasets Using Machine Learning

	Algorithm	Accuracy	Precision	Recall	F1 Score	ROC
CRC Datasets						
Dataset 1 (PRJEB7774)*	Random Forest	0.69	0.20	0.04	0.06	0.50
	XGBoost	0.67	0.37	0.30	0.32	0.56
	MLP	0.76	0.72	0.34	0.44	0.64
Dataset 2 (PRJEB27928)*	Random Forest	0.71	0.68	0.88	0.76	0.70
	XGBoost	0.65	0.66	0.75	0.70	0.64
	MLP	0.71	0.75	0.75	0.72	0.71
Dataset 3 (PRJEB12449)*	Random Forest	0.69	0.71	0.60	0.65	0.68
	XGBoost	0.60	0.59	0.58	0.58	0.60
	MLP	0.74	0.72	0.72	0.72	0.74
Dataset 4 (PRJEB10878)*	Random Forest	0.53	0.57	0.79	0.66	0.53
	XGBoost	0.55	0.58	0.59	0.57	0.54
	MLP	0.45	0.47	0.40	0.42	0.45
IBD Datasets						
Dataset 1 (PRJEB1220)*	Random Forest	0.68	0.71	0.76	0.73	0.66
	XGBoost	0.65	0.69	0.72	0.70	0.64
	MLP	0.64	0.66	0.73	0.70	0.63
Dataset 2 (PRJNA400072)*	Random Forest	0.72	0.73	0.97	0.83	0.49
	XGBoost	0.56	0.72	0.40	0.74	0.61
	MLP	0.68	0.74	0.86	0.79	0.55

*These are unique accession numbers assigned to datasets in public genomic databases

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Abstract ID: 3267 / Poster Number: 222.16P

Phage platform: A breakthrough solution for antimicrobial resistance

Presenting author: Mr. Varun Rathi, Indian Institute of Technology, Ropar, India

Co-authors: *Atharva Poundarik, Prakash R. Nayak*

By 2050 AMR could lead to 10 million deaths/year globally[1]. AMR will reduce global GDP by 4% and push 24 million people into extreme poverty by 2050[2]. Phages has resurgence of interest, with ongoing research and optimization is required for expanding their application in targeting AMR. This aim is to develop a phage-based platform through systematic and scalable methodology. The platform leverages scientific source of selection, optimized isolation and enrichment protocols and testing lyophilized formulation to enhance stability and shelf life. Library has been prepared against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, for rapid screening against rapidly emerging AMR strains. Phage cocktail evaluated in statistically powered trial showing promising efficacy. Phages with lytic efficiency of upto 85% against *S. aureus*, 90% *E. coli* and 85% against *P. aeruginosa* have been purified and stored for further study. Adapted phages against AMR *E. coli* and *S. aureus* showing 85% lytic efficiency, maintaining <10 PFU/mL reduction over a year using cryopreservants. Lyophilized phages showed active lytic activity upon resuspension. Phages have been further characterized based on host ranges, burst sizes, electron microscopy, genome sequencing and stability study. *In vivo* trial showed promising efficacy, significantly reducing AMR *E. coli*-related infections, with lesion scoring was comparable to those treated with antibiotics based on sensitivity profiling, highlighting the targeted nature of phage therapy as an alternative to broad-spectrum antibiotics. This data underpins the potential of phage cocktails with its varied spectrum of employment. Platform can be used for prophylaxis, environment sanitation, water treatment, agriculture and veterinary interventions.

Abstract ID: 1238

Phage therapy challenges: Addressing resistance in Pseudomonas aeruginosa strains from cystic fibrosis patients

Presenting author: Dr. Federica Briani, Università degli Studi di Milano, Milano, Italy

Co-authors: *Shila Shehu, Alessandra Bosc, Allegra Laricchia, Jimena Nieto Noblecia, Francesca Forti, Lisa Cariani, Marco Cafora, Anna Pistocchi,*

Introduction: Phage therapy is a promising option for treating antibiotic-resistant *Pseudomonas aeruginosa* infections in people with cystic fibrosis (CF) by exploiting phages' ability to kill bacteria. A major drawback in the implementation of phage therapy is the widespread phage resistance in *P. aeruginosa* (Pa) isolates from airway infections in CF patients (referred to as Pa CF isolates) [1].

Objective: To address this challenge, we aimed to assemble a collection of natural and modified phages specifically tailored to infect a panel of 36 Pa CF isolates, among which many were resistant to most, or all, phages of our laboratory collection.

Method: New Pa phages were isolated from wastewater and assessed for their safety and suitability for human use. Six natural phages were used for *in vitro* evolution through UV mutagenesis or iterative infection cycles (i.e., the Appelmans protocol [2]), either as single phages or as mixtures of different phages, i.e. phage cocktails.

Findings: Several evolved phages with broader host-range than the parental ones were isolated (Fig. 1). Genome sequencing showed that UV-evolved phages typically carried few mutations in genes encoding proteins involved in host adsorption, while Appelmans-evolved phages resulted from multiple mutations and recombination events, making it more challenging to identify the molecular mechanisms behind their altered host range. Natural and mutated phages were combined to develop new phage cocktails theoretically capable of infecting all Pa CF isolates in our strain collection.

Conclusion: Overall, our results confirm that phage resistance in CF isolates can be overcome through *in vitro* random evolution. However, new phage cocktails displayed a narrower host range than theoretically anticipated, possibly due to interference among phages composing the cocktails and/or cross-resistance. Additional studies are underway to address this issue.

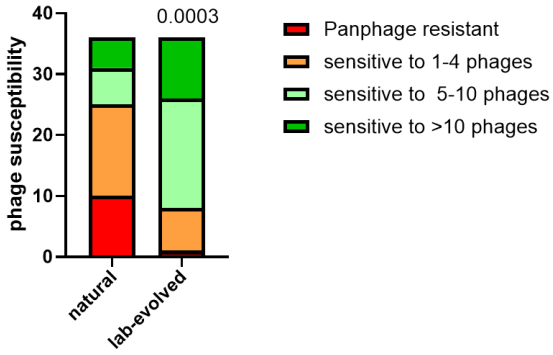


Figure 1: *Phage susceptibility of PA CF isolates (N = 36) considering only natural (left column) or also lab-evolved (right column) phages.*

Abstract ID: 3722

Phage-bacteria interaction networks of Pseudomonas aeruginosa clinical isolates and jumbo and non-jumbo phages reveal interactions between prophages, defense systems and lytic phages

Presenting author: Dr. Ana Georgina Cobian Guemes, University of California San Diego, San Diego, United States

Introduction: *Pseudomonas aeruginosa* is the predominant microorganism involved in phage therapy requests at the Center for Innovative Phage Applications and Therapeutics, University of California, San Diego, accounting for 37% of all inquiries.

Objective: In response to this high demand, we developed a specialized phage library, comprising six jumbo phages and forty-four non-jumbo phages. This library was evaluated for its efficacy against a diverse set of 100 clinical strains of *Pseudomonas aeruginosa* to select phages with the broadest host range and identify interactions between bacteria and phages.

Methods: Our bacteria collection included strains from patients with cystic fibrosis and those without, as well as epidemic strains, and spanned the spectrum from extensively drug-resistant, multidrug-resistant, to sensitive strains. Several mechanisms prevent phages from killing the bacteria host, which presents a challenge for phage therapy interventions. To refine the search for phages with the most extensive host range, suitable for phage therapy applications, and to decode prevalent patterns in phage infection strategies, more than 3,500 Phage-Bacteria Interaction Networks were performed by spotting phage dilutions in 0.3% top agar bacteria plates (Figure 1). This interactions matrix was paired with genomic information to unravel the complex interactions between bacteria and phages. Over 100 strains of Pseudomonas aeruginosa and 35 phages were sequenced using Illumina to provide a detailed genomic landscape. Phage genomes were assembled, then annotated using PHANOTATE through BV-BCR. Bacterial defense mechanisms were identified using Defense Finder. Furthermore, prophages were predicted using PHASTER.

Findings: We identified three phages with a broad host range with the following percentages of lysis: phage Tri180, 56%; phage Good Vibes, 76%; phage Clover, 60%; and phage Teru, 63%. We did not identified a significant difference between jumbo and non-jumbo phages host range. We identified prophages in the bacteria genomes that may prevent the infection of these bacteria with the tested lytic phages.

Conclusion: Leveraging this comprehensive dataset with machine learning, we aimed to decode the intricate bacteria-phage interactions. This approach—merging genomic sequencing, network analysis, and computational techniques—intends to deepen our understanding of phage-host dynamics, paving the way for more effective phage therapy solutions.

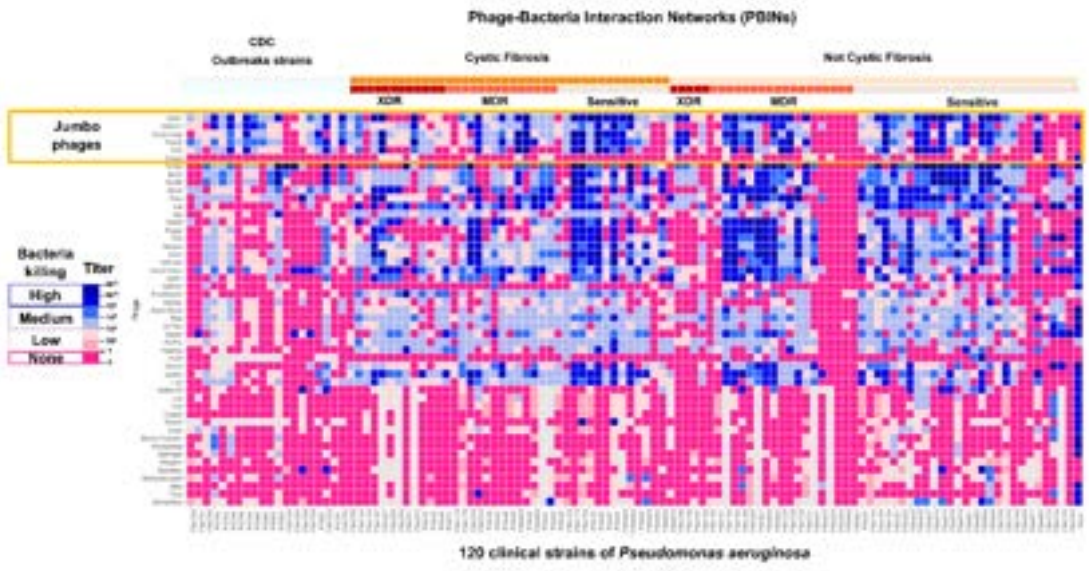


Figure 1: Phage-Bacteria-Interaction-Network of *Pseudomonas* phages. Titer of each phage (n=35) on each bacteria strain (n=110) was calculated by spot testing of phage dilutions. Plates were incubated at 37C over 18 hours.

Abstract ID: 2966 / Poster Number: 317.15P

Phages in the dark: Unveiling the viral diversity in cave environments

Presenting author: Prof. Martina Cappelletti, University of Bologna, BOLOGNA, Italy

Co-authors: *Bernardo Barosa, Andrea Firrincieli, Daniele Ghezzi, Paolo E. Costantini, Alberto Danielli, Giacomo Broglia,*

Phages are regarded as the most abundant biological entities on Earth, with an estimated biomass of approximately 200 Mt[1]. They exhibit notable diversity, varying in virion structure (e.g., tailed, enveloped, and filamentous), genetic material (single- or double-stranded), and gene content. Through their lytic activities, roles in horizontal gene transfer (HGT), and the rewiring of host metabolism during infection, phages modulate the ecology and evolution of microbial communities. Therefore, they are key contributors to ecosystem functioning and have important implications for biogeochemical cycles, for instance, by mediating carbon fluxes[2]. Despite this, their taxonomic diversity, abundance, and distribution in understudied environments remains to be elucidated in further detail.

Cave systems, given their isolation from the surface and the lack of light-driven photosynthetic processes, are highly oligotrophic environments known to host an unexpected microbial diversity. In these systems, microorganisms often depend on chemotrophy and mutualistic networks to sustain the development of complex communities, being at the foundation of the food webs inside caves[3,4]. However, knowledge of the distribution and ecological significance of phages in caves and their interactions with resident microbial communities remains limited and restricted to few studies[5].

To address these questions, we performed whole metagenomic shotgun sequencing using both short-read (Illumina) and long-read (Oxford Nanopore) strategies, coupled with metagenomic data obtained from public databases. In total, we created and analyzed a dataset including 154 metagenomes, representative of cave systems worldwide, and encompassing diverse cave types (i.e., quartzite, limestone, sulfidic, and basaltic caves) and sample matrices (e.g., biofilms, soil, and water). This large-scale approach allowed us to construct a thorough catalogue of viral and microbial genomes, providing insights on the diversity and ecological roles of phages in cave systems. Our findings expand the understanding of the Earth's virosphere and unravel the contribution

of phages in evolutionary processes and environmental colonization of microbes in caves.



Abstract ID: 3217 / Poster Number: 218.16P

Pharmacomicrobiomics in Colorectal Cancer: Drug-Gut Isolates and Host Interactions

Presenting author: Ms. Sara Mennella, Ca' Foscari University of Venice, Department of Molecular Science, Venice, Italy

Co-authors: *Kubra Oztat, Alessandro Bregalda, Amir Nabinejad, Flavio Rizzolio, Sabrina Tamburini*

The gut microbiome significantly impacts colorectal cancer (CRC) by affecting cancer development, progression, and treatment efficacy. Emerging pharmacomicrobiomics research highlights a two-way interaction where bacterial metabolites modulate tumor behavior and inflammation, while anticancer drugs influence microbial activity and host immunity. This study evaluated the interactions between five CRC drugs (oxaliplatin, 5-fluorouracil[5-FU], irinotecan, regorafenib, and VS1), human gut isolates, and host. Eight bacterial strains—six newly isolated from one healthy donor (three *Escherichia coli* strains and one *Enterococcus faecium* were identified) and two established commensals (*E. coli* 25922 and *Lactobacillus rhamnosus* GG)—were exposed to 5-fold drug serial dilutions, starting from 200 µM, under aerobic and anaerobic conditions. Among the tested drugs, regorafenib and 5-FU showed antibacterial activity at 200µM. Regorafenib inhibited the 82% of bacterial growth of one unknown bacterial isolate, while 5-FU inhibited the 50% of bacterial growth of three isolated *E. coli* and 76% of the unknown one. Drug cytotoxicity was also assessed on two cancer (HT-29, Colo205) and one normal (MRC5) human cell lines and seven patient-derived colon organoids. Almost all drugs affected both cancer cells, with an IC50 below 4.5µM, except for VS1 that exhibited an IC50 value 10-times higher, and colon organoids, which exhibited variable responses depending on the drug and the patient. While almost all drugs affected cells and organoids, they had minimal impact on gut bacterial communities. This study emphasizes the importance of microbiome-tumor-drug interactions and highlights the need for future studies to advance the development of CRC personalized treatments.

Abstract ID: 3218 / Poster Number: 220.16P ||||| Ovarian cancer remains a significant global health challenge, with emerging evidence highlighting the role of tumor-associated microbes in cancer progression and treatment, underscoring the importance of pharmacomicrobiomic studies in understanding drug-microbiota interactions. This study evaluated the effects of three chemotherapeutics, carboplatin (3.125–200 µM), paclitaxel (3.125–200 µM), and doxorubicin (0.781–50 µM), on ovarian cell lines (three cancer: SKOV3, Ovcar3, Kuramochi; one normal: FT190), three newly isolated tumor-associated bacteria and two commensal reference strains (*Escherichia coli*-25922, *Lactobacillus rhamnosus*-GG), using 5-fold serial dilutions. Minimum Inhibitory Concentrations (MICs) for standard antibiotics (ampicillin, tetracycline, gentamicin, vancomycin) were also determined for each bacterial strain. Cytotoxicity tests revealed a strong effect on ovarian cell viability, 90% for all three: doxorubicin at 50 uM and paclitaxel and carboplatin at 200 uM. In contrast, tumor-associated bacteria (*E.coli-SGB10068, Streptococcus pneumoniae-SGB8138, Pseudomonas aeruginosa-SGB12320*) and reference strains (*E. coli*-25922 and *L. rhamnosus*-GG), exhibited resistance to chemotherapeutics (MICs > 200 µM). However, *E. coli*-25922 showed 76% of growth inhibition at 50 µM doxorubicin under aerobic conditions. In addition, antibiotic testing demonstrated resistance of *P. aeruginosa* to ampicillin and vancomycin at 1 mg/mL, but susceptibility to gentamicin and tetracycline <40µg/mL. *E. coli-SGB10068* displayed resistance to ampicillin (1 mg/mL) while *E. coli*-25922 was susceptible (MIC:0.04 mg/mL). These findings highlight that chemotherapeutics effectively target ovarian cancer cell lines, but show limited impact on tumor-associated bacteria, potentially influencing cancer progression. Future studies should explore integrating microbiota-targeted strategies to enhance chemotherapy efficacy and antibacterial efficacy on tumor-associated microbes.

Abstract ID: 2416 / Poster Number: 316.15P

Phase-variable restriction-modification systems – an epigenetic regulator of Porphyromonas gingivalis pathogenicity

Presenting author: Ms. Sara Sharaf, University of Aberdeen, Aberdeen, United Kingdom

Co-authors: *Karolin Hijazi, Debbie Wilkinson, Elaina Collie-duguid, Marco Oggioni, Yasmeen Althari, Jin Pu*

Introduction: *Porphyromonas gingivalis* is a keystone bacterium implicated in periodontitis and associated with other systemic inflammatory diseases. A subset of strains possesses phase-variable restriction-modification systems (pv-RMS), an epigenetic regulatory mechanism. The specificity (*hsdS*) gene within pv-RMS undergoes recombination, generating allelic variants that may influence bacterial gene expression (**Figure 3**).

Objective: This study investigates the role of epigenetic regulation through Type 1 pv-RMS in modulating *P. gingivalis* virulence and immune evasion.

Methods: *P. gingivalis* strain possessing a pv-RMS was cultured under conditions that resulted in enrichment of a specific allele. Allele screening of *hsdS* in Type 1 pv-RMS strain was conducted post restriction digestion of amplicons inclusive of the specificity gene. Four distinct alleles were identified through their variable sizes using capillary electrophoresis. Phenotypic assays were employed to assess allele variant pathogenicity, using cell lines CAL-27 and THP-1 derived macrophages as models of invasion and immune evasion, respectively. Allele-enriched populations were co-cultured with these cells for 4 different time points and data was acquired as fluorescence measurements using confocal microscopy/ImageJ for both cellular assays. Cell lysis and bacterial load quantification of *16S P. gingivalis* via RT-qPCR was used as further verification of cellular invasion. All experiments included *P. gingivalis ATCC 33277*, *P. gingivalis W83* and negative controls.

Findings: pv-RMS strains rearranged into different alleles preferentially depending on the growth condition. One of four *hsdS* alleles (*hsdSA)* exhibited the highest cell invasion potential as measured by Corrected Total Cell Fluorescence and bacterial loads (copies/µl) compared to the other allele variants of *hsdS*. Another allele (*hsdSD*) displayed enhanced capability to evade phagocytosis.

Conclusion: This study implicates pv-RMS in regulation of pathogenicity in *P. gingivalis*. These findings suggest alternative mechanisms that modify pathogenicity in periodontal bacteria, which should be considered when developing therapeutics and investigating the role they play in associated systemic conditions.

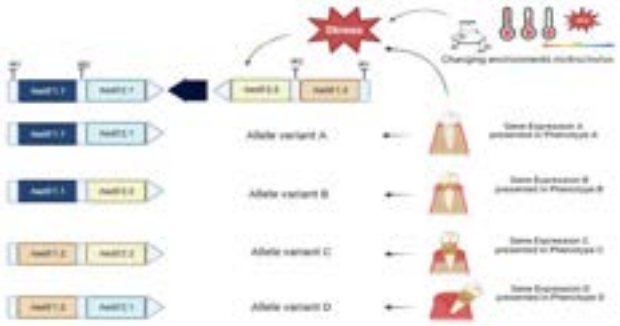


Figure 3: Visual representation of Type 1 pv-RMS phasevarions in Porphyromonas gingivalis (created on Biorender).

Abstract ID: 2703 / Poster Number: 139.17P

Phenotypic and genotypic characterization of Acinetobacter calcoaceticus-baumannii complex including drug susceptibility profile

Presenting author: Prof. Varsha Gupta, GMCH Sector 32, Chandigarh, India

Co-authors: *Nikki Baxi, Lipika Gautam, Shivani Sharma*

Introduction: *Acinetobacter calcoaceticus-baumannii* complex(*Acinetobacter cbc*) is a ubiquitous bacterium known to be implicated in hospital-acquired infections. Owing to the rise in antimicrobial resistance, *A. baumannii* has become a significant challenge for healthcare facilities making infections difficult to treat.

Objective: To investigate the phenotypic and genotypic traits of *Acinetobacter* isolates.

Methods: This longitudinal observational study was done analysing 178 *Acinetobacter* isolates from first clinical samples. Phenotypic characterizationand antibiotic susceptibility testing was done using standard criteria. Genotypic analysis was done for targeted resistance genes:bla_{TEM}, bla_{SHV}, bla_{OXA-48}, bla_{CTX-M},$bla_{IMP,}$ $bla_{VIM,}$ $bla_{OXA-23,}$ $bla_{KPC,}$ $bla_{GIM,}$ $bla_{OXA-51,}$ $bla_{PER-1,}$ bla_{INT-1} and bla_{NDM}.

Findings: Majority of the isolates were from endo-tracheal aspirates (41.11%), followed by other samples.43.47% of the samples were from intensive care unit (ICU), 53.53% from wards and 3% from the OPD. Mortality rate was 46% in the ICU patients and 11% in patients from wards. Susceptibility to carbapenem, piperacillin-tazobactam and ampicillin-sulbactam was 15%, 39% and 17%respectively. Colistin susceptibility was 95.45%. 86.66% of the isolates were multi-drug resistant(MDR) and 5.55% were pan-drug resistant (PDR). Genotypic analysis detected $blaOXA_{51\text{-}like}$ in 97%, *blaNDM*in 45.45%, *blaPER-1* and *int1* genes in 20% and *blaTEM*in less than 10% of the isolates. None of the isolates harboured the genes *blaCTX-M* and *blaSHV*.

Conclusion: *Acinetobacter cbc*has an unlimited capacity for antibiotic resistance development. In our study, majority of the isolates were MDR and few isolates were PDR, underlying the importance to understand the phenotypic and genotypic characteristics of antimicrobial resistance in *Acinetobacter cbc*.

Abstract ID: 3657 / Poster Number: 169.15P

Pheromone profiles and microbial insights: exploring the microbiome of sand lizard femoral glands

Presenting author: Dr. Mariusz Krupiński, University of Lodz, Department of Industrial Microbiology and Biotechnology, Lodz, Poland

Co-authors: *Katarzyna Janik-Superson, Joanna Michalska-Madej, Sebastian Piskorski, Alejandro Ibáñez*

Introduction: Lizards heavily rely on pheromones for communication, marking territories and mating behaviors, facilitated by femoral glands (FGs) that secrete chemical compounds FGs harbour rich microbial communities, but the specific role of these remains unknwon.

Objective: Our project aims to investigate the role of symbiotic bacteria in chemical communication among sand lizards (*Lacerta agilis*). Key objectives include identifying a stable core microbiome and bacterial groups influential in mating behaviours.

Method: Microbial DNA were isolated from FGs swabs of 87 individuals (30 females and 57 males), sampled in 2022 and 2023 mating seasons, captured in 9 sampling sites in Poland. The methodology was based on high-throughput sequencing (NGS) using the Illumina NovaSeq 6000. Amplicon libraries were prepared using the prokaryotic 16S rRNA minibarcode.

Findings: FGs of males and females shared 630 common bacterial genera. The microbiome of males was almost twice as rich in the number of detected genera (N=1168) compared to females (N=699). As many as 534 unique genera were found in males, which were not detected in females. Females had a unique 69 genera. In males, the most common genera were: Pseudomonas, Sphingomonas, Paracoccus, Methylorubrum, Fodinibacter; Nocardioides, Variovorax, Rhodococcus, Bradyrhizobium, Mycobacterium, Pedobacter, Massilia, Arthrobacter, Hymenobacter, Dietzia, Curtobacterium and Aeromicrobium. In females, Methylorubrum, Pseudomonas, Sphingomonas, Massilia, Curtobacterium, Mycobacterium, Rhizobium and Fodinibacter were detected most frequently. The set of bacterial genera differed also between sampling sites.

Conclusion: The microbiome of male lizards is richer compared to females. The composition of the lizard microbiome seems to be influenced by their microbiome micro-habitat.

Abstract ID: 2599 / Poster Number: 267.16S

Photoantimicrobials and photoanticancer agents derived from the genera Penicillium and Talaromyes – potential game changers in the combat against multiresistant microorganisms and cancer?

Presenting author: Dr. Pamela Vrabl, University of Innsbruck, Innsbruck, Austria

Co-authors: *Markus Meitinger, Angelika Seeber, Vanessa Kern, Madgalena Hintner, Martin Kirchmair, Carmen Bendetta, Friederike Glauch, Bianka Siewert*

Introduction: Photodynamic therapy (PDT) is a promising alternative approach to combat multiresistant microbes and cancer. On a meta-level, PDT comprises up to three elements: (1) a light-activated photosensitizer (PS), (2) light exposure, and (3) aerobic conditions to produce singlet oxygen (PDT type II). Under anaerobic conditions or with different PS, other reactive oxygen species like superoxide radical anion or hydroxyl radical are produced (PDT type I). An excess of radical oxygen species can selectively kill microorganisms or cancer cells. PDT has received increasing interest due to its targeted approach and the possibility of a localized treatment with comparable little side effects, while there is a low risk of resistance development. One bottle-neck is still the available repertoire of suitable photosensitizers. Natural photosensitizers have typically been sourced from plants, but recent research indicates that fungi are a yet untapped source of these compounds. If we can develop methods to produce effective photosensitizers from fungi on a large scale, similar to penicillin, it could significantly enhance our ability to combat antibiotic-resistant microbes and cancer.

Objectives: This ongoing study aims to (1) investigate the genera *Penicillium* and *Talaromyces* for potent fungal photosensitizers that exhibit selective cytotoxicity against microorganisms or cancer cells, and to (2) determine cultivation parameters for selected fungal strains to enhance the production of promising photosensitizers.

Methods: About 20 species were selected at the start of the project, which are known producers of anthraquinones, and subjected to a variety of growth conditions in petri dishes but also submerged cultures employing the OSMAC approach (e.g. variations in media, temperature, light conditions). For bioactivity-guided isolation, crude extracts of these cultures were tested for their photocytotoxic activity against cancer cell lines (nasopharynx, bladder, hypopharynx, esophageal, and nasal septum carcinomas) and their photoantimicrobial activity against microorganisms (e.g. *Candida albicans*,

Escherichia coli, and *Staphylococcus aureus*). In addition, the extracts were analyzed by UHPLC-HRMS/MS to allow dereplication and/or a first annotation of putatively interesting metabolites. To enhance the yield of promising photosensitizers, selected fungal producers were cultivated in various liquid media and under different cultivation conditions.

Findings: The first findings of this project revealed several photoactive raw extracts against pathogens but also cancer cells. So far, one of the most promising results was obtained with *Talaromyces islandicus* CBS 117284 and *T. stipidatus* CBS 37548, which showed a photocytotoxic effect at concentrations as low as 0.625 µg/mL and 12.6 µg/mL, respectively, under blue light (λ = 478 nm, H = 5.3 J/cm^2) for 15 minutes. While for these organisms the bioactivity-guided isolation is still in progress, several metabolites responsible for these phototoxic effects were putatively annotated in the purified fractions. Initial efforts to stimulate in *T. islandicus* an increased photosensitizer production in submerged cultures were positive.

Conclusions: The initial findings support our hypothesis that fungi from the genera *Penicillium* and *Talaromyces* can produce effective photosensitizers. These compounds demonstrate activity against microorganisms and/or cancer cells. Importantly, we've shown that photosensitizers can be produced in submerged cultures, which is crucial for future large-scale production in bioreactors.

Abstract ID: 2042 / Poster Number: 109.17P

Phylogenetic analysis of *Faecalicatena contorta* species from human gut microbiota – Distribution of short-chain fatty acids pathway

Presenting author: Dr. Christiane Bouchier, Institut Pasteur, Paris, France

Co-authors: *Damien Rei, Gérald Touak, Dominique Clermont*

The human gut microbiota is dominated by obligately anaerobic bacteria among which members of the phylum *Firmicutes* such as the family *Lachnospiraceae* are abundant. The metabolism of carbohydrates by the gut microbiota is a key process supplying nutrients and energy to the host. Due to their capability to produce short-chain fatty acid (SCFA) during glucose fermentation, some *Lachnospiraceae* members including the genus *Faecalicatena* are recognized as potentially beneficial gut microbiota member.

Based on a targeted-selecting approach, we isolated from a stool of a healthy adult, a strain phylogenetically close to the type strain of *Faecalicatena contorta* described in 2017. Here, we report the complete genome sequence of this *F. contorta* isolate. Using genomic analysis, its genome was compared to those of other *F. contorta* strains with a focus on the SCFA production pathway. The main result was that different arrangements of synthesis butyrate pathway genes were detected and this within the same species.

This study will increase our knowledge of a bacterium of great interest for the gut microbiota and make available for other studies a complete genome in the Genbank database. To date, less than10 genomic sequences are publicly available as scaffold assemblies.

Abstract ID: 1667

Phylogenetic position and metabolic capacities of large “pillotinaceous” spirochetes in termite guts

Presenting author: Dr. Sebastian Cristian Treitli, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Co-authors: *Undine Sophie Mies, Renate Radek, Patricia Zinnhardt, Reuter Lisa, Katja Platt, Natalie Röhr, Andreas Brune*

Termite guts harbor diverse assemblages of spirochetes that are either free-living or in association with various flagellated protists. Some of these spirochetes are unusually long (up to 100 µm) and possess a large cell diameter and numerous periplasmic flagella. Based on these and other ultrastructural features, previous studies have described several species exclusively on morphological traits and classified them in the family “Pillotinaceae”. Although such “pillotinaceous” spirochetes are quite abundant in certain termite hosts, they have not been cultured, and nothing is known about their metabolic properties and phylogenetic position.

To address these questions, we isolated single cells of large spirochetes from the gut contents of various termites, amplified their genomic DNA using single-cell techniques, and used their 16S rRNA gene sequences to link individual phylotypes to their corresponding morphotypes by electron microscopy and fluorescence *in situ* hybridization (FISH). For selected representatives, we sequenced the amplified genomes to investigate their metabolic potential.

Phylogenetic analysis revealed that “pillotinaceous” spirochetes do not form a separate family but represent three distinct, well-supported clades in the family Breznakiellaceae, which harbors the majority of termite gut spirochetes. Based on phylogeny and FISH, it seems that *Incisitermes tabogae* and *I. schwarzi* harbor only one phylotype, while *Kalotermes flavicollis* and *Reticulitermes santonensis* harbor at least two distinct species. A comparison of the ultrastructural features of large spirochetal cells in gut homogenates with previous morphological descriptions confirmed that the phylotypes from *I. tabogae*, *I. schwarzi* and *K. flavicollis* belong to the genus *Hollandina*, the phylotypes from *K. flavicollis* and *R. flavipes* to the genus *Pillotina*, and phylotypes from *R. flavipes* to the genus *Clevelandina*. Functional genome analysis revealed that the *Pillotina* species from *K. flavicollis* encodes an array of glycosyl hydrolases (GHs) involved in cellulose and hemicellulose degradation, and a large number of ABC sugar transporters that may be involved in the uptake of the resulting mono- and oligomers. By contrast, the *Hollandina* species from *I. tabogae* lacks most of the GHs found in *Pillotina*

but encodes a complete Wood–Ljungdahl pathway and a *nif* operon, suggesting that its metabolism is focused on reductive acetogenesis and nitrogen fixation. Our results provide the first molecular information on large “pillotinaceous” spirochetes and highlights their potential functions in the termite gut.

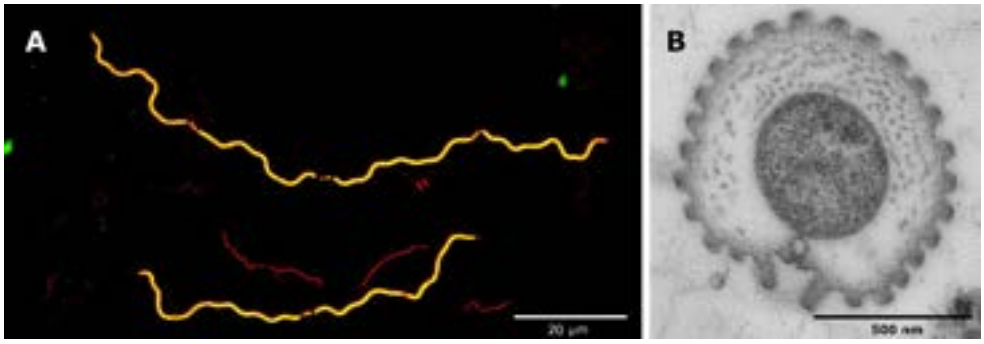


Figure 1: **A** – FISH image of large spirochetes from *K. flavicollis*. Cells of *Pillotina* sp. hybridize with both the general Spiro-36 probe (red) and the specific Pil-197 probe (green). **B** – Cross section through a large spirochete from *K. flavicollis* showing the typical morphology of *Pillotina*.

Abstract ID: 1971 / Poster Number: 224.15P

Phylogeny and comparative genomics of Arthromitus gen. nov. and other lineages of segmented filamentous bacteria in the order Lachnospirales

Presenting author: Ms. Panagiota Folla, Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany

Co-authors: *Sebastian Cristian Treitli, Evgenii Protasov, Undine Sophie Mies, Joana Kästle Silva, Katja Platt, Andreas Brune*

More than 170 years ago, Joseph Leidy described microbial filaments colonizing the hindgut of varying arthropods as members of the genus *Arthromitus*. Later recognized as endospore-forming bacteria, they were hypothesized to constitute a life stage of *Bacillus cereus*. Meanwhile, we know that the segmented filamentous bacteria (SFB) in arthropod guts belong to the order *Lachnospirales* (*Clostridia*) and are distinct from similar morphotypes found in mammalian intestines. However, their diversity and physiology remain uncharted, mostly due to the lack of cultured representatives. Phylogenetic analysis of the 16S rRNA genes in long-read amplicon libraries revealed that the gut microbiota of termites, cockroaches, millipedes, and scarab beetle larvae comprises several family-level lineages of SFB in the order of *Lachnospirales*. Based on phylogenomic analysis of 107 metagenome-assembled genomes (MAGs) from various termites, we formally describe the first representatives of these lineages. The filamentous morphotypes were assigned to the respective taxa via whole-genome amplification of capillary-picked single-filaments and RNA-FISH. Preliminary results of a comparative genome analysis suggest a divergent distribution of carbohydrate-active enzymes (CAZymes) and amino acid degradation pathways, indicating that the primary energy sources differ between taxa. We are currently conducting a comprehensive genome annotation to elucidate the major metabolic pathways and cellular processes that characterize each of the lineages.

Abstract ID: 1513 / Poster Number: 295.17P

Physiological and genomic potential of extremotolerant chemolithoautotrophic Ralstonia sp. U7_2 for CO2 utilization and conversion to valuable products

Presenting author: Mr. Plaban Kumar Saha, National Institute of Technology Dutgapur, Durgapur, India

Co-authors: *Swatilekha Sarkar, Mouli Bhattacharyya, Pinaki Sar, Plaban Kumar Saha*

The global carbon cycle has been significantly disrupted by anthropogenic emission of carbon-dioxide (CO_2), resulting in severe environmental challenges. Biological approaches for CO_2 utilization and valorization, particularly by chemolithoautotrophic microorganisms, have gained recent interests. In this study, the chemolithoautotrophic *Ralstonia* sp. U7_2, isolated from deep subsurface igneous rock beneath the Deccan Traps, India, was characterized for its ability to grow at elevated CO_2 levels (up to 10 mmol/L), utilize up to 7 mmol CO_2/L culture and produce valuable biomolecules. The strain could grow in presence of wide ranges of temperature (30℃-70℃), pH (pH 3-11), salinity (0-10% w/v NaCl), and desiccation (up to 250 days). Response surface methodology estimated the optimal growth of strain U7_2 at 56°C temperature, 8.5 mmol/L CO_2 concentration, 7.6 pH, and 2% salinity (w/v NaCl). *Ralstonia* sp. U7_2 demonstrated versatility in using inorganic substrates as electron donors (hydrogen, sulfide, nitrite, etc.) and oxygen, nitrate, sulfate, etc. as electron acceptors. Whole-genome sequencing of the strain revealed a robust genetic framework, including genes for CO_2 fixation pathways (CBB and rTCA cycles) and inorganic substrate oxidation (hydrogen, thiosulfate, nitrite, etc.). The genome analysis also indicated the presence of putative genes linked to survival under extreme conditions, as well as synthesis of exopolysaccharides, polyhydroxyalkanoates, short-chain fatty acids, antibiotics, and other biomolecules. Notably, the strain could produce various volatile fatty acids (acetic, propionic, butyric and heptanoic acid) at high CO_2 concentration. This study highlighted the physiological and genetic potential of extremotolerant *Ralstonia* sp. U7_2 for CO_2 utilization and bioconversion to valuable products.

Abstract ID: 1787 / Poster Number: 430.15P

Physiology and Transcriptional Analysis of ppGpp-Related Regulatory Effects in Streptomyces diastatochromogenes 1628

Presenting author: Xuping ShenTu, China Jiliang University, Hangzhou, China

Co-authors: *Kai Sun,*

ppGpp is a ubiquitous small nucleotide messenger that mediates cellular self-protective responses under environmental stress. However, the mechanisms of ppGpp that control transcription and other metabolic processes depend on the species, and ppGpp regulates the same process via different mechanisms. The level of ppGpp is regulated by RelA/SpoT homolog (RSH) enzymes that synthesize and hydrolyze the alarmone. Here, we constructed a ppGpp0 strain and monitored the effects of ppGpp on the transcriptional level, physiology, and secondary metabiotic production in the antibiotic producer *Streptomyces diastatochromogenes* 1628. The results showed the cell division and growth of ppGpp0 increased by measurement of gene transcription and DCWs. The utilization of nitrogen was affected depending on the nitrogen type with a significantly higher DCW of the ppGpp0 mutant in the medium supplied with the yeast extract and a lower growth rate in the inorganic nitrogen ammonium salt. The ppGpp-mediated stringent response could not affect the usage of carbon resources. More importantly, ppGpp0 inhibited the expression of antibiotic clusters and the production of toyocamycin and tetramycin P. The antibiotic resistance was also significantly downregulated in the ppGpp0 mutant. In conclusion, this study showed detailed changes in ppGpp-mediated stringent responses on *S. diastatochromogenes* 1628 cell growth, nutrient utilization, morphological characteristics, antibiotic production, and resistance, which will provide insights into the role of ppGpp in Streptomyces.

Abstract ID: 2612 / Poster Number: 249.15P

Pigment and extracellular enzyme profiling of a bacterial strain Roseateles amylovorans bsSlp3-1T

Presenting author: Ms. Darya Guliayeva, Institute of Microbiology of the National Academy of Science, Minsk, Republic of Belarus

Co-authors: *Anastasiya Sidarenka*

Introduction: *Roseateles* species are widespread in the aquatic environment and have a number of biotechnological properties. For instance, they produce carotenoids and are capable of hydrolyzing various organic compounds.

Objective: The aim of this study is the bioprospecting of the strain *Roseateles amylovorans* bsSlp3-1^T, isolated from a freshwater reservoir (Minsk, Belarus), for the potential production of pigments and extracellular enzymes.

Method: The whole-genome sequencing was performed using Illumina MiSeq and Oxford Nanopore MinION technologies. Amylase and cellulase activities were measured using Megazym assay kits (K-CERA and K-CellG5). Protease activity was determined by azocasein assay.

Findings: Functional analysis of the genome of *R. amylovorans* bsSlp3-1^T (accession number CP104562) revealed the presence of key genes of spirilloxanthin biosynthesis (*crtC, crtD, crtF*), genes encoding alpha-amylase, glucoamylase, endoglucanase, ClpP protease, esterase and cutinase. In vitro studies revealed that *R. amylovorans* bsSlp3-1^T produced the pink pigment in minimal salt medium with sodium pyruvate, casein hydrolysate and glucose (single carbon source) at low temperatures (4–10°C) after incubation for 2–4 weeks. Amylase production by the strain was confirmed on starch medium (hydrolysis zone of 32–36 mm), with amylase activity measuring 0.415 CU/ml (40°C). *R. amylovorans* bsSlp3-1^T was capable of hydrolysing cellulose, casein and gelatin, with cellulose and protease activity measuring 0.024 U/ml (40°C) and 17.9 U/ml, respectively. The degradation of poly-Ɛ-caprolactone (PLC) was detected upon cultivation of *R. amylovorans* bsSlp3-1^T in R2A or minimal salt medium with PLC films. All findings indicate that *R. amylovorans* bsSlp3-1^T is biotechnologically promising and requires further investigation.

Abstract ID: 3044 / Poster Number: 414.16S

Plant diversification boosts soil microbial networks and arbuscular mycorrhizal fungal community in vineyards, with stronger effects in arid regions

Presenting author: Dr. Ana Vanessa Aguilar Paredes, Scuola Superiore Sant'Anna, Pisa, Italy

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Keywords: soil diversity, soil microbial networks, cover crops, vineyards, sustainable agricultural practices, climate change, agroecology

Organic agronomical management can alter the distribution and relative dominance of soil microbial communities, resulting in positive effects on biodiversity and agroecosystem functionality. In organic systems, sustainable agricultural practices, such as plant diversification by cover crops, may further promote soil microbial diversity and improve soil chemical parameters, particularly in woody crops like vineyards, leading to ecosystem services. However, the effects of these practices on symbiotic fungi, such as arbuscular mycorrhizal fungi (AMF), prokaryotes, and their interactions can vary across climatic regions. This study aimed to investigate how cover crops influence soil AM fungal and prokaryotic communities, and how these changes are associated with soil chemical parameters. Chilean vineyards, which have long incorporated cover crops, were studied across four climatic zones. Under drought conditions, the relationship between cover crops, AM fungal diversity, was found stronger. Additionally, networks between AMF and prokaryotes were more complex in abiotic stressed zones. Soil parameters was linked to the abundance of specific AMF and prokaryotes found in the hubs of soil networks. This study allow to better understand the mechanisms involved in the sustainability and resilience of viticulture against environmental challenges and provides insights into the Chilean microbial diversity.

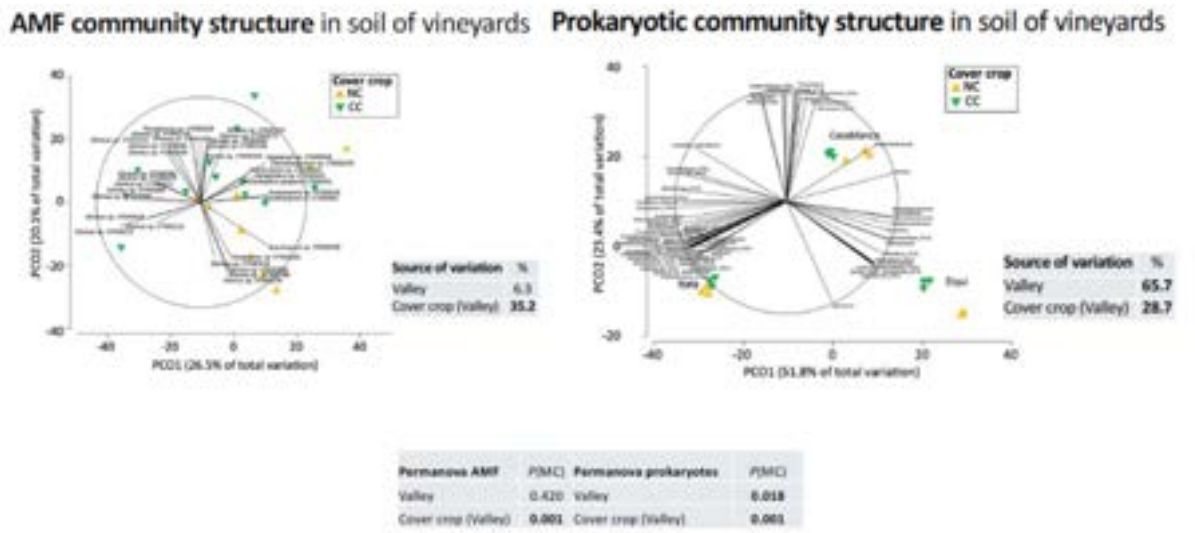


Figure 1: AMF and prokaryotic community structure in soil of vineyards.

Abstract ID: 860 / Poster Number: 384.17P

Plant growth-promoting bacteria inhibit fungal plant pathogens

Presenting author: Dr. Josephine Giard, Heriot-Watt University, Edinburgh, United Kingdom

Co-authors: *Angela Feechan, Stephen Euston, Michele Partipilo, Pablo Ivan Nikel, Amedea Perfumo, Leena Kerr, Ross Alexander*

Climate change brings severe agricultural challenges as crops are exposed to increased levels of both abiotic (drought, waterlogging) and biotic stresses (fungal diseases). A large body of literature now shows that by enriching soils with bacteria possessing plant growth-promoting (PGP) characteristics, plant resilience against pathogens can be improved. However, to identify beneficial bacteria, it is important to first investigate their PGP potential. Here, we assessed the PGP potential of selected (novel) soil bacteria of local (UK) and global origin, including Pseudomonas, Mucilaginibacter, and Paenibacillus species. Employed approaches included assessment of the production of PGP metabolites, solubilisation of (in)organic compounds, enzyme activity, tolerance of stressors, biofilm formation, and nitrogen fixation. We found that all investigated bacteria possess some but not all of the PGP properties. Ps. protegens DTU 9.1, Ps. koreensis C63, and Ps. koreensis D70 showed the greatest PGP potential while M. gossypiicola and M. frigoritolerans were the least active. Subsequently, we assessed the potential of these bacteria to inhibit the growth of several different fungal pathogens (Botrytis cinerea, Ramularia collo-cygni, and Zymoseptoria tritici). We found that a majority of bacteria were able to inhibit the growth of at least one of the fungal pathogens. Analysis of genomic sequencing data suggests that some of these bacteria produce novel antifungal compounds. We are currently working to purify and identify these novel antifungal compounds, and to test the bacteria's potential to be used in biocontrol strategies to prevent and treat plant diseases.

Abstract ID: 4112 / Poster Number: 379.17P

Plant-growth-promoting potential of bacteria from organic and chemically-treated red raspberry roots and rhizosphere

Presenting author: Dr. Olja Medić, University of Belgrade – Faculty of Biology, Belgrade, Serbia

Co-authors: *Ivana Marić, Iva Rosić, Marina Anteljević, Ivan Nikolić, Tamara Ranković, Slaviša Stanković, Tanja Berić*

Raspberries are commercially important fruit crops in Serbia and all temperate regions. The excessive use of synthetic fertilizers in agriculture poses a serious threat to the environment and health of all living organisms. Application of beneficial plant-growth-promoting (PGP) bacteria is an eco-friendly alternative that will ensure crop productivity in an efficient and safe manner.

The objective of this research was to determine the plant-growth-promoting potential of bacteria from organic and chemically-treated red raspberry (*Rubus idaeus* L.) roots and rhizosphere.

Isolation of bacteria was performed by a high-throughput microtiter plate dilution method. Bacterial isolates were screened for five PGP traits – phosphate solubilization, production of siderophores, indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, and ammonia.

Total of 482 bacterial isolates were obtained from chemically-treated and 351 from organic root and rhizosphere samples. Among the isolates from chemically-treated samples, 35.5% were phosphate solubilizers, while this trait was detected in 29.6% of isolates from organic samples. Out of these, 115 isolates from chemically-treated and 60 from organic samples were able to produce siderophores. IAA production was detected in 55 isolates, ACC deaminase was produced by 47 isolates, and 14 were able to produce ammonia. Nine isolates from chemically-treated and five from organic samples were positive for all five tested PGP traits.

Both chemically-treated and organic red raspberry roots and rhizosphere present a valuable source of PGP bacteria. Fourteen bacterial isolates with multifaceted PGP activities were selected as most promising candidates for potential applications to improve the growth and yield of raspberry.

Abstract ID: 3411 / Poster Number: 237.17F

Plasmid copy number analysis reveals universal rules governing plasmid biology

Presenting author: Mrs. Paula Ramiro-Martinez, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

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Introduction: Plasmids –autonomously replicating DNA molecules– are pervasive in the microbial world, where they fuel evolution as key vehicles for gene transfer. Plasmids are as diverse as their hosts and exhibit a broad range of replication and mobility strategies, genetic repertoires, host ranges, sizes, and copies per cell. While many of these characteristics have been extensively characterized, the factors determining plasmid copy number (PCN) are still poorly understood. PCN is essential for both plasmid biology and evolution because it affects the plasmid's stability, the metabolic cost to the host organism, and the gene expression levels. Understanding PCN is crucial for gaining insights into plasmid evolution.

Objective: This study aimed to comprehensively analyze the copy number of natural plasmids for the first time. Method: Here, we leveraged extensive DNA sequencing data to comprehensively analyse the copy number of >6300 plasmids from different Gram-positive and Gram-negative bacteria genera.

Findings: We found that PCN is highly variable and tightly associated with plasmid lifestyles. Moreover, we uncover a universal scaling law that links copy number and plasmid size across phylogenetic boundaries, indicating that pervasive constraints orchestrate the PCN-size trade-off. Strikingly, this correlation suggests that despite huge variability in plasmid sizes and PCNs, the total DNA per plasmid is constant and proportional to chromosome size in all bacterial species analyzed. In other words, independently of plasmid replication type or size, any given plasmid comprises ~2.5% of the cellular nucleotide pool.

Conclusions: Altogether, our work pioneers large-scale analyses of PCN while uncovering the fundamental rules that govern plasmid biology.

Abstract ID: 2537 / Poster Number: 246.15P

Polyethylene terephthalate (PET) primary degradation products affect c-di-GMP-, cAMP-signaling and quorum sensing (QS) in Vibrio gazogenes DSM 21264

Presenting author: Dr. Christel Vollstedt, University of Hamburg, Hamburg, Germany

Co-authors: *Lena Preuß, Malik Alawi, Albert Dumnitch, Anja Poehlein, Wolfgang Streit*

Introduction: The plastisphere offers microorganisms a new niche. Due to the increasing plastic pollution in the oceans, it is becoming increasingly important to understand the microbial ecology of the plastisphere and the associated effects.

Objective: Since Vibrio species are a common representative of the plastisphere, we investigate the effects of different plastic degradation products on our model organism *Vibrio gazogenes* DSM 21264. DSM 21264 possesses a previously described PET-Esterase (PET6), whose role in nature is still unclear.

Method: Gene expression in DSM 21264 under different conditions was investigated by transcriptome analyses. Growth experiments and promoterfusion studies were performed to support the data.

Findings: During initial attachment and biofilm formation, the surface doesn't yet appear to have a major influence on the expression of PET6. However, the transcription and enzyme production of PET6 is sufficient to release the primary degradation product BHET and TPA in µM amounts after 24 hours of incubation on PET film.

Interestingly, the expression of *pet6* increases with higher amounts of BHET in planktonically grown cultures. High levels of BHET also disrupt key signalling circuits involved in bacterial quorum sensing (QS), c-di-GMP and cAMP-CRP signalling, resulting in a lack of biofilm formation, synthesis of prodigiosin and altered colony morphology. While BHET had a broad effect, TPA mainly affected bacterial QS by attenuating the expression of the CAI-I autoinducer synthase gene.

Conclusion: These observations imply a potential role of BHET and TPA as nutritional signals in DSM 21264 that may affect its growth and survival in the plastisphere.

Abstract ID: 3981 / Poster Number: 363.15F

Polyoxomolybdate Ionic Liquids: Advanced Coatings for Mitigating Microbiologically Influenced Corrosion

Presenting author: Mrs. Mariella Malefioudaki, CSIC-Universidad de Zaragoza, Zaragoza, Spain

Corrosion in marine environments presents significant challenges across various industries, contributing to global economic losses estimated at $50–80 billion annually. A major factor in this degradation is microbiologically influenced corrosion (MIC), primarily driven by sulfate-reducing bacteria (SRB) and methanogenic archaea (MA). Conventional corrosion mitigation strategies, including cathodic protection and biocides, often fail to prevent the colonization of corrosive microbial communities, requiring the development of alternative, broad-spectrum antimicrobial strategies to prevent such biofilm formation. Recently, molybdate has emerged as a potential alternative to traditional biocides and nitrate. This study explores polyoxometalate ionic liquids (POM-ILs) as protective coatings for brass coupons. Two prototype polyoxomolybdate-based POM-ILs, $[(CH_3(CH_2)_6)_4N]_2[Mo_6O_{19}]$ and $[(CH_3(CH_2)_6]_4N]_4[Mo_8O_{26}]$, demonstrated strong antimicrobial activity and excellent resistance to acidic conditions. Electrochemical impedance spectroscopy and electron microscopy studies confirm the protective capabilities of POM-IL coatings under extreme corrosive conditions. The coatings formed a hydrophobic, acid-resistant, and biocidal protective layer, effectively preventing microbial colonization and corrosion. These findings highlight the potential of POM-ILs as next-generation solutions for mitigating MIC, offering a synergistic approach to corrosion protection and microbial control in harsh environments.

Abstract ID: 1746 / Poster Number: 049.17P

Polyphosphate affects protein aggregation and cellular energetics in dormant bacteria

Presenting author: Ms. Shreya Ramesh, VIB-KU Leuven, Leuven, Belgium

Co-authors: *Celien Bollen, Wouter Duverger, Natalie Verstraeten, Joost Schymkowitz, Frederic Rousseau, Jan Michiels*

Entering a transiently dormant state before antibiotic treatment allows bacteria to survive the stressor and cause recurring infections. We have shown that as cells enter dormancy, they sequester their proteins into liquid-like condensates, and decrease their cellular ATP levels. However, as the dormancy deepens, these condensates undergo a phase transition to form solid, β-sheet rich aggregates. In contrast to the aggregates, condensates are easy to dissolve, which is necessary for the cells to recover and regrow. Therefore, the formation and dissolution of the condensates, and the change in cellular ATP levels are crucial for bacterial dormancy. Understanding the key regulators of these processes might reveal potential targets to prevent antibiotic failure.

Long chains of inorganic phosphate called polyphosphates (polyP) have been shown to keep proteins in a more soluble intermediate state. Additionally, the phosphoanhydride bond of polyP may act as an energy reservoir. We, therefore, investigated whether polyP has a role in the two abovementioned crucial processes governing cellular dormancy using different microscopy and spectroscopy techniques.

We observed that polyP delays the phase transition of the condensates, whereas a lack of polyP causes β-sheet enrichment within the condensates, accelerating their maturation. We also observed a delayed ATP replenishment in the absence of polyP, unveiling a potential role for polyP as an energy currency. Combined, our results reveal a crucial dual role for polyP, as a scaffold and energy reservoir, in dormant bacteria.

Abstract ID: 2161

Polypropylene biodegradation; identification of a novel metabolic pathway for the assimilation of branched chain aliphatic polymers

Presenting author: Dr. Joseph A. Christie-Oleza, University of the Balearic Islands, Palma, Spain

Co-authors: *Theo Obrador-Viel, Maria del Mar Aguiló-Ferretjans, Robyn J. Wright, Vinko Zadjelovic, Balbina Nogales, Rafael Bosch*

Theo Obrador-Viel[1], Maria del Mar Aguiló-Ferretjans[1], Robyn J. Wright[2], Vinko Zadjelovic[3], Balbina Nogales[1], Rafael Bosch[1], **Joseph A. Christie-Oleza[1]**

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Polypropylene (PP) is the second most widely produced plastic globally, accounting for nearly 19% of total polymer production, surpassed only by polyethylene (PE). Both polymers are highly resistant to degradation due to their high crystallinity, hydrophobicity, and the absence of efficient enzymes targeting their C-C polymer backbones. However, abiotic weathering can oxidize and fragment these polymers, generating diverse aliphatic compounds that microbes can assimilate and mineralize. While PE degradation products are processed via the beta-oxidation pathway, no metabolic pathway for PP degradation products had been identified until now.

In this study, we isolated bacteria capable of growing on weathered PP, confirmed by carbon consumption analysis and isotopic signature tracking. Proteogenomic characterization revealed a novel metabolic pathway enabling these bacteria to decarboxylate the methyl branches characteristic of PP, facilitating biodegradation. Targeted mutagenesis further validated the pathway's role in processing PP-derived compounds as well as mitigating their toxicity. Comparative metagenomic analyses of biofilms on PP versus other environmental compartments highlighted the enrichment of this pathway in PP-associated plastispheres, establishing the pathway as a reliable biomarker for PP biodegradation.

This work presents the first mechanistic understanding of the metabolic requirements for PP degradation, offering critical insights into the microbial assimilation of recalcitrant plastics and advancing our knowledge of plastic biodegradation pathways.

Abstract ID: 3776 / Poster Number: 354.17P

Pomegranate and citrus by-products: microbial fermentation for innovative functional food applications

Presenting author: Assoc. Prof. Lucia Vannini, University of Bologna, Cesena (FC), Italy; Interdepartmental Centre for AgriFood Industrial Research, University of Bologna, Cesena (FC), Italy

Co-authors: *Fatemeh Shanbeh Zadeh, Beatrice Cellini, Emiliana Giordano, Francesca Patrignani, Rosalba Lanciotti*

Pomegranate and citrus processing produce large amounts of waste, up to 65% and 78%, respectively. These discarded residues are rich in valuable bioactive compounds. Microbial fermentation offers a promising biotechnological approach to recover and convert them into functional ingredients suitable for nutrient-rich food applications.

This study aims to screen and select lactic acid bacteria (LAB) and yeast strains for fermenting clementine and pomegranate by-products (BPs) to obtain functional food ingredients.

The growth performances of the strains and the effects of fermentation on antioxidant activity, total phenolic content (TPC) and volatile compounds (SPME/GC-MS) of the matrices were considered.

The results revealed that several yeasts rapidly grew within 24–48 hours and generally led to higher TPC. LAB adapted within three days and some of them significantly enhanced antioxidant activity. More than 185 volatiles were identified highlighting enhanced flavor and aroma through fermentation and in some cases reducing off-flavors.

Overall, these findings underscore the importance of strain selection and the potential for optimizing fermentation parameters to enhance both clementine and pomegranate BPs into functional food ingredients.

This work is partly funded under the National Recovery and Resilience Plan Mission 4 Component 2 Investment 1.3 – Call for tender No.341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code PE00000003, Concession Decree No.1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title “ON Foods – Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods”

Abstract ID: 2891 / Poster Number: 262.15P

Porphyromonas gingivalis HmuS protein, functioning as a de-chelatase, influence bacterial virulence

Presenting author: Mr. Patryk Cierpisz, University of Wroclaw, Wroclaw, Poland

Co-authors: *Teresa Olczak, Michał Śmiga*

Introduction: Periodontal diseases are chronic inflammatory conditions affecting tooth-supporting tissues, often leading to gum recession and tooth loss. *Porphyromonas gingivalis*, a key pathogen in chronic periodontitis, lacks a functional heme biosynthesis pathway and relies on heme as a primary source of iron and protoporphyrin IX for survival and virulence. To acquire heme, the bacterium utilizes the Hmu system composed of a hemophore-like protein (HmuY), an outer membrane transporter (HmuR), and four proteins (HmuSTUV) with unclear functions.

Objective: The mechanism of iron removal from heme in *P. gingivalis* is unknown. HmuS, a putative chelatase with homology to the CobN protein, is hypothesized to function as a de-chelatase involved in this process. This study aimed to explore the activity of the HmuS protein and its role in *P. gingivalis* growth and virulence.

Method: UV-visible spectroscopic methods were used to characterize the enzymatic activity of HmuS. To analyze the importance of HmuS for *P. gingivalis* growth and virulence, the phenotype of the *hmuS* deletion mutant strain (Δ*hmuS*) was characterized. The influence of the *hmuS* gene deletion on global gene expression was examined using RNA sequencing (RNA-seq).

Findings: HmuS acts as a de-chelatase and extracts iron from heme. Deleting the *hmuS* gene induces changes in the expression of 80 genes, significantly decreases the ability to form biofilm by *P. gingivalis,* and decreases the activity of cysteine proteases, gingipains.

Conclusion: These results highlight the critical role of HmuS activity in iron acquisition using a heme source, ultimately supporting *P. gingivalis* survival and virulence potential.

Abstract ID: 2897 / Poster Number: 263.15P

Porphyromonas gingivalis produces a functional HemH homolog important for its virulence

Presenting author: Michał Śmiga, PhD, University of Wrocław, Wrocław, Poland

Co-authors: *Teresa Olczak*

Introduction: *Porphyromonas gingivalis* contributes to dysbiosis in the oral microbiome, leading to periodontal diseases. As a heme auxotroph, it acquires heme from human hemoproteins. Despite encoding the three final enzymes in the heme biosynthesis pathway, including ferrochelatase (HemH), their role in *P. gingivalis* remains unknown[1,2].

Objective: *P. gingivalis* can grow in a medium without heme but supplemented with protoporphyrin IX (PPIX) and iron, indicating preservation of a ferrochelatase activity. This study aimed to determine the role of HemH in heme formation and its importance for *P. gingivalis* virulence.

Methodology: The function of HemH was analyzed theoretically using a comparison with closely related homologs and experimentally by assessing the impact of the *hemH* gene deletion on *P. gingivalis* phenotype and global gene expression using RNA sequencing (RNA-seq).

Findings: *P. gingivalis* HemH and its homologs in other *Porphyromonas* species exhibit low homology to classical *E. coli* HemH protein, but possess conserved key amino acid residues essential for ferrochelatase function. *P. gingivalis* HemH is important for growth in the presence of PPIX and iron. Moreover. deletion of the *hemH* gene affects the expression of ~120 genes, many of which are involved in heme acquisition and bacterial virulence.

Conclusion: HemH is important for *P. gingivalis* growth and serves as an alternative mechanism to obtain heme. It may support *P. gingivalis* survival and virulence potential, likely playing a similar role in other *Porphyromonas* species.

Abstract ID: 473 / Poster Number: 240.17P

Portable Lab for Shipping (POLS): A Rapid Onboard Detection System for Microbial Contaminants in Ballast Water

Presenting author: Dr. Agni Hadjilouka, European University of Cyprus, Nicosia, Cyprus

Co-authors: *Eleni Varda, Lazaros Konstantinou, Emmanouil Loulakis, Theofylaktos Apostolou, Lazaros Dougiakis, Antonios Inglezakis, Constantinos Loizou*

The discharge of ballast water, used to maintain ship stability and navigational balance, has been recognized as a significant contributor to marine pollution. This water often contains diverse microorganisms, some of which can disrupt local ecosystems. In response to international regulations on ballast water treatment, this study developed a rapid, cost-effective, and accurate onboard method to help ship crews monitor regulatory compliance in real time. This research focused on the development of a Portable Lab for Shipping (POLS) method for the detection of microbial contaminants in ballast water. The method targets two microorganisms regulated by the International Maritime Organization (IMO): Escherichia coli and Enterococcus spp. The detection platform integrates potentiometric analysis, biosensor technology, the Bioelectric Recognition Assay (BERA), and a custom algorithm for data analysis. The POLS system exhibited high sensitivity and selectivity in detecting E. coli and Enterococcus spp. in ballast water, achieving a detection limit of 10^2 CFU/100 mL for both microorganisms and accuracies of 84.5% and 93.6%, respectively. By combining portability, efficiency, and real-time analysis, this study introduces a novel method for onboard assessment of ballast water microbial quality, addressing a critical need in marine environmental management. Offering a viable alternative to conventional laboratory-based methods, the POLS system delivers timely and reliable results in just three minutes, immediately notifying the end-user via an Android or iOS device, thereby significantly enhancing regulatory compliance across the shipping industry.

Abstract ID: 2101

Possible protection measures against microbially influenced corrosion on the inside of foundation structures of offshore wind power systems

Presenting author: Dr. Nicole Adam-Beyer, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

Co-authors: *Carolin Skottke, Mark Schmidt, Claus-Henning Solterbeck, Christian Niepel, Udo Niepel, Gerrit Haake, Jana Schloesser, Mirjam Perner*

Iron corrosion is a widespread and well-known problem in the maritime industry. It is estimated that corrosion processes cause financial losses of around US $ 2.5 trillion worldwide. Microbially influenced iron corrosion (MIC) accounts for up to 20% to 40%, posing a massive problem. This applies in particular to the wind energy sector, as wind farms are characterized by a relatively short lifespan of 30 years compared to other alternative energies, with the economic break-even point being 15-20 years. An improvement in corrosion resistance therefore has a direct effect on economic efficiency. Different foundation structures exist in offshore wind farms with the monopile structure being the prevailing type. It is a single steel-made, cylindrical and hollow pile that is driven into the seabed, trapping seawater, and thus exhibiting only a limited exchange with the environment. Microbial activity and biogeochemical processes inside the monopile, which influence the MIC, are highly impacted by these special conditions**.** So far, no data has been published on the inside of monopiles and the effects of MIC therein. Commonly used corrosion protection measures only address electrochemical corrosion.

In an interdisciplinary project we aim at developing effective, environmentally friendly and economically feasible protection measures against MIC in offshore monopiles. For this purpose, a solid understanding of the environmental conditions, microbial communities and mechanisms involved in MIC inside the monopiles is necessary. Thus, we carried out in situ incubations inside a monopile in the North Sea, incubating commonly used mild steel at 6 and 21 m water depth, accompanied by measurements of biogeochemical parameters. First results show a strong seasonal stratification of the water column inside the monopile with the absence of oxygen and accumulation of biogenic methane and hydrogen sulfide (up to millimolar concentrations) in bottom waters at certain times of the year. Despite storm-related ventilation events of the water column, RNA-based taxonomic profiles of biofilms from steel samples incubated at 21 m exhibited an enrichment in sulfate reducing Bacteria and methanogenic Archaea,

demonstrated to be relevant players in MIC under anoxic conditions. Steel-associated communities at 6 m water depth, hallmarked by oxic conditions, were enriched in Fe-oxidizing Bacteria. Current and future work involves ex situ and further in situ incubations of suitable coated steel and steel alternatives as well as the development of locally applied inhibition measures to identify the most efficient measures for MIC inhibition inside monopiles.

Abstract ID: 3078 / Poster Number: 325.16F

Post-Cultivation Processing Alters the Biological Properties of microalga Chlamydomonas reinhardtii

Presenting author: Ms. Tamara Vujović, Ruđer Bošković Institute, Zagreb, Croatia

Co-authors: *Marija Miloš, Lucija Vranjković, Tina Paradžik, Mirela Ivančić Šantek, Rozelindra Čož-Rakovac, Ivančica Strunjak-Perović*

Introduction: Green microalga *Chlamydomonas reinhardtii* has been widely used as a model microorganism in biotechnology. However, studies investigating this alga as a potential source of bioactive compounds are still scarce due to the difficulties that often occur during post-cultivation processing, especially dewatering of the harvested microalgal biomass.

Objective: To determine whether the biological properties of *C. reinhardtii* are influenced by the post-cultivation processing methods, we used six different drying strategies on the harvested microalgal biomass. The biological properties were investigated using several *in vitro* assays.

Methods: Wet microalgal biomass was oven-dried at 40 °C, 50 °C, 60 °C, 80 °C, or 100 °C, or freeze-dried. Dry biomass was used to prepare extracts that were further concentrated using solid-phase extraction and solvents of different polarities. Antidiabetic, antilipidemic, and neuroprotective properties of two *C. reinhardtii* fractions (methanol – MeOH and dichloromethane – DCM) were evaluated using enzyme inhibition assays. Additionally, the microdilution technique was used to test antimicrobial susceptibility.

Results: Our findings showed that different drying methods used during post-cultivation processing caused significant variations in the *C. reinhardtii* biological properties. Also, results varied between the two tested fractions, with overall results showing that *C. reinhardtii* methanolic fractions have greater biological potential compared to DCM fractions.

Conclusion: Optimization of the post-cultivation processing parameters is one of the possible solutions for the lack of studies regarding microalgal bioactive compounds. Specifically, these parameters have a great effect on the biological properties of *C. reinhardtii*.

Abstract ID: 3254 / Poster Number: 377.16P

Potential of kaolin, riboflavin and skimmed milk as natural substancies for UV protection and formulation of Erwinia amylovora specific bacteriophages

Presenting author: Dr. Andjelka Prokic, University of Belgrade – Faculty of Agriculture, Belgrade, Serbia

Co-authors: *Milan Ivanović, Jelena Adamović, Nebojša Nedić, Nevena Zlatković, Katarina Gašić, Aleksa Obradović*

Phage therapy is intensively studied alternative for control of fire blight of pome-fruits caused by *Erwinia amylovora* (Ea). However, phage efficacy in vivo is affected by diverse factors, such as UV irradiation limitting persistence and causing direct damage of the viral DNA. Therefore, in order to prolong Ea phages survival on plant surfaces UV-protective formulations are needed.

This study aimed to evaluate the protective effect of three natural compounds: kaolin, riboflavin and skimmed milk, on vitality of Ea phage particles after time-depended exposure to UV light *in vitro*.

The bacteriophage ΦEa10 was formulated by mixing phage suspension ($1x10^5$ PFU/ml) with 0.5, 5 and 10% of potentially protective substance, in wells of 96 multiwell plate in ratio 1:1, prior to exposure to UV light (λ=254 nm). The phage titer was determined after 30, 60, 120 and 300s of irradiation by plaque assay on NYA medium using susceptible *E. amylovora* strain KBI 52 as a host.

Significant difference in viability of the non-formulated and formulated phages was observed for all three compounds at applied concentrations. Riboflavin at 5 and 10% enabled longest persistence of phages, decreasing phage titer for 0.4 and 0.3 log PFU/ml, respectively after their exposure to UV source for 300s. The same concentrations of kaolin reduced titer for 1.03 and 0.52 log PFU/ml and skimmed milk formulations for 1.07 and 0.9 log PFU/ml, respectively.

Results indicate that formulation of *Ea* phages can increase their UV-persistence and stability and improve efficiency in fire blight biocontrol.

This research was supported by the Science Fund of the Republic of Serbia, GRANT No. 7421, Innovative solutions in phage-mediated biocontrol of fire blight – InnovaPhage.

Abstract ID: 1774 / Poster Number: 045.15P

Potential pathogenicity of Bacillus mycoides strains from active layer soils in Siberian tundra

Presenting author: Dr. Yung Mi Lee, Korea Polar Research Institute, Incheon, South Korea

Co-authors: *Woohyun Kim, Jehyun Jeon, Hae Woong Choi, Gyeongseo Suk, Mincheol Kim, Min Jung Kwon*

The rapidly warming Arctic has raised concerns about the possible emergence of pathogenic microorganism. Despite the wide distribution and known pathogenicity in the human and fish, the virulence of *Bacillus mycoides* which belongs to the *Bacillus* cereus group remains poorly understood. In this study, we investigated the virulence of *B. mycoides* strains isolated from active layer soils collected in Siberia, Russia, by analyzing physiological characteristics, including growth response to temperature and spore formation, as well as genome sequencing and temperature-dependent (10°C, 20°C, and 37°C) virulence-related phenotypes. All strains were capable of growing up to 37°C and forming endospores. The genomes of *B. mycoides* ranged from 5.8 Mb to 6.3 Mb and harbored virulent factor genes involved in exotoxin production, such as *hblACD* and *hlyIII* encoding hemolysin BL and hemolysin III, respectively Additionally, genes encoding exoenzymes, including *colA* (collagenase), *inhA* (protease InhA protein), and *smcL* (sphingomyelinase), were identified. Phenotypic tests confirmed the activity of protease, collagenase, and hemolysin. Notably, protease and hemolytic activity were temperature-dependent, with the highest activity observed at 20℃. Future research into the mechanisms of these virulence factors, their temperature-specific responses and their roles in pathogenicity through *in vivo* studies will provide a comprehensive understanding on the pathogenic potential of *Bacillus mycoides* under warming Arctic conditions.

Abstract ID: 1171 / Poster Number: 050.16P

Potential Therapeutic Role of Levilactobacillus brevis in Inflammatory Diseases: Anti-Inflammatory and Barrier-Enhancing Effects in Cellular Models

Presenting author: Ms. Su-Jin Min, Konkuk University, Seoul, South Korea

Co-authors: *Hyun-Joo Yoon, Hye Ji Jang, Na-Kyoung Lee, Hyun-Dong Paik*

This study aimed to investigate the potential of *Levilactobacillus brevis* strains to alleviate Rheumatoid arthritis (RA) by improving gut health.

RA is a chronic autoimmune disease characterized by inflammatory responses. Alterations in the gut microbiota composition are recognized as significant contributors to RA pathogenesis. This has highlighted the potential of probiotics as critical contributors to therapeutic strategies for RA management.

The anti-inflammatory effects of *L. brevis* strains were evaluated using two cell models: LPS-stimulated RAW 264.7 macrophages for systemic inflammation and SNP- and LPS-stimulated HT-29 cells for intestinal inflammation and motility. The observed effects were confirmed using PCR, ELISA, and Western blot analyses.

Treatment with these strains reduced nitric oxide production in LPS-stimulated RAW 264.7 macrophages, suppressed pro-inflammatory cytokine expression, and increased anti-inflammatory cytokine expression. Moreover, the levels of prostaglandin E_2 (PGE_2) and leukotriene B4 (LTB4), critical mediators in inflammatory diseases such as arthritis, were notably decreased. These strains effectively suppressed the nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and mitogen-activated protein kinase (MAPK) pathways, which are critical regulators of inflammatory responses.

In HT-29 cells, treatment with these strains resulted in decreased nitric oxide production under SNP stimulation. Under LPS stimulation, the expression of pro-inflammatory cytokines such as IL-8 was suppressed. Additionally, the expression of tight junction and mucin genes was increased. These effects collectively contributed to improved intestinal barrier function.

In conclusion, *L. brevis* strains demonstrated anti-inflammatory properties and protective potential against intestinal inflammation and intestinal motility, suggesting their potential use as therapeutic agents for inflammatory diseases.

Abstract ID: 2445

Potentially harmful culturable bacteria and their antibiotic resistance on macroplastic debris in a sewage-contaminated lagoon from South-West Madagascar

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Plastic debris in aquatic environments hosts a variety of microorganisms, including potentially harmful pathogens. Recent studies have highlighted that plastics may also host antibiotic-resistant bacteria (ARB) and be a novel niche that facilitates the spread of diseases and resistance genes in the environment. Coastal lagoons, exploited by artisanal fisheries, are under growing anthropogenic pressure and face intense urban pollution, e.g. wastewater containing potentially harmful fecal bacteria, and plastic debris. This makes them a suitable site to study the interaction between plastic debris and potentially pathogenic bacteria and assess the microbiological risk of plastic pollution for marine resources biodiversity and human health.

In this study, we collected 113 macroplastics (MAP) and 50 seawater samples in and outside of the Toliara lagoon, the largest coastal lagoon in Madagascar, during both dry and wet seasons. Culturable bacteria were isolated by using selective culture media followed by MALDI-ToF analysis to identify the abundance and diversity of potentially pathogenic species in each matrix. Antibiotic resistance profiles were obtained following the European Committee on Antimicrobial Susceptibility guidelines (2023), measuring the Multiple Antibiotic Resistant (MAR) index.

Overall, 186 isolates encompassing 20 genera were identified on macroplastics, *Vibrio, Enterococcus* and *Staphylococcus* being the most prevalent. MAP showed a consistently higher number of culturable bacterial species than nearby seawater. The dominant species on macroplastics was *Vibrio alginolyticus* (22824 ± 47895 cells/g). *Staphylococcus aureus* was the most abundant cultivated bacteria in the seawater with 8,2 ± 32,2 cells/ml. Antibiotic resistance was significantly higher in macroplastic-associated culturable bacteria compared to those found in seawater, with a MAR index of 0,43 ± 0,17 and 0,34 ± 0,27 respectively. Notably, we observed systematic resistances to broad-spectrum antibiotics such as penicillins (penicillin G, ticarcicillin, ampicillin) in *Enterobacterales* and resistance to erythromycin and imipenem in all the *Enterococcus*

isolates from macroplastics. Moreover, two macroplastic isolates of *Escherichia coli* demonstrated multi-drug resistance capability by resisting >17/22 antibiotics.

These findings underscore the role of macroplastics as a fomite for potentially harmful living bacteria and to contribute to the global issue of antimicrobial resistance in coastal marine systems.

Abstract ID: 817 / Poster Number: 151.16P

Precise Regulation of Lysogeny in Escherichia coli Using Synthetic λ Bacteriophages Armed with CRISPR-Cas Systems

Presenting author: Prof. Sang Jun Lee, Chung-Ang University, Anseong, South Korea

Co-authors: *Ho Joung Lee, Song Hee Jeong*

The specificity of bacteriophage host interactions is primarily dictated by the host cell surface receptors. Incorporating *cas* genes and guide RNAs into phage genomes enhances their host specificity and regulatory capabilities. In this study, we engineered a temperate λ bacteriophage model equipped with CRISPR-Cas systems to detect specific target genes in *Escherichia coli* host cells. Our results demonstrate that synthetic λ phages harboring Cas12f1-sgRNA effectively inhibit lysogeny formation. Building on our earlier discovery that truncated sgRNAs eliminate mismatch tolerance in the CRISPR-Cas12f1 system, we further showed that synthetic phages armed with Cas12f1 and truncated sgRNAs can discriminate single-nucleotide variations in the host genome. This ability enables precise determination of whether the host cell undergoes complete lysis or lysogeny. These findings highlight the potential of CRISPR-Cas12f1-equipped synthetic bacteriophages as powerful tools for selectively regulating microbial hosts by recognizing target DNA sequences with exceptional resolution.

Abstract ID: 3345 / Poster Number: 178.17P

Preliminary characterization of the de novo cysteine biosynthetic pathway in Pseudomonas aeruginosa

Presenting author: Marta Mellini, PhD, Roma Tre University, Rome, Italy

Co-authors: *Claudia Ridolfi, Marta Beccarini, Livia Leoni, Giordano Rampioni*

Introduction: New therapeutic options are urgently needed to contrast infections caused by multidrug resistant (MDR) bacteria. Extensive researches are ongoing to identify novel drug targets. Recently, the involvement of the *de novo* cysteine biosynthetic pathway in relevant aspects of bacterial physiology, such as virulence and antibiotic tolerance, have been described in different bacteria. This has led to the proposal of this pathway as a promising drug target. The *de novo* cysteine biosynthetic pathway remains largely uncharacterized in the bacterial pathogen *Pseudomonas aeruginosa*, despite the presence of genes putatively encoding for the corresponding enzymes in its genome.

Objective: To characterize the *de novo* cysteine biosynthetic pathway in *P. aeruginosa,* evaluating its impact on virulence and antibiotic tolerance.

Method: *P. aeruginosa* PAO1 mutants lacking genes possibly involved in the *de novo* cysteine biosynthetic pathway were generated and characterized for their ability to: *i)* grow in different media in the presence or absence of cysteine; *ii)* produce relevant virulence factors; *iii)* resist to antibiotics and oxidative stress.

Findings: Specific genes were essential for *P. aeruginosa* growth in media lacking cysteine. Interestingly, mutations in these genes also caused growth defects in cysteine-containing standard laboratory media and media mimicking the *in vivo* infection environment. Despite no impact in antibiotic/oxidative stress resistance have been observed, we identified mutations causing decreased virulence factors production without affecting growth.

Conclusion: This investigation supports the relevance of the cysteine biosynthetic pathway in *P. aeruginosa* pathophysiology, encouraging further characterization of this process for its possible exploitation as a drug target.

Abstract ID: 2672 / Poster Number: 370.16P

Presence of Escherichia coli and Enterobacteriaceae counts in cured cheeses: evaluation of microbiological quality in samples of cured cheeses

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Co-authors: *Alba Martínez-Laorden, Celia Arraiz-Fernández*

Ensuring food safety and quality in cheese production is vital to protect public health and maintain consumer confidence. Dairy products, such as cheese, can become contaminated with Enterobacteriaceae, such as Escherichia coli, which can cause serious gastrointestinal infections. To address this situation, microbiological control and compliance with good manufacturing practices throughout the production process is essential.

The aim of this experiment is to detect the presence of Escherichia coli in cheese from Navarra, the Basque Country and La Rioja, as well as to quantify Enterobacteriaceae counts to assess the level of bacterial contamination.

A total of 60 cured cheeses (54 raw milk and 6 pasteurised milk cheeses) were evaluated. Microbiological analysis was performed using a medium for the count of enterobacteria and a specific medium for the isolation of E. coli. In addition, the identification of E. coli strains was confirmed by PCR techniques.

Results reveled than 8 samples exceeded 1 log CFU/g for Enterobacteriaceae, with counts ranging from 1.3 to 6.73 log CFU/g. Escherichia coli was detected in 12 out of the 60 cheese samples tested, and 3 cheese made from raw milk showed counts above 4 log CFU/g.

These findings highlight the importance of maintaining strict hygiene measures during cheese production to ensure safety and quality. The presence of E. coli in 20% of samples and high enterobacteria counts in 5% of the samples emphasize the need to maintain good hygiene and control practices during production to ensure the quality and safety of the cheese.

Abstract ID: 3933 / Poster Number: 200.17P

Prevalence and characterization of Staphylococcus aureus and Methicillin-resistant strains in preschool children attending Immunization Clinics in Sri Lanka

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Co-authors: *M.N.P. Handapangoda, Nilakshi Barua, Jun Yang, W.S. Lee, L.P.A.L.P. Ruwansiri, U.P.R.U. Dissanayake, D.N. Sapugahawatte, S.T. Kudagammana, B.N. Dissanayake, Veranja Liyanapathirana,*

Introduction: *Staphylococcus aureus* (SA) is commonly carried in the anterior nares prior to infection. Carriage of methicillin-resistance in Staphylococcus aureus (MRSA) can complicate treatment options, increasing the risk of severe health outcomes and healthcare costs.

Objective: This study investigated the prevalence and antimicrobial resistance of SA and MRSA in 375 children aged 2 to 5 years, recruited from immunisation clinics in the Teaching Hospital Peradeniya and the Yatinuwara area between June 2023 and January 2024.

Method: Anterior nasal and nasopharyngeal swabs were systematically collected and stored at -80 ºC. SA was cultured on conventional media and the identity confirmed with MALDI-TOF (Bruker). The antimicrobial susceptibility (AST) of the MRSAs was determined by disk diffusion assay according to Clinical and Laboratory Standards Institute (CSLI) procedures, 2023. Demographic data were collected using interviewer-administered questionnaires.

Findings: The median age of the study group is 54 months (IQR: 36-60), with 50.1% (188/375) males and 49.9% (187/375) females. A total of 25.6% (98/375) participants were positive for SA, with 14.9% (56/375) MRSA. Of 56 MRSA, 8.9% (5/56) were resistant and 16.1% (9/56) exhibited intermediate susceptibility to ciprofloxacin, 1.8% (1/56) was resistant to Trimethoprim-sulfamethoxazole; 62.5% (35/56) of the MRSAs were resistant to erythromycin, 3.6% (2/56) were resistant to clindamycin;7.1% (4/56) were resistant to gentamycin. 1.8% (1/56) were resistant, and 1.8% (1/56) exhibited intermediate susceptibility to chloramphenicol, respectively. 3.6% (2/56) were resistant to tetracycline.

Conclusion: A high prevalence of SA (25.6%) and MRSA (19.9%) was identified among young children, raising concerns about antibiotic resistance and enhancing infection prevention.

Abstract ID: 433 / Poster Number: 031.16P

Prevalence and Genetic Characterization of Campylobacter, Salmonella and Listeria monocytogenes in Raw Meat in Beijing China 2021-2023

Presenting author: Mr. Xiaochen Ma, Beijing Center for Disease Prevention and Control, Beijing, China

Co-authors: *Penghang Zhang, Yanlin Niu*

Introduction: Foodborne pathogens such as Campylobacter, Salmonella, and Listeria monocytogenes are major contributors to foodborne illnesses worldwide. Understanding their prevalence, genetic diversity, and co-contamination patterns in raw meat is critical for improving food safety and public health strategies.

Objective: Understand the contamination of raw meat in Beijing by foodborne pathogens and emphasize the necessity of strengthening control measures throughout the entire food supply chain.

Method: A total of 1,052 raw meat samples, including poultry, pork, beef, and mutton, were collected from markets in Beijing. Pathogens were isolated using standard culture-based methods. Whole-genome sequencing (WGS) and bioinformatics analyses were conducted to determine sequence types (STs), clonal complexes (CCs), virulence gene profiles and pathogenicity islands. Statistical analyses were used to assess microbial detection rates, co-contamination patterns, and pathogen correlations.

Findings: The prevalence rates of Campylobacter, Salmonella and Listeria monocytogenes in raw meat were 20.34%, 24.71% and 52.28%, respectively. Poultry meat exhibited the highest contamination rates for Campylobacter (35.49%) and Salmonella (38.40%), while Listeria monocytogenes was widely detected in all meat types, showing the highest prevalence in pork (66.19%). Co-contamination was observed in 33.65% of the samples, with significant correlations between Campylobacter and Salmonella in poultry ($p = 0.0205$) and pork ($p = 0.0195$). Genetic analyses revealed high diversity among isolates, with distinct clonal complexes and serotype-ST associations.

Conclusion: The findings provide valuable insights into the molecular epidemiology of these pathogens, informing the development of targeted interventions to reduce foodborne illness risks and improve food safety.

Abstract ID: 1985 / Poster Number: 105.17P

Prevalence and Mechanisms of Colistin Resistance in Acinetobacter baumannii Isolates from Bahrain: Evaluation of Diagnostic Methods and Clonal Relationships

Presenting author: Ms. Zainab Salman, Arabian Gulf University, Manama, Bahrain

Co-authors: *Zainab Saleh, Mohd. Shadab, Nouf Alrashed, Mohammad Shahid*

Background: *Acinetobacter baumannii* (*A. baumannii*) is a pathogen linked to multiple nosocomial infections, particularly in ICUs. Its multidrug resistance has become a global concern. Colistin, the last-resort antibiotic for treating MDR strains, faces growing resistance due to various mechanisms, creating a significant challenge.

Objective: This study aims to assess colistin resistance in *A. baumannii* isolates, evaluate the consistency of AST methods, and explore resistance mechanisms and clonal relationships, marking the first investigation of its kind in Bahrain.

Method: A total of 101 A. baumannii isolates were collected from four hospitals in Bahrain, comprising 25 carbapenem-sensitive (CSAB) and 76 carbapenem-resistant (CRAB) isolates. AST was performed using BMT, CAT, E-Test, and disc diffusion methods. PCR was done to identify mcr1 and mcr4 genes, while BOXA1R PCR assessed clonal relationships. Sequencing results are pending.

Results: Of the 101 isolates, 14 (13.9%) were colistin-resistant by BMT. Among these, 12 (85.7%) showed resistance in CAT, none on E-test, and 3 (21.4%) on disc diffusion. PCR detected the mcr-4 gene in 10 (71.4%) of the resistant isolates. BOXA1R PCR revealed significant clonal relationship among the resistant isolates.

Conclusion: This study highlights a high prevalence of colistin resistance in A. baumannii isolates in Bahrain, with most resistant isolates harbouring the mcr-4 gene, indicating a plasmid-mediated resistance mechanism. The findings also emphasize the superior sensitivity of CAT over E-test and disc diffusion for screening colistin resistance, suggesting its potential for resource-limited settings. Ongoing sequencing will provide insights into additional resistance mechanisms and aid interventions.

Abstract ID: 1598 / Poster Number: 175.16P

Prevalence of adlb gene in different Staphylococcus aureus genotypes isolated from bovine mastitis in eight countries

Presenting author: Dr. Paola Cremonesi, National Research Council, Lodi, Italy

Co-authors: *Valentina Monistero, Alessandra Gazzola, Alicia Romanò, Mario Vittorio Luini, Hans Ulrich Graber, Paolo Moroni, Giulia Dilio*

Background: *Staphylococcus aureus* is one of the major mastitis pathogens in dairy farms. The diffusion and persistence of specific *S. aureus* genotypes within herds and cows is linked to particular pathogenic and contagious properties. The association of genotype B (GTB) with high within-herd prevalence of intramammary infection has been recently demonstrated. The contagiousness of *S. aureus* GTB has been associated to the *adlb* carriage, a gene located in the GTB-specific staphylococcal cassette chromosome.

Objective: The aim of this study was to investigate the *adlb* presence in a large collection of *S. aureus* strains, belonging to different genotypes and isolated from dairy herds in 8 countries.

Method: The *adlb*-targeted real-time PCR was performed on 120 *S. aureus* strains isolated from bovine mastitis in Argentina, Brazil, Colombia, Germany, Italy, the United States, South Africa, and Tunisia. The genotypes of these strains had been already investigated by ribosomal spacer (RS)-PCR in a previous study.

Findings: Each country showed a specific genotypic pattern, and only 5% of the strains were positive for *adlb*. All the 5 GTB strains had been isolated from Italy, and carried the gene. Only one more strain isolated in Germany and included in the widely disseminated genotypic cluster R, namely GTR^{VI}, harbored the *adlb*.

Conclusion: Our study confirmed that *adlb* gene is strongly associated to *S. aureus* GTB, and supported the potential transfer of this gene among different genotypes, such as GTR^{VI}. Its detection can be helpful for identifying harmful strains and predicting their contagiousness in dairy cattle.

Abstract ID: 3003 / Poster Number: 077.16P

Prevalence of Redondoviridae in periodontitis patients and their association with oral bacteria

Presenting author: Ms. Sonja Gostimirovic, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

Co-authors: *Jovana Mitrovic, Darija Vukovic, Jelena Cvetanovic, Verica Aleksic Sabo, Ivana Gusic, Petar Knezevic*

Introduction: *Redondoviridae* is a newly described family of DNA viruses predominantly found in human oro-respiratory tract. Redondoviruses have been identified in both healthy individuals and those with certain diseases, where significant association was established in case of periodontitis. Periodontitis is a highly prevalent inflammatory disease of oral cavity with oral bacteria as key factor to disease progressing.

Objectives: This study aims to determine the prevalence of redondoviruses in periodontitis and its correlation with periodontitis-associated bacteria.

Methods: Buccal swabs were collected from patients with periodontitis (n=70) and healthy individuals (n=25) for analysis. The samples were examined by qPCR assays with primers targeting putative capsid region of redondoviruses and *rpoB* gene for red complex bacteria *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*.

Findings: The prevalence of redondoviruses in periodontitis patients was 70 % while in healthy individuals 32 % ($p = 0.0031$). Among periodontitis group most prevalent oral bacteria was *T. forsythia* (87.1 %), followed by *P. gingivalis* (85.7 %) and *T. denticola* (84.3 %). In healthy individuals the highest prevalence was observed for *T. denticola* (44.0 %), followed by *P. gingivalis* (36.0 %) and *T. forsythia* (36.0 %). Periodontitis association was determined for all analyzed bacterial species ($p < 0.05$) and a statistically significant correlation between redondoviruses and red complex bacteria was determined ($p < 0.05$).

Conclusions: Prevalence of redondoviruses was significantly higher in periodontitis patients than healthy controls, and positive association with red complex bacteria was established. Redondoviruses may serve as a reliable marker for periodontitis.

Abstract ID: 288 / Poster Number: 128.16P

Preventing bacterial adhesion to skin by altering their physicochemical cell surface properties specifically

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The adhesion of bacteria to surfaces is associated with physicochemical and biological interactions.

The present investigations provide new results about the differential adhesion levels of skin bacteria using a representative 3D skin model which mainly relies on the different physicochemical properties of the respective surfaces. Modulation of the adhesion of bacteria and thus their colonization, may occur by adjusting the physicochemical properties of the epidermal and bacterial surfaces.

Lewis acid and hydrophobicity were the most strongly correlated parameters with the antiadhesion properties of the tested compounds.

Modulation of physicochemical properties appears to be the primary driver of reduced Staphylococcus aureus adhesion in this study, with no significant changes observed in the expression of genes associated with classical adhesion pathways.

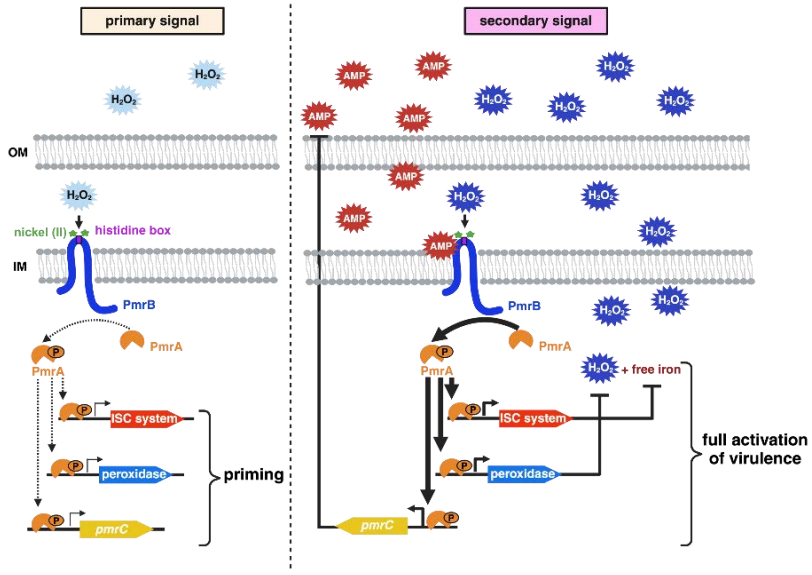
Abstract ID: 525

Priming of a signal transduction system confers full activation of virulence upon Acinetobacter baumannii

Presenting author: Dr. Hoan Ngo, Seoul National University, Seoul, South Korea

Co-authors: *Jinki Yeom*

All living organisms must adapt to environmental stresses to survive. In bacteria, the two-component system (TCS) enables signal transduction to modify global gene expression under stress conditions. The pathogenic bacterium *Acinetobacter baumannii* requires the PmrA/PmrB TCS for virulence. We now report that the sensor PmrB responds to oxidative stress through multiple histidine residues with nickel cofactor, activating the regulator PmrA. Activated PmrA induces expression of genes encoding proteins for iron-sulfur cluster assembly and peroxidase, thereby mitigating oxidative stress damage. Notably, priming PmrB with sub-lethal reactive oxygen species (ROS) dramatically enhances both oxidative stress and antimicrobial peptide defense systems, enabling full virulence of A. baumannii. ROS sensing by PmrB proves crucial for *A. baumannii* survival and virulence in innate immune cells and in mice infected via the airway route. Our findings reveal how detection of sub-lethal stress signals enables pathogens to display their full virulence potential and resist stresses during infection (Summary Figure).



Summary Figure: Model for the priming effect by H_2O_2 under control of the PmrA/PmrB system in *A. baumannii*. (Left panel) During infection, bacteria usually encounter low concentration of H_2O_2 (1 – 10 μM) in blood stream or tissues. At this low

concentration, H_2O_2 acts as a prime signal that could be detected in the periplasmic space by the sensor kinase PmrB by its histidine box and nickel. Upon receiving the prime signal, PmrB activates the response regulator PmrA by phosphorylation. Once activated, PmrA up-regulate genes related to oxidative stress defense system (e.g ISC system and peroxidase) and antimicrobial resistance (PmrC) awaiting up-coming stresses. (Right panel) Upon bacterial exposure to lethal dose of H_2O_2 or antimicrobial peptides (AMP) (e.g colistin and LL-37), the previously primed PmrA/PmrB system detects those signals and up-regulate the defense system at a much stronger level, compared to non-primed one, that enable bacteria to resist oxidative stress and develop antimicrobial resistance and ultimately survive inside the host.

Abstract ID: 1873 / Poster Number: 369.17P

Probiotic potential of Lacticaseibacillus paracasei strains isolated from Caciocavallo Pugliese cheese

Presenting author: Dr. Anna Licitra, National Research Council of Italy (CNR), Bari, Italy

Co-authors: *Daniele Chieffi, Stefano Sallustio, Marco Montemurro, Antonella Garbetta, Carlo Giuseppe Rizzello, Vincenzina Fusco*

Among Lactic Acid Bacteria (LAB), the bacilli of the *Lacticaseibacillus casei*-group are emerging for their ability to exert health benefits. In the present work, we used the recently developed selective medium MRS-Vancomycin maltose (MMV) agar to isolate *Lacticaseibacillus casei*-group bacilli from different steps of the production of Caciocavallo Pugliese cheese, a pasta filata ripened cheese produced in the Apulia region. Forty-five/65 isolates (69.2%) from MMV were identified as *L. paracasei*, thus confirming the suitability of this agar medium in the isolation of *Lacticaseibacillus casei*-group members.

In order to evaluate their genetic diversity, the 45 *L. paracasei* were typed by RAPD- and REP-PCR. Overall, 10 different RAPD profiles (I-X) and 9 different REP profiles (a-i) were obtained thus allowing the selection of 10 different *L. paracasei* strains that were subjected to whole genome sequencing. The adhesion assay to Caco-2 cells and search of the genetic determinants of probiotic features revealed the probiotic potential of the strains. The safety assessment, based on the genomic analysis, performed by searching for virulence and antibiotic resistance genes, as well as on the phenotypic evaluation, by testing the hemolytic activity allowed us to identify the *L. paracasei* strains as safe potential probiotic microorganisms for application in food industries, although in vivo validation and investigation of health effects have to be performed.

Abstract ID: 3214 / Poster Number: 172.17P

Probiotic-based anti-virulence strategies against Staphylococcus aureus infections

Presenting author: Ms. Weizhe Wang, University of Copenhagen, Copenhagen, Denmark

Co-authors: *Hanne Ingmer, Stephanie Fulaz, Ifigeneia Kyrkou, Martin S. Bojer, Jørgen Leisner*

Staphylococcus aureus is an opportunistic pathogen colonizing about one-third of the human population and causing various infections. The increasing prevalence of antibiotic-resistant strains underscores the need for alternative treatments. The *S. aureus* accessory gene regulator (*agr*) quorum sensing (QS) system, which controls virulence factor expression through the production and sensing of autoinducing peptides (AIP), offers a promising target. Probiotics such as *Lactiplantibacillus* spp. are known to inhibit *S. aureus* though immune modulation, competitive adhesion, and antimicrobial production. Interesting, the identification of *agr*-like systems in *Lactiplantibacillus* spp. (*lamBDCA*) suggests the potential for probiotics to engage in anti-virulence interactions against *S. aureus*.

This study characterized the *lamBDCA* locus in *L. plantarum* LMG 13556 and *L. paraplantarum* CIRM-BIA 1870, exploring their effects on *S. aureus* QS. Co-cultivation with *L. paraplantarum* significantly inhibited *S. aureus* QS and hemolysin production, whereas *L. plantarum* had a weaker effect. Disruption of the *lamBDCA* locus in *L. paraplantarum* abolished its inhibitory activity, implicating its AIP in cross-species interference with *S. aureus agr*. Transcriptomic analysis revealed that *lamBDCA* regulates genes involved in stress response and metabolism, further highlighting its functional role.

Our findings demonstrate that *L. paraplantarum* suppresses *S. aureus* virulence through QS interference, highlighting the role of QS in the probiotic properties of *L. paraplantarum* and presenting a promising probiotic-based strategy to combat antibiotic-resistant *S. aureus* infections.

Abstract ID: 2900 / Poster Number: 201.16P

Procapsid structure of Staphylococcus aureus phage phi812

Presenting author: Mrs. Maryna Zlatohurska, CEITEC Masaryk University, Brno, Czech Republic; D. K. Zabolotny Institute of Microbiology and Virology, Kyiv, Ukraine

Co-authors: *Zuzana Cienikova, Tibor Füzik, Pavel Plevka*

Staphylococcus aureus phage phi812 is a lytic virus with a broad host range, including antibiotics-resistant and biofilm-forming strains. To fill the gaps in knowledge about phi812 head assembly, this work aimed to determine the three-dimensional structure of intermediate procapsid II. We used cryogenic electron microscopy (cryo-EM) and single particle analysis to reach the goal.

Phage particles were obtained from phi812-infected *S. aureus* 30 minutes post-infection. The cryo-EM showed the empty isometric shells slightly larger than mature capsids. The initial icosahedral reconstruction resulted in a 3D density map with a resolution of 3.6Å. 3D classification followed by 3D reconstruction yielded three 3.0-3.2Å structures with varying compositions and occupancy of the minor capsid proteins.

This study reveals the structure of phi812 intermediate procapsid II, enhancing understanding of its head assembly process.

This project has received funding through the MSCA4Ukraine project, which is funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the MSCA4Ukraine Consortium as a whole nor any individual member institutions of the MSCA4Ukraine Consortium can be held responsible for them.

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Abstract ID: 1492 / Poster Number: 293.17P

Production and Biochemical Characterization of Thermostable Protease

Presenting author: Dr. Belma Nural Yaman, Biomedical Engineering, Faculty of Eng and Arch, Eskisehir Osmangazi University, Eskişehir, Turkey

Co-authors: *Rabia AYDIN, Dilan BARUT YAVAŞ, Ahmet ÇABUK*

Introduction: Enzymes are crucial enzymes that catalyze metabolic events essential for the survival of life. Proteases, like numerous other enzyme categories utilized throughout diverse industries, are often sourced from microorganisms. This is due to the considerable benefits of microbial enzymes compared to those derived from plants, animals, or chemical processes. These advantages include exceptionally high catalytic activity, the elimination of undesirable by-products, enhanced stability, reduced cost, and the capacity for large-scale production in a single process. Bacterial proteases have been determined to be more efficacious than animal and fungus proteases.

Objective(s): thermostable and thermo active protease production by thermophilic microorganism for industrial study.

Method: Thermophilic isolate coded C3-1 strain was isolated from hot spring located in Naşa, Kütahya. Protease was produced by C3-1 in protease production medium and harvested by centrifugation. The supernatant was used to determine the enzyme activation based on Anson method. Then biochemical characterization including thermostability, optimal pH, optimal temperature, and the influence of heavy metals and denaturants on protease activity were examined.

Findings and Conclusion: The enzyme activity of the enzyme diluted at a 1/50 ratio at a reaction temperature of 37 °C was measured as 698.104 U/ml·min. The ideal reaction temperature was established at 40 °C, and the optimal pH of the buffer was identified as 11. The activity of the protease, which remains active up to 60 °C, is enhanced by Cu^{2+} metal. Nevertheless, as other metals did not induce a notable reduction in enzyme activity, it can be concluded that the enzyme exhibits metalloprotease properties. This study concludes with the production of a thermostable protease exhibiting high activity, appropriate for industrial applications.

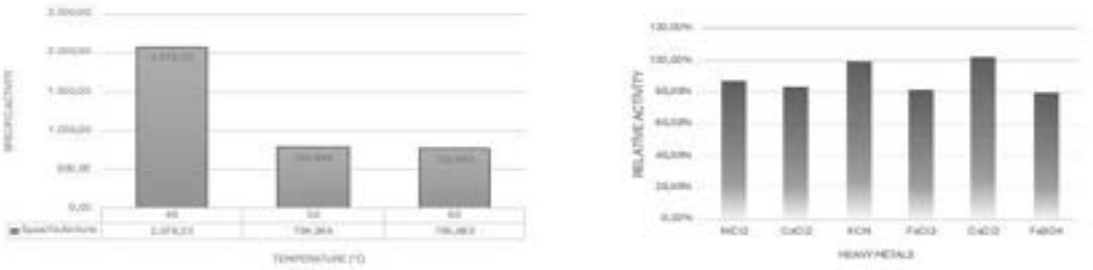


Figure 1: Attractive results of thermostable protease

Abstract ID: 3696 / Poster Number: 348.17P

Production of bioethanol using pineapple agro-industrial waste as a substitute carbon source

Presenting author: Ms. Nicole Rojas Soto, Universidad Nacional de Costa Rica, Heredia, Costa Rica

Co-authors: *Daniel Jiménez Soto, Jorengeth Abad Rodríguez Rodríguez, Silvia Mau Inchaustegui*

Bioconversion of lignocellulosic biomass into specific constituents can be used in biorefining to produce a variety of bio-products, such as second-generation biofuels. The study aimed to evaluate the production of bioethanol from Costa Rican marine yeast using seawater and pre-treated lignocellulosic pineapple residues as substrate.

Lignocellulosic waste from pineapple was pre-treated with sulfuric acid, the resulting slurries were enzymatically hydrolyzed. Different %v/v mixture proportions of non-detoxified liquid pineapple hydrolysate were evaluated. The concentrations of hexoses, pentoses and ethanol were determined by HPLC. Statistical analysis was performed to select the optimal percentage of carbon source.

The results demonstrated that the non-detoxified liquid fraction of 50/50 % v/v medium composed of pineapple hydrolysate and YPG medium with seawater was the most effective and was selected for fermentation. Fermentation with a 50/50 % v/v medium produced 12.92 g L^{-1} of ethanol, while fermentation using only YPG medium with seawater produced 10.13 g L^{-1} of ethanol. Productivity (Q_p) in YPG with seawater was 0.10 g / L h. Similarly, a productivity of 0.10 g / L h was obtained with the 50/50 % v/v medium.

The study demonstrated that the 50/50 % mixture of liquid fraction of pineapple agro-industrial waste provides a sustainable alternative carbon source for ethanol production.

Abstract ID: 2607 / Poster Number: 290.16F

Production of enveloped virus-like particles for enzyme encapsulation in yeast

Presenting author: Mr. Felix Fronek, TU Wien, Vienna, Austria

Co-authors: *Adrian Köber, Matthias Steiger, Matthias Schittmayer-Schantl, Ruth Birner-Grünberger*

Subcellular compartmentalization allows eukaryotic cells to perform complex, multistep, or energetically unfavorable reactions efficiently by avoiding side reactions and establishing chemical gradients.

The generation of functionalized particles that contain a lipid bilayer enables the benefits of this compartmentalization to be utilized in synthetic biology approaches, thereby facilitating the efficient use of substrates, including primary or even toxic metabolites.

In this study, enveloped virus-like particles (VLPs) were produced in the yeast *Saccharomyces cerevisiae* by heterologous expression of the HIV-1 Gag polyprotein, controlled under the inducible *gal1* promoter. Purification and enrichment of VLPs were performed by density gradient ultracentrifugation.

We demonstrated that we can efficiently load these VLPs with proteins of interest, such as lactate dehydrogenase (LDH) and green fluorescent protein (GFP), by post-translational fusion to the Gag protein. In addition, we verified that the lipid bilayer provides a protective barrier for the proteins contained within the VLPs, monitored by protease digestion. Furthermore, the LDH targeted into the VLPs exhibited significantly higher enzymatic activity in the presence of a detergent. Proteomics data confirmed the high efficiency of the loading system, and that membrane proteins, such as Gal1, are highly abundant in our VLPs.

This could be exploited for substrate and product transport as well as for the generation of (electro-)chemical gradients to fuel various transport reactions. By further refining the system, the functionalized VLPs have the potential to act as a cell-free production system, providing the aforementioned advantages of compartmentalization while protecting the encapsulated enzymes.

Abstract ID: 2555 / Poster Number: 324.16F

Production of Xanthan Gum from Kitchen and Dairy Waste

Presenting author: Dr. Biswa Prasun Chatterji, Assam down town University, Guwahati, India

Co-authors: *Binita Barman, Rituparna Debnath, Sonalika Kar*

Introduction: Xanthan gum is an industrially valuable product, produced by fermentation of sugar containing media by *Xanthomonas campestris*. It is widely used in various commercial products such as cosmetics, toothpaste and foods like jam, jelly, ketchup etc. The problem in industrial fermentation of xanthan gum is high cost of raw material i.e. sucrose.

Objective: This present study focuses on fermentative production of xanthan gum by recycling of kitchen and dairy waste, eventually cutting down the cost of production.

Methods: The pure culture of *X. campestris* was obtained from NCIM, India. Control media containing malt extract (0.3g/L), glucose (1g/L), yeast extract (0.3g/L), peptone (0.5g/L) was fermented by *X. campestris* NCIM 2961 for 48 hours at 30℃ to get a yield of 3.9g/L. Xanthan gum was precipitated with isopropyl alcohol and dried.

Experimental media was prepared by adding water to onion peel (100g) and inedible part of Kohlrabi (100g) and blended, then filtered the aqueous extract to adjust the pH at 6.8 and autoclaved. The dairy waste was obtained as liquid from a local dairy and used for making alternative media without adding any chemicals. Now the media were inoculated by *X. campestris* NCIM 2961 and incubated at 30℃ for 48 hours. Xanthan was precipitated by Isopropyl alcohol.

Findings: The yield of xanthan gum from dairy waste was 3.26g/L, from kohlrabi 3.8g/L, and from onion peels 3.05g/L.

Conclusion: Thus, we successfully fermented aqueous extract of kitchen waste and dairy waste to yield xanthan gum by an eco-friendly method to recycle solid food and dairy waste.

Abstract ID: 2258 / Poster Number: 253.17P

Production, chemical and genomic insights into exopolysaccharides synthesized by Haloarcula japonica strain SST1 isolated from a subterranean salt deposit in Armenia

Presenting author: Dr. Hovik Panosyan, Yerevan State University, Yerevan, Armenia

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(48.5%), low protein content (4.32 %), minimal nucleic acids (0.97%), and a significant level of uronic acids (10.8%). When molasses was used as a substrate the EP production yield was 304.8 mg L^{-1} with the carbohydrate content 20.8%. Structural analysis revealed that the EPS is a heteropolymer composed of mannose, galactose and glucose. Genomic analysis identified genes involved in the activation of monosaccharides, biosynthesis, assembly and secretion of EPSs.

Conclusion: The utilization of molasses positions *H. japonica* strain SST1 as a promising and cost effective EPS producer, making it suitable for circular bioeconomy.

Abstract ID: 1590 / Poster Number: 040.15P

Profiling pathogenic protozoa associated with sewage in Valencia City using 18S rRNA

Presenting author: Dr. José Luis Alonso Molina, Universitat Politècnica de València, València, Spain

Co-authors: *Alba Fornés, Pilar Suárez, Pau Rubio, Carmen Ivorra, Dayhan González-López, Felipe Cabarcas, Juan Alzate*

Introduction: Sewer systems transporting wastewater provide unique habitats with a distinct and coherent microbial community not resembling other ecosystems. Waterborne parasitic protozoan infections have a worldwide distribution and are a source of epidemic and endemic human diseases in developed and developing countries.

Objective: This study used 18S rRNA amplicon-based next-generation to facilitate pathogenic or non-pathogenic protozoa detection and identification in sewage-related environments.

Method: We used 18S rRNA and shotgun techniques to profile pathogenic or non-pathogenic protozoa and pathogenic bacteria, respectively, from samples of sewer system transport in Valencia City.

Findings: Protozoa identified by 18S rRNA sequencing in the analyzed sewage samples included Cryptosporidium, Naegleria, Acanthamoeba, Vermamoeba, Rhogostoma, Hartmanella, Blastocystis, and Vahlkampfia. Blastocystis showed the highest relative abundance and richness. The most abundant Blastocystis subtypes appear to be ST1 and ST2, accompanied by ST3, ST4, ST6, ST7, and ST8. Sequencing of 18S rRNA amplicons revealed highly diverse free-living amoeba (FLA) communities in the sewer system. We detected several bacterial pathogens of significant public health concern across the different sites of the sewer system, such as Legionella pneumophila, Helicobacter pylori, Pseudomonas aeruginosa, and Mycobacterium avium. Positive associations were commonly reported between FLA and multiple opportunistic pathogens, such as L. pneumophila, M. avium, and P. aeruginosa, as FLA can serve as hosts to support their growth (Hu et al. 2025).

Conclusion: Protozoa molecular characterization gives new insights and should encourage further research, as industrialized countries are not exempt from protozoa circulation, and a better survey is necessary.

Abstract ID: 2826 / Poster Number: 072.16P

Promoter Fusions and Their Limitations for

Monitoring Gene Expression Heterogeneity

Presenting author: Mrs. Naisa Vetter, University of Tübingen, Tübingen, Germany

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Promoter fusions are widely used as proxies for the expression of specific genes of interest and serve as valuable tools for analyzing single cells within communities. In this study, we employed promoter fusions (P*cap-yfp*) to investigate the highly heterogeneous expression of the *cap* operon, which encodes enzymes responsible for the capsular polysaccharide (CP) biosynthesis in *Staphylococcus aureus*. However, at the single-cell level, the expression of the reporter gene *yfp* did not correlate with the expression of the native *cap* genes or CP synthesis, as assessed through a combination of immunofluorescence, YFP intensity from promoter-reporter fusions, and smRNA-FISH.

Promoter fusions integrated into the chromosome exhibited significantly higher expression and reduced heterogeneity compared to the native *cap* operon. These differences were independent of the chromosomal localization of the P*cap-yfp* construct. Swapping the ribosomal binding site (RBS) of our system revealed that a strong RBS not only enhanced translation but also influenced transcription. Despite using identical RBSs, the P*cap-yfp* constructs still exhibited increased mRNA levels compared to native *cap* mRNA. Since transcript stability remained unchanged, we conclude that intrinsic mRNA properties determine transcriptional behaviour.

Therefore, careful consideration is necessary when using promoter fusions to analyze transcriptional heterogeneity at the single-cell level.

Abstract ID: 2880 / Poster Number: 435.16P

Promoting microbial literacy in a low-income district of the city of Sevilla

Presenting author: Dr. Rafael R. de la Haba, University of Sevilla, Sevilla, Spain

Co-authors: *Alicia García-Roldán, Dáša Straková, Cristina Galisteo, Iván Delgado-Sánchez, María José León, Cristina Sánchez-Porro, Antonio Ventosa*

Microbial life, either beneficial or harmful, permeates almost every environment on Earth. Contrary to the common societal misconception that equates "microbes" with pathogens, the vast majority of microorganisms are advantageous. They play essential roles in sustaining life and are indispensable for human health, environmental preservation, and numerous biotechnological applications. Despite their importance, public understanding of critical issues like antimicrobial resistance (AMR), a looming global health threat, remains alarmingly low.

Acknowledging the prevalent negative perception of microorganisms, our initiative aimed to highlight their relevance while raising awareness about the risks associated with AMR. To achieve this, we organized a series of outreach activities, including interactive talks and accessible wet-lab experiments, specifically designed for pre-university students. Notably, this project targeted an often-overlooked demographic: students from marginalized, low-income communities. Our efforts focused on a high school located in the excluded district of “Las 3000 Viviendas” in Sevilla (Spain), a suburban area characterized by socioeconomic challenges.

The outcomes of the project were highly encouraging. Participants demonstrated increased curiosity about microbial life, heightened interest in pursuing scientific careers (particularly among female students), and improved awareness of public health issues. Additionally, the program contributed to higher vaccination rates, better hygiene practices, reduced antibiotic misuse, and greater adoption of preventive measures against sexually transmitted diseases. These effects extended beyond the students, potentially influencing their families and wider community.

Abstract ID: 901 / Poster Number: 475.15S

Proof of concept: Next-generation coral probiotics

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Coral reefs house approximately 30% of the world's marine biodiversity and sustain the livelihoods of one billion people by providing goods and services. Corals engage in symbiosis with Symbiodiniaceae, photosynthetic microalgae that live inside coral cells and fuel their metabolism. Rapidly increasing sea surface temperatures drive extensive coral bleaching (i.e., expelling symbiotic algae) and mortality globally.

Microbial therapies are emerging as a promising option for coral conservation. The efficacy of probiotics in promoting coral health and preventing mortality under thermal stress has been well demonstrated. However, current probiotics are poorly retained in coral host, requiring repeated and continuous inoculations for the beneficial effects to be maintained.

We believe this challenge can be solved by isolating and subsequently applying evolutionarily young endosymbiotic bacteria, genomically identified through expansion of insertion sequences and proliferation of pseudogenes among other genome-wide evolutionary changes. According to evolutionary biology theories, such bacterial endosymbionts start to depend on the host and may establish tight, stable, and long-term relationships with the host. The use of evolutionarily young endosymbionts as coral probiotics can therefore minimize the need and cost for reapplication.

We have successfully cultured next-generation coral probiotics, specifically novel lineages of *Ruegeria*, showing their localization in the proximity of symbiotic algae, their higher efficacy than congenic probiotics in enhancing host thermal tolerance threshold through laboratory experiments, and their presence and abundance in many natural corals. We further performed field trials, using ex situ coral inoculations in nursery before outplanting and then 8-month monitoring after outplanting, and demonstrated their strong incorporation and retention in host tissue and their capacity for mitigating the bleaching effects of a natural heat stress event during July-August 2024. We conclude that next-generation probiotics are valuable for coral conservation and they are ready for application in combination with coral restoration.

Abstract ID: 729 / Poster Number: 271.17P

Propidium Monoazide-Treated, Cell-Direct, Quantitative PCR for Detecting Viable Chloramphenicol -Resistant Escherichia coli and Corynebacterium glutamicum Cells

Presenting author: Bumkyu Lee, PhD, Jeonju University, Jeonju, South Korea

With the rapid development and commercialization of industrial genetically modified microorganisms (GMMs), public concerns regarding their potential effects are on the rise. It is imperative to promptly monitor the unintended release of viable GMMs into wastewater, the air, and the surrounding ecosystems to prevent the risk of horizontal gene transfer to native mi-croorganisms. In this study, we have developed a method that combines propidium monoazide (PMA) with a dual-plex quantitative PCR (qPCR) approach based on TaqMan probes. This meth-od targets the chloramphenicol-resistant gene (CmR) along with the endogenous genes D-1-deoxyxylulose 5-phosphate synthase (dxs) and chromosomal replication initiator protein (dnaA). It allows for the direct quantitative detection of viable genetically modified E. coli and C. glutamicum cells, eliminating the requirement for DNA isolation. The dual-plex qPCR targeting CmR/dxs and CmR/dnaA demonstrated excellent performance across various templates, including DNA, cultured cells, and PMA-treated cells. Repeatability and precision, defined as RSDr% and bias%, respectively, were calculated and found to fall within the acceptable limits specified by the European Network of GMO Laboratories (ENGL). Through PMA–-qPCR assays, we determined the detection limits for viable chloramphenicol-resistant E. coli and C. glutamicum strains to be 20 and 51 cells, respectively, at a 95% confidence level. Notably, this method demonstrated superior sensitivity compared to Enzyme-Linked Immunosorbent Assay (ELISA), which has a detection limit exceeding 1000 viable cells for both GM bacterial strains. This approach offers the potential to accurately and efficiently detect viable cells of GMMs, providing a time-saving and cost-effective solution.

Abstract ID: 2742 / Poster Number: 143.17P

Propolis for the combined treatment against Staphylococcus aureus

Presenting author: Mrs. Irene Fernández Babiano, University Institute of Biosanitary Research of Extremadura (INUBE), Badajoz, Spain

Co-authors: *Maria Coronada Fernández Calderón, Belen Mendoza Chamizo*

Staphylococcus aureus is one of the principal causes of infection associated with clinical environments. Biofilm formation is a significant virulence factor of this pathogen as it reduces the efficacy of antibiotic treatment and host immune response. This factor, together with the increase in antibiotic resistance, requires the search for new antimicrobial agents against *S. aureus*. Natural products such as propolis, that contain a large number of bioactive molecules, constitute a reservoir to be explored.

In this work, the chemical profile and the antibacterial and anti-biofilm capacity of a Spanish propolis against *S. aureus* strains, alone or in combination with conventional antibiotics, were studied. The effect of sub-inhibitory concentrations on growth and biofilm formation was also evaluated. In addition, its principal mechanism of action and the eventual damage to the host were studied.

Spanish propolis has a promising antibacterial activity alone and shows a synergistic effect in combination with vancomycin. Propolis principally affects to membrane integrity, and seems to activate the regulation of genes associated with biofilm formation. Furthermore, it is a safe compound for biosanitary applications because it exhibits high antioxidant capacity and low toxicity to human cells. Thus, our results support the potential use of propolis as an adjuvant in the control of *S. aureus* infections associated with biofilm formation.

Abstract ID: 3194 / Poster Number: 082.16P

Proteomic and Metabolomic Profiling of Archaeal Extracellular Vesicles from the Human Gut

Presenting author: Ms. Viktoria Weinberger, Medical University Graz, Graz, Austria

Co-authors: *Barbara Darnhofer, Himadri Thapa, Polona Mertelj, Regis Stentz, Emily Jones, Gerlinde Grabmann, Rokhsareh Mohammadzadeh, Christina Kumpitsch, Torben Kuehnast, Thomas Weichhart, Thomas Koecher, Harald Koefeler, Simon Carding, Stefan Schild, Christine Moissl-Eichinger*

Microorganisms thriving in the human gastrointestinal microbiome have developed numerous mechanisms for microbiome-host and microbe-microbe interaction, including the formation of extracellular vesicles (EVs). However, the interactive mechanisms of members of the human archaeome remained largely unexplored.

Here, we report the capability of two *Methanobrevibacter* strains *M. smithii*, *Candidatus* M. intestini and *Methanosphaera stadtmanae*, to produce extracellular vesicles. To enhance our understanding regarding the characteristics of this pioneering discovery we employed multi-omics techniques, co-incubation experiments with human cell lines, and initial measurements including concentration, size, and content analysis encompassing protein, lipid, DNA, and RNA.

While the size (~130 nm) and morphology of these archaeal EVs were comparable to bacterial EVs, proteomic and metabolomic analyses revealed unique traits, including the increased abundance of adhesin-like proteins potentially used for adhesion, or potential signaling molecules relevant for host interaction. Additionally, the archaeal EVs contained glutamic acid and choline glycerophosphate, which could be involved in gut-brain signaling.

Vesicles from all studied species were efficiently taken up by human monocytes and induced the production of inflammatory cytokines in both macrophages (differentiated THP-1 cells) and epithelial HT-29 cells. Human cell lines show different cytokine responses upon exposure to EVs from the different archaeal strains. *Cand*. M. intestini induced a substantial excretion of the proinflammatory cytokine IL-8 in epithelial HT-29 cells.

The identification of archaeal EVs and their components provides novel insights into the complex interactions between the archaeal and bacterial microbiome and the host, potentially offering new paths to modulate vesicle-mediated impacts on host health.

Abstract ID: 2757 / Poster Number: 426.15P

Pseudomonas bharatica CSV86T: a promising host for heterologous protein expression and periplasmic localization

Presenting author: Mr. Braja Kishor Saha, INDIAN INSTITUTE OF TECHNOLOGY BOMBAY, MUMBAI, India

Pseudomonas strains are currently being explored for heterologous protein expression due to certain limitations in widely used *E. coli* strains. *Pseudomonas bharatica* CSV86^T, a novel soil isolate, preferentially utilizes complex aromatic compounds over simple carbon source like glucose and glycerol. Strain CSV86^T grows rapidly ($\mu = 0.54\ h^{-1}$) with a short lag phase on naphthalene as sole source of carbon and energy. A host specific *nah* promoter was chosen based on high promoter strength (LDF score = 6.03) and its ability to exhibit strong yet leaky expression of the reporter enzyme. Carbaryl hydrolase (CH) enzyme from *Pseudomonas* sp. C5pp with a novel signal peptide (Tmd+Sp) was cloned under *nah* promoter in pSEVA234. Recombinant CH was found to be translocated to periplasmic space of strain CSV86^T. Activity of CH was found to be 2814 nmol min^{-1} mg^{-1} in periplasmic fraction as compared to 90 nmol min^{-1} mg^{-1} in cytoplasmic fraction. CH was purified from periplasmic fraction of strain CSV86^T in a single step Ni-NTA affinity chromatography to near homogeneity with 16.2-fold. Kinetic properties (K_m = 83 µM & k_{cat}/K_m = 0.58) were found to be similar to that of native enzyme. Further, ring cleaving dioxygenases like catechol 1,2-dioxygenase, catechol 2,3-dioxygenase and gentisate dioxygenase (GDO) were cloned under *nah* promoter with Tmd+Sp at the N-terminus. GDO was successfully expressed in CSV86^T and found to be translocated into periplasmic space. GDO was partially purified from total cell-free extract by ammonium sulfate precipitation followed by ion exchange chromatography and kinetic properties were determined.

Abstract ID: 3391 / Poster Number: 335.17P

Psychrobacter sp. TAE2020 as a source of biosurfactants with antibiofilm activity

Presenting author: Caterina D'angelo, PhD, University of Naples Federico II, Naples, Italy

Co-authors: *Angela Casillo, Maria Michela Corsaro, Diana Olimpo, Maria Luisa Tutino, François Coutte, Philippe Jacques, Ermenegilda Parrilli*

Background: Microbial biofilm formation poses persistent challenges in environmental, industrial, and medical settings, imposing significant health and economic burdens. In medical settings, biofilms are the cause of chronic, nosocomial, and medical device-related infections. One of the most common microorganisms involved in implant-associated infections is *Staphylococcus epidermidis*, an opportunistic pathogen that forms biofilms on the surface of indwelling medical devices. Traditional treatments for biofilm-related infections are often ineffective, prompting the exploration of biological anti-biofilm compounds as novel therapeutic strategies. The use of surface-active molecules such as biosurfactants to prevent the early stages of *S. epidermidis* biofilm formation presents a promising control strategy. The exploitation of biodiversity is pivotal in the search for new anti-biofilm agents and in this contest, the Polar marine bacteria, represent an untapped reservoir of biodiversity since they can synthesize a broad range of bioactive compounds, including anti-biofilm molecules with surfactant capabilities.

Objective: This work aims to expand and deepen the biotechnological potential of Antarctic marine bacteria as a sustainable source of novel antibiofilm compounds active against *S. epidermidis*. Specifically, the study focuses on the identification and purification of a novel anti-biofilm molecule with strong surfactant capability from the Polar marine bacterium *Psychrobacter* sp. TAE2020 (TAE2020).

Methods: As a purification step, TAE2020 cell-free supernatant was subjected to the foam fractionation method, to enrich solutions of surface-active molecules. The resulting foam (TAE2020_FOAM) was subjected to ultrafiltration and the permeate fraction (P_FOAM), enriched with low molecular compounds was subjected to adsorption chromatography using Amberlite XAD-2 resin. The active compound(s) were eluted with methanol and designated as the Psychrotin fraction. Various analytical biochemical methods were used to characterize this fraction's composition (LC-MS, GC-MS).

Results: The Psychrotin fraction exhibited strong surfactant activity, reducing surface tension of water from 72,9 ± 0.5mN/m to 34.69 ± 0.42 mN/m and demonstrated significant antibiofilm activity against *S. epidermidis*. GC-MS analysis on the Psychrotin fraction revealed saturated and unsaturated long-chain fatty acids (C14:1, C15:0,C16:1,C16:0,C17:0,C18:2,C18:1,C18:0) and amino acids, including leucine, isoleucine, proline, aspartic acid, glutamic acid, and alanine.

Conclusions: Our findings highlight the potential of *Psychrobacter* sp. TAE2020-derived biosurfactants as novel, environmentally friendly agents for the prevention and treatment of biofilm-associated infections. Further characterization of these molecules may pave the way for innovative therapeutic applications in the biomedical field.

Abstract ID: 1012 / Poster Number: 416.16F

Putative Plastic Degrading Communities within New Zealand's Geothermal Environments

Presenting author: Mr. Nikolai Pavlov, The University of Auckland, Auckland, New Zealand

Co-authors: *Jessica Wallbank, Syrie Hermans, Gavin Lear*

Polyethylene terephthalate (PET) and many other plastic polymers become amorphous at temperatures above 70°C, which makes them more susceptible to microbial biodegradation. Microorganisms capable of surviving in hot environments and producing thermostable enzymes may, therefore, play crucial roles in plastic biodegradation. However, their presence in geothermal areas remains understudied.

We conducted 16S rRNA gene sequencing of microbial consortia within geothermal springs at Kuirau Park, Rotorua, Aotearoa-New Zealand, to examine the distributions of putative plastic-degrading taxa. We selected 35 sites with varying pH (4-10) and temperature (20-80°C) conditions, where the most abundant putative plastic-degrading taxa belonged to the genera Flavobacterium, Pseudomonas, Sphingomonas and Rheinheimera, which combined, were present in 24 out of the 35 sites examined. We found no clear spatial patterns in the putative plastic degrading taxa distribution but identified higher microbial richness within sites with moderate thermophilic conditions (30-40°C). Temperature appeared to have a more significant effect on the distribution of microbial taxa compared to pH, as the relative abundances of microbial taxa had a stronger Spearman correlation with temperature values (>30°C). Additionally, five sites selected due to the presence of high concentrations of plastic-degrading taxa underwent shotgun metagenomic sequence analysis, revealing genes associated with the metabolism of aromatic compounds and various plastic polymers, including PCL, PLA, PU, PET, PES, PVA, and LLDPE.

Our study confirms a wide presence and distribution of putative plastic-degrading microorganisms and genes in geothermal environments. It highlights the potential of geothermal springs for future research into microbial plastic degradation.

Abstract ID: 2806 / Poster Number: 007.17S

Quantification of Plant Growth-Promoting Rhizobacteria (PGPRs) in Agricultural Soil through Real Time PCR

Presenting author: Dr. Caterina Agrimonti, University of Parma, Parma, Italy

Co-authors: *Ilenia Iosa, Nelson Marmiroli*

Introduction: In the last decades microbial-based biostimulants have received increasing attention in the agriculture practices because they promote plant growth and crop yield reducing the utilization of chemical fertilizers and, consequently the negative environmental impact. Biostimulants include fungi, microalgae and rhizobacteria (PGPRs). These last colonize the rhizosphere improving uptake of nutrients, plant resistance to biotic and abiotic stresses, soil fertility and crop yield. However, the variability and complexity of the soil environment affect the persistence and the efficacy of PGPRs: this makes necessary reliable methods for their detection and quantification, in order to understand their shelf-life and interaction with plant rhizosphere. Quantitative real-time PCR (qtPCR) is fast, sensitive and does not require bacteria cultivation; therefore is a suitable method to analyse a large number of samples.

Objectives: The aim of this work was the development of a qtPCR method to quantify five PGPR species of a microbial consortium (*Burkholderia ambifaria*, *Bacillus amyloliquefaciens*, *Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Rahnella aquatilis)* in rhizospheric soils. This method was utilized to evaluate the growth and survival of these species in an experimental field sown with two varieties of wheat (Bramante and Svevo), in presence and absence of the soil improver biochar Figure 1).

Findings: Through literature search and *in silico* sequence analyses, a set of primer pairs which selectively tag three bacterial species (*B. ambifaria*, *B. amyloliquefaciens* and *R. aquatilis)* was retrieved and utilized in qtPCR assays on soil samples of the field experiment schematized in Figure 1. The results showed that the targeted bacteria have colonized and grown into the soil, reaching a maximum of growth between 15-20 days after inoculum. Results also showed that biochar had a positive effect on PGPR growth.

Conclusions: The qtPCR was an effective method to trace the fate of supplied bacterial species in the soils after their inoculation in different conditions (for example: presence or absence of improvers.).

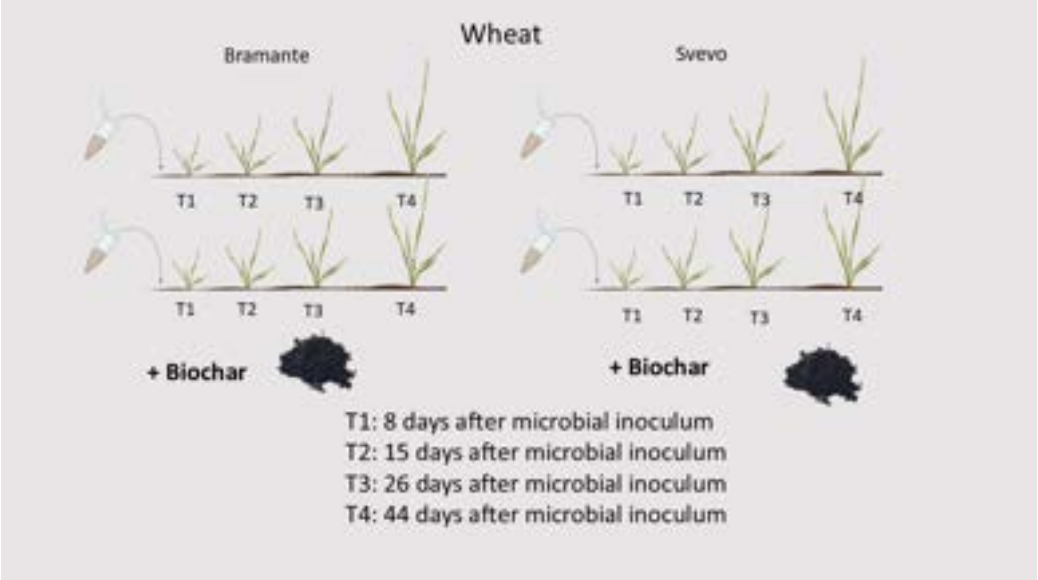


Figure 1: *Diagram of the field experiments on which the qtPCR assays were conducted*.

Abstract ID: 2975 / Poster Number: 399.15P

Quinone cross-feeding between gut bacteria linked to widespread occurrence of partial menaquinone biosynthesis pathways

Presenting author: Ms. Morgane Roger-Margueritat, TIMC, UMR 5525, CNRS, Grenoble Alpes University, VetAgro Sup, Grenoble INP, Grenoble, France

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Background: The gut microbiota is a complex community of microorganisms that can be directly linked to the host health. The gut is thus the place for all kinds of interactions between members of this community, and between the microbiota and the host. Cross-feeding is a particular mode of exchange of metabolites between producing donor bacteria and recipient bacteria that are unable to synthesize the metabolites. This exchange is known to contribute to the diversity and balance of the gut microbiota community. Thus the identification of novel cross-feeding interactions and the elucidation of the underlying evolutionary processes hold considerable promise in promoting human health. Quinones are small lipophilic molecules that are key for electron transfer chains and the bacterial metabolism. They have recently been identified as potential candidates for cross-feeding.

Objectives: The aim of this study was to confirm the existence of quinone cross-feeding, while assessing its prevalence in the human gut microbiota and the underlying evolutionary mechanisms.

Methods: Genomic annotations using Hidden Markov Model profiles for sequence similarity search were performed to detect quinone biosynthetic pathways for nearly 1500 bacterial species from the Unified Human Gastrointestinal Genome dataset (UHGG; Almeida et al., 2021). Biochemical analysis and heterologous complementation experiments were used to confirm the functionality of the identified quinone pathways. Further experimental analyses were performed directly in representative gut species to validate the quinone cross-feeding. A novel method that couples High Performance Liquid Chromatography and tandem Mass Spectrometry was also developed for the quantification of the diversity of quinones present in complex gut microbiota samples. Maximum likelihood phylogenetic trees were obtained to investigate the distribution and evolution of the quinone pathways of interest.

Results: Our bioinformatic analyses revealed the menaquinone (MK) pathway as the most abundant quinone pathway found in the UHGG dataset. Strikingly, partial MK pathways were preferentially found in species present in the human gut microbiota, and this was consistently observed across major phyla. Our hypothesis was that these partial pathways could enable the production of MK from intermediate products of the MK pathway, and could thus sustain a cross-feeding for MK production. The functionality of the genes constituting partial MK pathways in recipient bacteria was experimentally validated and the cross-feeding of quinone precursors was confirmed. Our data show that quinone biosynthesis depends on cross-feeding (from other bacteria and from the host) for MK biosynthesis. Phylogenetic analyses revealed that the first steps of an ancestral MK biosynthetic pathway were repeatedly lost in several bacterial phyla, leading to the observed auxotrophy for several gut bacteria. This could constitute a parallel adaptation of the bacteria to the gut environment. The newly developed quinone quantification method is currently used for the confirmation of quinone cross-feeding in vivo and the detection of quinone content in dysbiotic gut microbiota.

Abstract ID: 620

Race for iron: An exploiter's perspective on siderophore-mediated networking in bacterial communities

Presenting author: Dr. Aileen Krüger, Forschungszentrum Jülich GmbH, Jülich, Germany

Co-authors: *Johanna Wiechert, Seung-Hyun Paik, Bund Michelle, Dietrich Kohlheyer, Thomas Drepper, Julia Frunzke*

The trace element iron is a physiological requirement of almost every living organism as it plays important roles in numerous biological processes. Nevertheless, due to the extreme low solubility of Fe^{3+} in aerobic environments, microbes employ sophisticated strategies to manage homeostasis of this valuable resource. One strategy is the production and secretion of Fe^{3+}-complexing siderophores. Within this project, we assess competitive and cooperative strategies for the acquisition of iron through siderophores as public goods in defined microbial communities.

Corynebacterial species have been reported as frequently occurring natural siderophore exploiters in natural microbiomes, such as the nasal microbiome (1). Within this project, we investigate *Corynebacterium glutamicum* as model to explore the exploiter's perspective on siderophore-mediated networking. To establish defined microbial communities, we screened for different siderophore producers allowing *C. glutamicum* to overcome iron-limitation. These result indicated that *C. glutamicum* is capable of utilizing a chemically diverse set of xenosiderophores including *Escherichia coli* enterobactin, *Bacillus subtilis* bacillibactin and *Ustilago maydis* ferrichrome, while xenosiderophores like *Pseudomonas putida* pyoverdine cannot be used. Adaptive laboratory evolution experiments with *C. glutamicum* and spent medium from different siderophore producers resulted in the isolation of *C. glutamicum* strains with superior xenosiderophore utilization capabilities. Establishment of synthetic co-cultures grown in microfluidic devices confirmed the dependency of *C. glutamicum* on enterobactin production of *E. coli* under iron limiting conditions. This setup enables the examination of iron management strategies and their impact on the structure and dynamics of microbial communities, uncovering spatial variations and temporal dynamics in microbial interactions. However, analysis of defined communities also demonstrated that siderophore-mediated interactions are highly context-dependent, challenging the simplistic classification of siderophores as public goods.

(1) Zhao et al. (2024): *The ISME Journal*.

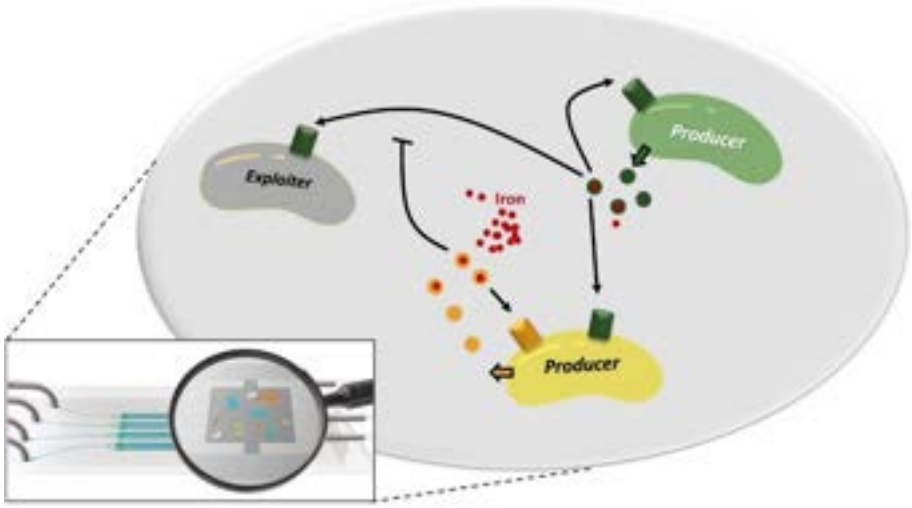


Figure 1: Schematic representation of siderophore-mediated bacterial interactions. Different siderophore-producing strains (green, yellow) influence iron-acquisition dynamics within a bacterial community. These interactions include the production of siderophores to scavenge iron (red) and the exploitation of xenosiderophores by non-producing strains (grey). This interplay highlights the complex balance of positive and negative effects associated with siderophores as a shared resource.

Abstract ID: 1832 / Poster Number: 420.15P

Rapid and Comprehensive Genomic Analysis of Bacterial Virulence and Antimicrobial Resistance in Circular Metagenome-Assembled Genomes Using the Novel Visualization Tool VicMAG

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Bacterial pathogens spread in clinical and environmental settings, and plasmids often mediate the transfer of virulence factor genes (VFGs) and antimicrobial resistance genes (ARGs) among bacterial communities. Metagenomic analysis with highly accurate long-read sequencing, such as PacBio HiFi sequencing, comprehensively identifies plasmids associated with virulence and antimicrobial resistance, proving effective for the wastewater-based surveillance of bacterial pathogens.

Several assembly tools are available for HiFi metagenomic sequencing reads, whereas no visualization tool is available for displaying the resulted multiple circular metagenome-assembled genomes (cMAGs) with functional gene annotations. Here, we introduce a Python-based tool, VicMAG, for effectively visualizing highly complexed cMAGs annotated with the updated databases of VFGs and ARGs.

Before showing the result, the following steps were applied to MAGs assembled by hifiasm-meta or metaMDBG: 1) Extract cMAGs from all MAGs. 2) Annotate and detect VFGs and ARGs by DFAST. 3) Identify chromosomes, plasmids, and prophage regions in cMAGs using geNomad. The resulted display by VicMAG distinctively highlighted VFGs and ARGs in chromosomes and plasmids, and allowed for the comparison of cMAG sizes based on the cubic root of nucleotide lengths. This visualization facilitated our understanding of the diversity of bacteria and plasmids associated with virulence and antimicrobial resistance. This tool is applicable to visualize multiple of circular genomes as well. VicMAG is now open for use on GitHub. Furthermore, we are developing this into a web tool, enabling users in the field of clinical and environmental microbiology to collectively analyze cMAGs and individually scrutinize them of interest.

Abstract ID: 2899 / Poster Number: 152.17P

Rapid and massive antimicrobial screening of natural isolates through a novel 3D printed replica plate device

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Antimicrobial resistance is one of the most significant global public health problems. This necessitates the search for novel antibiotics producers from yet understudied natural sources. The success rate of drug discovery has proven to be very low. Therefore new approaches encompassing rapid and massive screening of array of microorganisms are still needed. In this study, we developed a novel 3D printed replica plate device to screen a large library of halophilic bacterial isolates from several hypersaline habitats in Bulgaria. Halophilic bacteria were isolated by drop deposition (1 cell per 1 µL drop on average) on solid media with the use of the newly developed replicator. Then the formed colonies were screened against six human pathogens by a modified agar overlay assay. The antibacterial activity of ethyl acetate extracts from the positive strains was evaluated by the agar well diffusion assay. Over 6000 colonies were screened and 56 of them (0.93% success rate) demonstrated positive results. After confirmation of their antibacterial activity the number was reduced to 23 active isolates out of the initial 56 (41% success). All of these strains demonstrated growth inhibition of at least one of the tested pathogens while mostly inhibited Acinetobacter calcoaceticus (62% of the strains), Enterococcus faecalis (42%) and Staphylococcus epidermidis (35%). The quantitative data showed inhibition halo zones in the range of 15–30 mm. Initially, our large-scale screening approach still had limited success, but rapidly led to a collection of strains where the rate of detecting antimicrobial-producing bacteria increased 40-fold.

Abstract ID: 3685 / Poster Number: 189.17P

Rapid and Reliable Detection of Carbapenemase-Producing Klebsiella pneumoniae and Escherichia coli: A Comparison of VITEK2, CIM, and Carbapenemase Gene Analysis

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Background: Rapid and reliable detection of carbapenemase-producing *K.pneumoniae* and *E.coli* infections, which are a major public health problem worldwide, is crucial for infection control measures.

Objective: In this study we aimed to compare the carbapenemase results of the VITEK2 (bioMérieux,France) with the carbapenem inactivation method (CIM) and to investigate the presence of carbapenemase genes.

Methods: A total of 172 *K.pneumoniae* and 211 *E.coli* isolates from blood cultures were included in the study. Antibiotic susceptibilities were determined using the VITEK2 automated system. CIM testing was performed on isolates with MIC values >0.125 for ertapenem and meropenem, and marked as "carbapenemase +/-" by the automatic system. PCR was performed to detect carbapenemase genes.The genes responsible for carbapenemase production ($bla_{\text{OXA-48}}$, bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}) were investigated by PCR.

Results: Out of 172 *K.pneumoniae* isolates"carbapenemase +/-" was detected in 43% (n:74) by VITEK2. Among these, $bla_{\text{OXA-48}}$ was identified in 66.2% (n:49), bla_{KPC} in 8.1% (n:6) and bla_{NDM} in 5.4%(n:4). No genes were detected in 22.9%(n:17) of the isolates. Additionally, 83.8%(n:62) of these isolates were positive by CIM, while 16.2 (12%) were found negative. Of the 209 *E.coli* isolates, "Carbapenemase +/-" was detected in 0.9% (n:2). Although we could not detect any carbapenemase gene by PCR in these two isolates, CIM tests were positive. None of the isolates tested were found to harbor bla_{IMP} or bla_{VIM} genes.

The carbapenemase test results of VITEK2 automated systems are rapid and reliable for *K.pneumoniae* and *E.coli*.

Abstract ID: 819 / Poster Number: 076.17P

Raw Milk as a Reservoir of Carbapenem-Resistant and ESBL-Producing Bacteria: Insights from a Serbian Farm Study

Presenting author: Ivana Peric, PhD, University of Belgrade Faculty of Agriculture, Belgrade, Serbia

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Antimicrobial resistance (AMR) in milk samples, particularly bacteria resistant to carbapenems and producing extended-spectrum beta-lactamases (ESBLs), poses a serious and growing threat to global health as resistant bacteria can spread through the food chain. The aim of this study was to determine the route of transmission and prevalence of these resistant bacteria, focussing on the isolation and identification of the pathogenic bacteria.

A total of 65 cow's milk samples, 28 goat's milk samples and 13 swab samples were taken from farms in Serbia. The samples were inoculated on ESBL and Carba Smart agar (Merck, Germany), which were developed for the detection of ESBL-producing and carbapenem strains. The isolated pathogenic bacteria were identified using the MALDI-TOF Mass Spectrometry method. In addition, a Quantum BT-Cef (ProGnosis Biotech, Greece) rapid test was performed to detect specific antibiotic groups in 30 samples of milk.

Among the resistant strains observed in milk, *Pseudomonas* spp. dominated (40.48%), followed by *Stenotrophomonas maltophilia* (17.46%), *Acinetobacter* spp. (7.54%), *Ochrobactrum* spp. (5.56%) and *Aeromonas* spp. (4.37%). *Pseudomonas* spp. (35.90%), *Empedobacter brevis* (15.38%) and *Lacticaseibacillus paracasei* (7.69%) were most frequently present in swab samples. One cow's milk sample were positive for the presence of β-lactams.

The results show that raw milk is important source of multi-resistant bacteria and resistance genes. Therefore, it is necessary to identify possible sources and vectors for the transmission of antibiotic resistance genes. To solve these problems, regular milk controls, staff training to improve hygiene practises and controlled use of antibiotics are required.

Abstract ID: 700 / Poster Number: 072.15P

Reactive oxygen species affect gut microbial communities in a dose-dependent manner

Presenting author: Mx Hina Ayub, Kiel University and University Medical Center Schleswig-Holstein, Kiel, Germany

Co-authors: *Hannah Jebens, Malte Rühlemann, Mathilde Poyet, Mathieu Groussin*

Gut microbial communities are fundamental to human health, playing critical roles in digestion, nutrient metabolism, immune regulation, and protection against pathogens. Dysbiosis, or disruptions to these communities, has been linked to inflammatory bowel disease (IBD), a collection of chronic inflammatory disorders. Oxidative stress, a key contributing factor to inflammation, has been implicated in IBD. Reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) are byproducts of normal cellular processes and immune activity. Although ROS play important roles in cell signalling and defence, excessive levels can cause oxidative stress, leading to cellular damage. While significant progress has been made in understanding the role of ROS exposure in immune deregulation, their impact on the gut microbiome remains underexplored. To investigate these dynamics, we cultivated a stool sample from a healthy donor in a chemostat bioreactor designed to mimic conditions of the gastrointestinal tract. ROS were introduced through controlled H_2O_2 pulses and samples were collected twice a day for four weeks. Samples were analysed for their microbial community composition to assess the impact of oxidative stress on gut microbiome dynamics. Preliminary observations revealed a dose-dependent microbial response, with low ROS levels promoting resilience and recovery and higher levels delaying growth and increasing redox potential variability. These results provide valuable insight into how oxidative stress shapes growth patterns to potentially contribute to dysbiosis in inflammatory bowel disease (IBD). Ultimately, these findings are expected to enhance our understanding of gut microbial resilience to oxidative stress and inform therapeutic strategies for restoring microbial balance during inflammatory conditions

Abstract ID: 3388 / Poster Number: 430.17F

Realising meaningful development through microbial biotechnologies: the potential of realist evaluation based ethical frameworks

Presenting author: Dr. Kevin De Sabbata, Keele University, Keele, United Kingdom

Introduction: Microbial biotechnologies have great potential to contribute to development processes, but they also pose risks and require articulate infrastructures to produce benefits. In this regard, projects in this area might be able to lay successful claims on development funds, while not guaranteeing clear benefits for individuals and communities. Current ethical review systems seem unable to address this issue, as they use a reductionistic approach failing to consider contexts and mechanisms impacting the success of reviewed interventions.

Objective: This paper advocates for a realist evaluation approach to the ethical evaluation of microbial biotechnologies, showing how such an approach would allow for a better understanding of the impact and ethical implications of microbial biotechnologies for local communities, especially in Low-Middle Income Countries (LMICs).

Methodology

Literature based theory-building analysis.

Findings: Realist evaluation uses Context-Mechanisms-Outcomes (CMO) analysis, to assess interventions in light of the concrete context in which they take place. Literature in health services research, technology implementation, global health and development shows how this framework can have great potential in relation to ethical evaluation. Applying CMO analysis to the compliance assessment of key ethical principles in past ethically controversial cases involving microbial biotechnologies shows how such an approach would have allowed to avoid most of the problems emerged in such contexts.

Conclusion: Realist evaluation frameworks would be highly advantageous in relation to the ethical evaluation for microbial biotechnologies and would guarantee a fairer use of development funds in relation to biotechnologies.

Abstract ID: 463 / Poster Number: 143.16P

Reassembled S. cerevisiae-Derived Vacuolar Membranes Functionalized with AML-Targeting Peptides for Precision Drug Delivery in Human Leukemia Therapy

Presenting author: Ms. Yun Young Cho, Jeonbuk National University, Jeonju, South Korea

Co-authors: *Jiho Min, Yang-Hoon Kim*

Introduction: Developing targeted drug delivery systems is crucial for enhancing therapeutic efficacy and minimizing off-target effects in leukemia treatment. This study addresses these challenges by engineering a biocompatible platform using vacuoles derived from recombinant Saccharomyces cerevisiae(S. cerevisiae) with acute myeloid leukemia(AML)-targeting peptides. Normal vacuoles face stability issues due to hydrolytic enzymes within their interior. To overcome this, vacuolar membranes with AML-targeting peptides were reassembled, excluding the enzyme, creating a stable and effective drug delivery system.

Objective: The objective of this study is to develop reassembled vacuolar membranes derived from recombinant S. cerevisiae functionalized with peptides targeting AML, aiming to improve the delivery and therapeutic efficacy of Daunorubicin(DNR) in human leukemia cells.

Method: Reassembled vacuoles(ReV) from recombinant S. cerevisiae, functionalized with AML-targeting peptides and encapsulating DNR, were developed. They were characterized for size, stability, morphology, and chemical composition using NTA, FT-IR, and FE-SEM. Cytotoxicity, drug release, and apoptosis induction were assessed in HL-60 cells to evaluate therapeutic potential.

Findings: Peptide-functionalized ReV demonstrated improved targeting efficiency, structural stability, and precise drug release kinetics, achieving complete DNR release within 24 hours. Cytotoxicity assays showed significantly higher apoptosis rates in HL-60 cells compared to NorV, with reduced nonspecific cytotoxicity in RAW264.7 and HeLa cells. The incorporation of targeting peptides reduced off-target effects, highlighting the specificity of the platform.

Conclusion: Peptide-functionalized ReV represent an innovative and effective drug delivery platform for AML therapy, combining targeted delivery, enhanced drug stability, and minimized side effects. These findings pave the way for advanced precision treatments in human leukemia.

Abstract ID: 3167 / Poster Number: 444.16P

Recent education and role models are predictors of hand hygiene compliance among medical students

Presenting author: Assoc. Prof. Girbe Buist, University Medical Center Groningen, Groningen, Netherlands

Introduction: Hand hygiene is the most important measure to prevent health care-associated infections, but compliance of health care workers is generally low. When trying to improve compliance, medical students are an important population to target. Education is an important and attainable strategy to improve hand hygiene compliance of medical students.

Objective: We evaluated the knowledge and perceptions of medical students regarding hand hygiene and factors associated with compliance.

Methods: This was a cross-sectional study using an online questionnaire based on World Health Organization (WHO) questionnaires on knowledge and perceptions of hand hygiene. Self-reported compliance was based on the 'My five moments for hand hygiene' of the WHO and a multivariable regression analysis was done to assess factors associated with compliance. All medical students of the university of Groningen (the Netherlands) were approached.

Findings: The questionnaire was completed by 290 students. Mean overall self-reported compliance was 85.7% (SD 12.5) with compliance being the highest before performing an aseptic procedure (97.1%, SD 10.7) and after exposure to body fluids (97.9%, SD 6.6) and lowest after touching the patient's surroundings (67.4%, SD 26.2). Factors associated with self-reported compliance were nationality, compliance of health care workers, time to last education on hand hygiene and the importance fellow students attach to the student performing optimal hand hygiene.

Conclusion: Our study identified topics on which knowledge could be improved upon through education. Furthermore, our results suggest that education should be regularly repeated and that role models are important influencers of compliance.

Abstract ID: 48 / Poster Number: 260.17P

Recombinant Escherichia coli as a Biosensing Element of Single-Chamber Microbial Fuel Cell Biosensor for Continuous Hexavalent Chromium Measurement

Presenting author: Prof. Ying-Chien Chung, China University of Science and Technology, Taipei City, Taiwan

Co-authors: *Chih-Yu Chen, Jong-Tar Kuo, Chiu-Yu Cheng,*

Hexavalent chromium [Cr(VI)] has been proven to be carcinogenic, highly toxic and also listed as a hazardous substance by the US ATSDR. Currently, the analysis methods for measuring Cr(VI) include atomic absorption spectroscopy, inductively coupled plasma and ion chromatography techniques. These techniques are expensive and do not identify the bioavailable metals. A novel biosensor combining molecular biology and electrochemistry was developed. We constructed the ChrB gene with high Cr(VI) specificity, and the ChrA gene with high Cr(VI) tolerance in two pET28a vectors and then transferred they to Escherichia coli as a biosensing element of single-chamber microbial fuel cell (SCMFC). Results showed that there was no difference between the growth rate of the recombinant bacteria ChrA-ChrB-E. coli and E. coli. The applicable temperature and pH range of ChrA-ChrB-E. coli were 25–45oC and pH 5–8, respectively. The Cr(VI) forms (CrO42- and Cr2O72-) and oxygen-containing anions would not affect the activity of ChrA-ChrB-E. coli for Cr(VI) detection. SCMFC biosensor inoculated ChrA-ChrB-E. coli was developed for continuous Cr(VI) measurement. The optimal resistance and liquid retention time for the SCMFC biosensor were 800 Ω and 2 min, respectively. Under this condition, two good linear relationships were observed between Cr(VI) concentrations (0–0.5 mg/L and 0.5–200 mg/L) and the voltage outputs. The biosensor could accurately measure Cr(VI) concentrations (0.02–150 mg/L) in synthetic wastewater with low deviations (-1.73%–2.50%) in a continuous operation mode. Thus, the novel SCMFC biosensor has potential as a real-time and continuous device to monitor Cr(VI) in water.

Abstract ID: 2898 / Poster Number: 473.15S

Recombinant production of novel antimicrobial and antiviral peptides using Corynebacterium glutamicum and sustainable substrates

Presenting author: Dr. Christian Riedel, Ulm University, Ulm, Germany

Bioactive peptides with a wide range of activities are abundantly available in nature. For example, bacteriocins are antimicrobial peptides produced by bacteria to inhibit competitors in their natural environments. Some of these peptides and/or their natural producers are widely used as food preservatives. Likewise, peptides with antiviral activity can be readily detected and isolated from the peptidome of human body fluids and other sources. In light of the increasing problems with antibiotic resistances of many important human pathogens and the threat of pandemics of infection diseases caused by zoonotic pathogens, bioactive peptides may become interesting alternative therapeutics. To allow preclinical research, clinical application, and entry peptide antiinfectives into the market, efficacy of production and purity of the products need to be improved. Currently, industrial production of bioactive peptides is mostly performed using natural producer organisms or chemical synthesis. This bears limitations due to complex substrates, extensive downstream purification, high costs and low yields. One possible solution for these limitations is to shift production to recombinant biotechnological production hosts. Due to the intrinsic antimicrobial activity of antibacterial peptides, this is, however, not a trivial task. High level production requires efficient secretion into the culture supernatant to avoid contaminations with cellular components/peptides. Moreover, correct posttranslational modification is required for activity.

Here, we will report on some of our recent efforts to identify (novel) bacteriocins in environmental microbiomes and (meta)genomic data. This includes high throughput screenings and mechanistic studies using live bacterial biosensors. We will further highlight approaches to establish recombinant production of several bacteriocins and other bioactive peptides using the industrial workhorse organism *Corynebacterium glutamicum* with a view to shift production to sustainable substrates and industrial waste streams. Moreover, we will discuss problems associated with recombinant production and possible solutions.

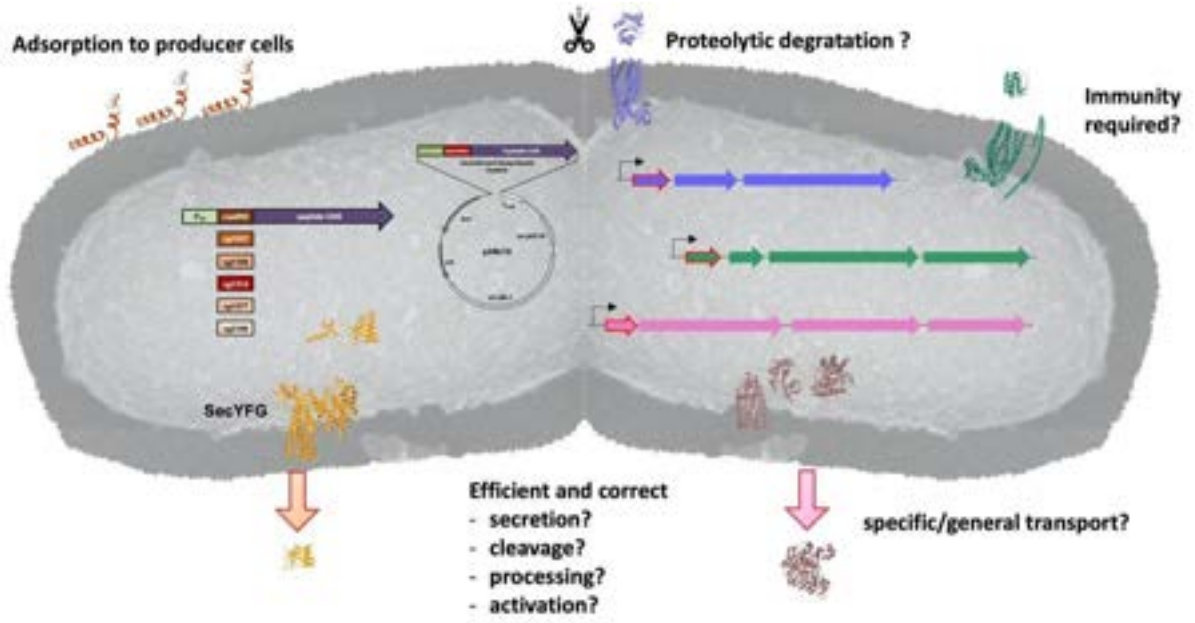


Figure 1: Challenges associated with recombinant production of bioactive peptides.

Abstract ID: 3603

Reconstructing the microbiome of in situ incubations uncovers essential microbes involved in polymer breakdown in Arctic deep-sea hydrothermal sediments

Presenting author: Dr. Katharina Sass, Christian Albrechts Universität zu Kiel, Kiel, Germany

Co-authors: *Hasan Arsin, Anita-Elin Fedøy, Ruth Schmitz-Streit, Wolfgang R. Streit, Ida H. Steen, Runar Stokke*

Deep-sea hydrothermal vents represent some of the most extreme environments on Earth, making them fascinating targets for marine bioprospecting and the discovery of novel biological resources. However, the full potential of microbial heterotrophic activity in these ecosystems is still being investigated. Previous metagenomic research has revealed that previously uncultured heterotrophic microbes play a key role in regulating the carbon flow in deep-sea hydrothermal sediments through the breakdown of organic matter. As a result, these environments are crucial for identifying new microbial solutions needed in the bioprocessing industry, where the breakdown of complex organic substances is a significant challenge.

In this study, we describe the development and enrichment strategy for novel *in situ* incubators designed to explore the degradation of natural polymers like chitin, as well as synthetic plastic polymers such as PET and HDPE. Sequencing data from *in situ* enrichments using sediment samples from the Jan Mayen vent field and the newly discovered Jøtul vent field revealed shifts in microbial community composition and pointed to specific microorganisms involved in polymer degradation.

Along with typical heterotrophic microbes, we identified potential novel chitin-degrading organisms within the uncultivated candidate phylum KSB1. These organisms are of particular interest since they have not been previously cultured, and their role in carbon metabolism had not been documented. Through bioinformatic analysis, we identified several chitin-degrading enzymes within the KSB1 phylum. After heterologous expression and purification, these enzymes demonstrated strong chitin-degrading activity, suggesting that KSB1 bacteria play an important role in the carbon cycle. Furthermore, *in situ* incubations with both chitin and plastic polymers showed an enhanced rate of plastic polymer degradation. Transcriptomic analysis enabled the reconstruction of microbiomes and provided insight into the underlying metabolic pathways.

These findings indicate that polymer-degrading microorganisms were successfully enriched *in situ* and play a vital role in the carbon cycling of deep-sea sediments. We propose that these microbes are key contributors to organic matter breakdown in hydrothermal vent ecosystems. Additionally, the novel microbial lineages identified here are likely adapted to industrial substrates, offering promising new avenues for enzyme discovery and biotechnological applications.

Abstract ID: 414 / Poster Number: 103.15F

Recovery of microbial communities in rewetted coastal shrub bogs in the Pocosin Lakes National Wildlife Refuge, NC, USA

Presenting author: Dr. Andrew St. James, Wake Forest University, Winston-Salem, United States

Introduction: Pocosin bogs form a unique class of subtropical wooded peatland found along the Atlantic coast in the Southeastern United States. These bogs store approximately 20% of the total peat in the continental United States and have been shown to accrete peat under significantly warmer temperatures than northern peatlands, including under increased drought conditions or light fires. Historic drainage of pocosin bogs for farming has resulted in the loss of nearly 70% of pocosin areas in coastal North Carolina. Recent studies have shown that rewetting of drained pocosins significantly reduces greenhouse gas losses and increases carbon sequestration, though the contribution of the microbial communities to these processes remains unknown.

Objective and Methods: The objective of this study is to compare metagenomic datasets from drained and rewetted pocosin areas within the Pocosin Lakes National Wildlife Refuge, NC, USA to evaluate structural and functional changes to the microbial communities therein.

Findings and Conclusion: Rewetting of drained pocosins was associated with (1) a decrease the overall microbial community diversity, (2) an increase the dominance of Acidobacteria, (3) a shift towards increased carbohydrate and lipid metabolism, and (4) an increase in the abundance of enzymes associated with Sphagnum degradation. When compared to pristine peatlands elsewhere, the shifts in the rewetted state indicate a recovery of the microbial community to a state that is characteristic of pristine conditions. We conclude that the microbial community in drained pocosin areas is capable of susbtantial recovery upon rewetting.

Abstract ID: 3056 / Poster Number: 433.15P

Recycling textile waste into fibres: Bioconversion of thermochemical PET hydrolysates from mixed textiles using cellulose producing Komagataeibacter spp

Presenting author: Ms. Suksham Suksham, Wood K plus – Kompetenzzentrum Holz GmbH, Linz, Austria

Co-authors: *Michael Egermeier, Anna Maria Erian, Hans Marx*

Global textile industry faces challenges from resource constraint, environmental deterioration, and surging textile waste. Converting textiles into recycled fibres offers a sustainable solution by promoting circular economy principles and reducing the demand of virgin material. Therefore, the development of sustainable textile recycling techniques that effectively treat blended textile waste would reduce textile waste incineration.

The objective is to recycle mixed textiles by degrading the polyethylene terephthalate (PET) into monomers ethylene glycol (EG) and terephthalic acid (TPA), and to utilize them as carbon sources to cultivate cellulose-producing bacteria.

Alkaline hydrolysis has been used to degrade the PET of a mixed textile. The obtained hydrolysate was quantified for EG and TPA, while the recovered cellulose was investigated for recycled fibre applications. The pathways to metabolize EG and TPA in Komagataeibacter species have been identified and Golden Gate Cloning was used to incorporate missing enzymes enabling the utilization of hydrolysates for cellulose biosynthesis. Adaptive laboratory evolution (ALE) has been performed to increase the strains tolerance towards and TPA and increase EG uptake rates.

Hydrolysis results for three different mixed textile wastes are shown, with their applicability for microbial conversion of the liquid fraction to produce bacterial cellulose. Two key enzymes were identified and introduced in Komagataeibacter to enable the utilization of EG as sole carbon source. Initial findings to increase the performance of both strains via ALE are shown.

Future investigations will include the metabolic engineering of Komagataeibacter for TPA utilization and increase bacterial cellulose production.

Abstract ID: 2609 / Poster Number: 141.15P

Re-defining type III cellulose synthase clusters: the cellulose synthase of Ancylobacter sp. STN1B

Presenting author: Sophie Marie Martirani-Von Abercron, PhD, CSIC, Granada, Spain

Co-authors: *Daniel Pacheco-Sánchez, Rocío Fernández-Gonzalez, Patricia Marín, Inés Castillo-Rodríguez, Silvia Marqués*

Cellulose is a key component of the extracellular matrix in several bacterial species. Its synthesis is mediated by cellulose synthase complexes, which are classified in three major types based on their accessory subunits. The well described type I system, characterised by the presence of the BcsD and BcsH scaffolding subunits essential for cellulose crystallinity, is present in known crystalline nanocellulose-producing strains. In contrast, type III systems lack these subunits as well as the porin moiety of the BcsC subunit, replaced by BcsK [1].

We have characterised the Bcs system of *Ancylobacter* sp. strain STN1B, which has a single type III cellulose synthesis cluster [2]. The strain produces abundant crystalline cellulose despite the absence of subunits essential for crystallinity.

We used *in silico* analysis, site-directed mutagenesis, protein-protein interaction assays, transcriptomics approaches and cellulose characterization to elucidate the role of the components of this type III cluster. Our results show that *bcsA*, *bcsB* and *bcsK* are essential for cellulose biosynthesis. Furthermore, the previously unassigned *bcsN* gene was also essential for cellulose synthesis. Four new genes downstream *bcsN*, encoding a periplasmic L-D transpeptidase, two highly expressed hypothetical proteins and an acyl-transferase, were also shown to be involved in cellulose synthesis. Two regulators, *bcsR* and *bcsT*, were also identified.

This study extends the known components of type III clusters by including 8 new flanking genes, and identifies key components responsible for cellulose synthesis, extrusion, regulation, and integration into the periplasmic space, providing new insights into the functional architecture of type III Bcs clusters.

Abstract ID: 3459 / Poster Number: 181.17P

Reduced phage susceptibility could be caused by modulation of host genome and altered surface in vancomycin intermediate Staphylococcus aureus

Presenting author: Frederik Pedersen, PhD, University of Copenhagen, Copenhagen, Denmark

Co-authors: *Jack Abrahamsson, Hanne Ingmer, Janine Bowring*

Vancomycin intermediate *Staphylococcus aureus* (VISA) is an unstable phenotype often associated with reoccurring infections and reduced treatment success. With limited treatment strategies, phage therapy is being explored as an alternative option. In VISA, resistance is acquired through a broad spectrum of mutations. Revertants that phenotypically lose resistance maintain a memory of the resistance genotype, as reversion occurs through acquisition of secondary mutations, allowing fast readaptation to a VISA phenotype. Both VISA and revertant mutations have been found to impact metabolism, gene expression, cell envelope and virulence, which begs the question how the susceptibility to phages are affected. Recently, a USA300-derived VISA demonstrated significant loss in phage susceptibility compared to the parental strain. We wanted to explore phage susceptibility in derived vancomycin sensitive revertants and assess if reduced phage susceptibility is caused by surface modulations. Here we show that reversion of a phage resistant VISA strain restored phage susceptibility to wildtype levels. Furthermore, we show that mutations in *arcC* acquired in the VISA phenotype may be associated with supressing phage infection. Using phase contrast microscopy and adsorption assays we determine if modulations to the cell wall could be contributing to increased phage resistance. We also investigate changes to the abundance of wall teichoic acid, a major cell wall component and the sole *S. aureus* phage receptor, by extraction. This data strives to offer new fundamental insights into phage biology, providing mechanistic building blocks for alternative therapy approaches when treating VISA by increasing understanding of the interplay between phages and its host.

Abstract ID: 640 / Poster Number: 353.17P

Redundancy of Citrate Chemoreceptors in the Phytopathogen Pectobacterium atrosepticum

Presenting author: Ms. Roberta Genova, Zaidin Experimental Station (CSIC), Granada, Spain

Co-authors: *Miguel Angel Matilla, Tino Krell*

Introduction: Phytopathogenic bacteria encode significantly more chemoreceptors than the bacterial average, suggesting the importance of chemotaxis in plant colonization and infection. However, information on the function of these receptors is scarce. Pectobacterium spp. are among the most relevant global phytopathogens, causing soft rot diseases in many agricultural important crops. Pectobacterium atrosepticum causes blackleg disease on potatoes and its model strain SCRI1043 harbours 36 chemoreceptors that are mostly of unknown function.

Objective: Elucidate the function of P. atrosepticum chemoreceptors and investigate their expression.

Methods: Purification of individual chemoreceptor ligand binding domains (LBDs), thermal shift assay-based ligand screening, isothermal titration calorimetry, quantitative chemotaxis capillary assays.

Findings: We identified five chemoreceptors that recognize citrate, a molecule abundantly present in the plant root exudates. Two of them recognize citrate and citrate/Mg2+ complexes at HBM-type LBDs with dissociation constants (KD) between 28 to 186 µM. The remaining three chemoreceptors recognize citrate but not citrate/Mg2+ at 4-helix bundle LBDs with KD values of 33 to 59 µM. Despite there being five citrate chemoreceptors, the magnitude of citrate chemotaxis is extremely low, suggesting the existence of regulatory mechanisms that are currently been investigated.

Conclusion: The presence of multiple citrate chemoreceptors likely broadens the response range of *Pectobacterium atrosepticum*, suggesting the importance of citrate in plant-bacteria communication. This study also highlights the usefulness of protein-based approaches in the study of complex chemotactic systems. Further insights into the regulation and function of these chemoreceptors will shed light on microbe–plant communication and on the virulence mechanisms of this phytopathogen.

Abstract ID: 1526 / Poster Number: 275.16F

Refinement of the in vivo mutagenesis system T7-DIVA for enhanced continuous directed protein evolution

Presenting author: Beatriz Alvarez, PhD, CNB-CSIC, Madrid, Spain

Co-authors: *Diego Crespo, Luis Angel Fernandez*

Introduction: We have previously developed the in vivo mutagenesis system called T7-DIVA (T7-targeted dCas9-limited in vivo mutagenesis) for the directed evolution of proteins. This system is based on a fusion of a base deaminase (BD, cytosine or adenine deaminase) with the T7 RNA polymerase (T7RNAP) that recognizes a T7 promoter placed towards the target gene. The fusion then will move along the gene and the base deaminase will create mutations in the gene. To block the progression of the fusion, dCas9 molecules guided by three crRNAs are used, yet the blockade is not total due to the high processivity of the system.

Objective: To optimize the T7-DIVA system for enhanced directed protein evolution.

Method: We have used the bases deaminases TadA3.1 and TadA3.155 that present dual activity cytosine and adenine deaminase for broadening the type of amino acid changes created. We have also used low-processivity variants of T7RNAP for a better control of the system. The new fusions were tested in *Escherichia coli* using URA3 as reporter gene to assess the mutagenic activity by plating on selective media. The type and distribution of mutations along the target gene were determined by sequencing methods.

Findings: Both TadA3.1 and TadA3.155 fused to the T7RNAP presented dual activity. Additionally, the processivity of the system was reduced using the T7RNAP variants increasing its control. All these elements were successfully combined for an optimized T7-DIVA system.

Conclusion: The T7-DIVA system was optimized using mutagenic fusions comprised of dual mutagenic activity enzymes and low-processivity T7RNAP variants.

Abstract ID: 2923 / Poster Number: 300.16S

Region-Specific Biomarkers in Azeitão and Nisa Cheeses: A Step Toward Authenticity and Tradition Preservation

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Co-authors: *Eugenio Parente, Maria Teresa Barreto-Crespo, Teresa Semedo-Lemsaddek*

The production of traditional Portuguese cheeses is a centuries-old practice that combines ancient techniques with local culture, creating a rich heritage passed down through generations. To promote these traditional foods and enhance their economic impact in rural areas, the Protected Designation of Origin (PDO) status was established. PDO-certification is granted to food products made within a specific geographical region, using locally sourced raw materials and traditional production methods. Portugal has over eleven PDO-certified cheeses, including varieties from *Azeitão* and *Nisa*. These cheeses are crafted in accordance with traditional methods and PDO regulations, without the addition of external microbiota during production. Consequently, the natural microorganisms present in the cheese-making environment play a pivotal role in shaping the distinct characteristics of each cheese. However, strict regulations and high value make these products vulnerable to fraud, highlighting the need for reliable identification and verification methods.

This research aims to characterize the microbiota of PDO cheeses using a metagenomic approach, with the goal of identifying eco-specific biomarkers unique to cheeses produced in the regions of *Azeitão* and *Nisa*.

Cheeses collected between 2016-22 from all PDO-certified producers, comprising four in *Azeitão* and two in *Nisa*, were included in this study. Total DNA was extracted from the samples, and the V3-V4 regions of the 16S rRNA gene and the ITS2 regions were sequenced using the Illumina MiSeq. The resulting FASTA sequences were processed in RStudio 4.2 using several specialized packages. The phyloseq package was employed for microbial data analysis and visualization, while DECIPHER and phangorn were used for sequence alignment and phylogenetic tree inference. Taxonomic assignments were conducted using the DADA2 package, relying on the SILVA v138.2 database for bacteria and the UNITE database for fungi. Additionally, the microbiomeMarker package was applied to identify eco-specific biomarkers. This comprehensive approach enabled a

detailed characterization of the microbiota in PDO cheeses from *Azeitão* and *Nisa*, facilitating the discovery of unique microbial markers specific to each region.

Discriminant analysis revealed that the primary factors influencing microbial diversity between samples were the cheese factories (p-value < 0.001) and the production region (p-value < 0.02). Regarding microbial communities, cheeses from *Azeitão* exhibited a higher prevalence of *Leuconostoc* and *Lactococcus* consistently over the years. In contrast, cheeses from *Nisa* not only showed similar prominence of *Leuconostoc* and *Lactococcus* but also a notably higher prevalence of *Lacticaseibacillus*. For fungal communities, both regions demonstrated a high prevalence of *Yarrowia*, *Geotrichum*, and *Kurtzmaniella*. Following the characterization of the microbiota, the identification of region-specific microorganisms was conducted, resulting in the detection of five biomarkers unique to Azeitão and four biomarkers specific to Nisa.

The preservation of product authenticity is essential in the traditional food industry, particularly given the significance of PDO status. Employing a metagenomics-based approach to identify specific microbial markers can enhance the validation process for PDO seal attribution, offering a dual advantage, benefiting both producers and consumers by safeguarding the quality and authenticity of these food products.

Abstract ID: 3317

Regulating the glucose-6-phosphate dehydrogenase encoding gene gsdA and its impact on growth and citric acid production in Aspergillus niger

Presenting author: Assoc. Prof. Matthias Steiger, TU Wien, Vienna, Austria; Austrian Centre of Industrial Biotechnology (acib), Vienna, Austria; Christian Doppler Laboratory for Sustainable Bioproduction with Fungal Systems, Vienna, Austria

Co-authors: *Susanne Fritsche, Valeria Ellena, Güler Demirbas-Uzel*

Introduction: Glycolysis in *Aspergillus niger* provides the precursors for citric acid production. Glucose-6-phosphate dehydrogenase (G6PD), encoded by the gene *gsdA,* is the first enzyme in the pentose phosphate pathway (PPP).

Objective: Despite its decisive position in the metabolic network the functional role of G6PD and its impact on citric acid synthesis and growth is not fully understood.

Method: An *A. niger* strain expressing a ptet-on regulated version of *gsdA* at the *pyrG* locus was constructed (Fig. 1). Under non-inducing conditions, the strain was not growing on glucose. On gluconate, a precursor for an intermediate of the oxidative PPP, growth was restored but delayed compared to the control strain expressing *gsdA* under the native promoter.

Findings: Furthermore, citric acid production was monitored in dependency of different *gsdA* induction levels using doxycycline. At low induction the yield on glucose was enhanced by 49 % compared to the control strain, albeit with reduced growth leading to overall lower citric acid titers. Without the native regulation of the *gsdA* gene, growth and citric acid production were time delayed. However, the yield of the *gsdA*-regulated strain was higher compared to the control after 120 h of cultivation and was positively influenced with an increasing proportion of gluconate in the medium.

Conclusion: The findings of this study underscore the dependency of growth and citric acid production on *gsdA* expression in *A. niger*.

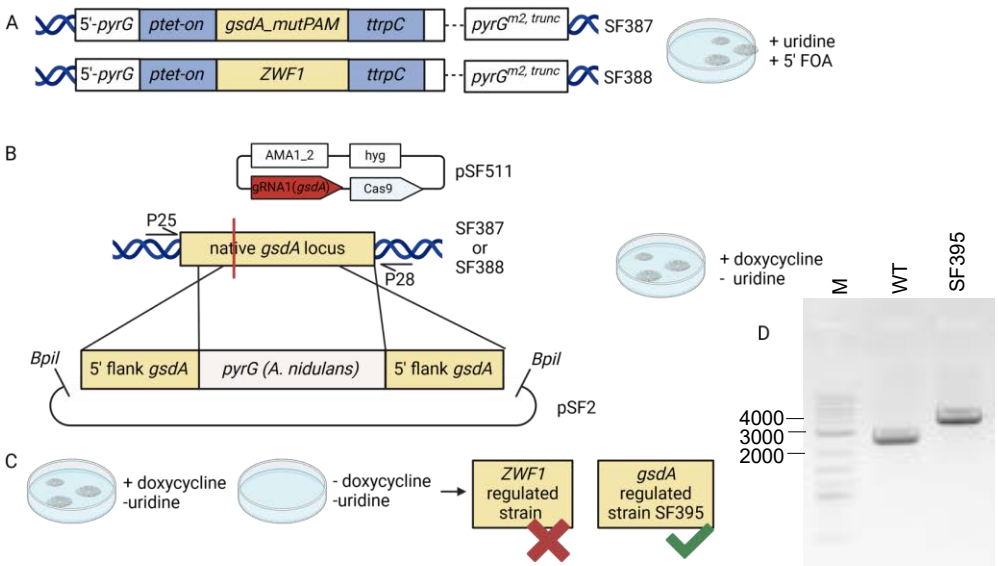


Figure 1: *Construction of a G6PD-regulated A. niger strain: (A) gsdA or ZWF1 expression-constructs tested, (B) the CRISPR/Cas9 strategy used for gene replacement, (C) phenotype of the gsdA regulated strain, (D) Gel electrophoresis of PCR fragments showing the replacement at the gsdA locus.*

Abstract ID: 1672 / Poster Number: 020.16P

Regulation of Biofilm Formation and Cell Adhesion in Chromobacterium violaceum: Role of RTX-Type Adhesins and LuxR Transcription Factors

Presenting author: Ms. Raquel Freire, Universidade de São Paulo, Ribeirão Preto, Brazil

Co-authors: *Beatriz Picinato, Tie Koide, José Da Silva Neto*

Biofilms are bacterial communities encased in an extracellular matrix, crucial for survival under adverse conditions. Adhesins play key roles in cell-cell and cell-surface adhesion and are secreted through specialized systems, such as the type 1 secretion system (T1SS). Several transcription factors, including those of the LuxR family associated with Quorum Sensing (QS) pathways, regulate these processes in bacteria. In *Chromobacterium violaceum*, a Gram-negative bacterium associated with human infections, the role of adhesins and regulatory pathways in biofilm formation remain poorly understood. This study investigates the regulatory mechanisms of biofilm formation and cell adhesion in *C. violaceum*, with a focus on the RTX adhesin CV_0311. Mutant strains for the RTX adhesin (CV_0311) and LuxR family transcription factors (CV_0305 and CV_0635) were characterized through autoaggregation, crystal violet biofilm staining, and fluorescence microscopy assays. The RTX adhesin CV_0311 was found to be essential for cell aggregation and biofilm initiation, as well as a T1SS (CV_0306-07-08) involved in its secretion. The transcription factors CV_0305 and CV_0635 exhibited distinct roles during different stages of biofilm formation. RNA-seq analysis revealed 173 and 425 differentially expressed genes in the ΔCV_0305 and ΔCV_0635 strains, respectively. Additionally, CV_0305 positively regulates the expression of CV_0311, while CV_0635 has a broader regulatory role, modulating pathways related to energy metabolism and cell motility. QS, via CviR, also positively regulates the CV_0311 adhesin and its associated T1SS. This study reveals a critical role of the RTX adhesin CV_0311 in biofilm formation in *C. violaceum* and highlights the complexity of its regulation.

Abstract ID: 3577 / Poster Number: 345.17P

Relationship between fish species and aquatic microbiota in Recirculating Aquaculture Systems (RAS) tested in a polyculture context

Presenting author: Salam Al Dakdouki, PhD, Laboratory Animal and Agroecosystems – L2A,University of Lorraine, Nancy, France

Co-authors: *Frédérique CHANGEY, Pascal FONTAINE*

Background: Recirculating Aquaculture Systems (RAS) are mainly indoor aquaculture systems used for fish monoculture. It allows to reuse 90-99% of water and to control rearing parameters (temperature, feed, etc.) for fish optimal growth. The functioning of RAS depends highly on the equilibrium between fish and microbial communities. Fish microbiota (gut, skin, gills) is diverse in microbial structure and composition and affected by intrinsic factors like fish species (mainly driven by gastrointestinal tract morphology and diet) and environmental factors such as temperature. As fish interact intimately with their environment, the relationship between the planktonic microbial community and the fish microbiota is strong, but what about in a context of polyculture of several fish species?

Objectives: The main aim of this study is to investigate how different fish species shifts the water microbial communities in RAS.

Methods: 3 rearing modalities were compared (i) **P**: monoculture of pikeperch (Sander lucioperca), (ii) **P_T**: polyculture of 50% pikeperch and 50% tench (Tinca tinca) and (iii) **T**: monoculture of tench. The experiment lasted 4 months. Water was sampled (at days 0, 30, 60, 90, 120). Microbial structure and composition were assessed by 16S rRNA gene metabarcoding approaches (Miseq V2-V4).

Results: β-diversity analysis shows differences in microbial structure of water between modalities and over time. At final time (D120), pCOA performed on metabarcoding results showed significant differences between the three microbiota (PERMANOVA <0.05) and that the microbiota in polyculture water is not simply the sum of the microbiota in monoculture water (Figure 1).

Conclusion: Our results show a difference in the structure of microbial communities between rearing modalities which allow to suggest the presence of a shaping effect of the fish species on microbial communities in RAS.

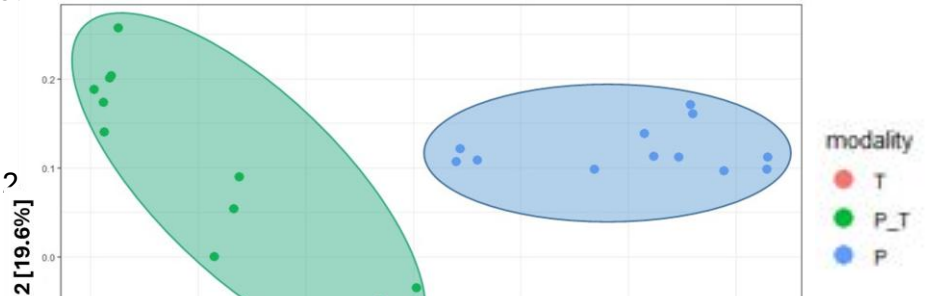


Figure 1: PCOa of water microbial communities' structure among modalities at D120 based on weighted Unifrac Distance.

Abstract ID: 2305 / Poster Number: 026.17F

Relative abundance of gut microbiota is associated with psychopathy scores in humans

Presenting author: Ms. Carolina Costa, Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Porto, Portugal

Co-authors: *Alexandre Figueiredo, Daniel Radford-Smith, Daniel Anthony, Fernando Barbosa, Benedita Sampaio-Maia, Philip Burnet*

Introduction: Psychopathy is a personality structure characterized by lack of empathy, deficient emotional responses, poor behavioural control, and an increased risk of antisocial and criminal behaviour. Despite its relevance, the biological mechanisms underlying psychopathy remain poorly understood, and the role of the microbiota has yet to be explored.

Objective: The current study assessed whether gut microbiota composition is associated with psychopathy scores.

Method: A total of 200 participants were recruited from the general population and completed the Self-Report Psychopathy Scale Short Form (SRP-SF) alongside additional self-report measures of empathy (Affective and Cognitive Empathy Questionnaire and Interpersonal Reactivity Index) and anxiety (State-Trait Inventory for Cognitive and Somatic Anxiety). Faecal samples were collected and microbiota composition was analysed by sequencing the V4 region of the 16S ribosomal RNA gene, followed by amplicon sequence variant (ASV) analysis. K-means clustering was performed in an untargeted approach to identify distinct psychopathy-related microbial profiles. Multiple pairwise comparisons were conducted to identify log fold changes in microbial abundance between clusters.

Findings: We identified four genera (*Prevotella*, *Allisonella*, *Rikenellaceae RC9 gut group*, and *Ruminococcaceae DTU089*) and two species (*Cloacibacillus evryensis* and *Blautia stercoris*) as being differentially abundant between clusters. The abundance of *Prevotella* and *Allisonella* showed positive significant correlations with total psychopathy scores. Furthermore, *Allisonella* and *C. evryensis* were independently and positively associated with total psychopathy scores in a linear model.

Conclusion: These findings suggest that gut microbiota composition is linked to subclinical psychopathic traits, highlighting potential novel pathways for understanding the biological basis of psychopathy.

Abstract ID: 945 / Poster Number: 041.16P

Repression of an oncogenic bacterial toxin via amino acid supplementation: an investigation using molecular analyses and colorectal cancer models

Presenting author: Dr. Sofia Sandalli, University of Glasgow, Glasgow, United Kingdom

Co-authors: *Emily Addington, Gillian Douce, Andrew Roe*

Many intestinal *Escherichia coli* of the B2 phylogroup produce a potent genotoxin called colibactin. In eukaryotic cells, the genomic aberrations induced by colibactin lead to cell cycle arrest, chromosomal instability, senescence, and can trigger the onset of colorectal cancer (CRC). Globally, CRC is the third most common and second deadliest malignancy with 2.2 million new cases expected by 2030. Given the strong interplay between CRC, diet, and the gut microbiome, we aimed to investigate the impact of dietary components on colibactin expression and subsequent downstream effects on CRC. Our results demonstrate the inhibitory effect of several proteinogenic amino acids (AAs) on colibactin production. The ability of L-AAs and corresponding D-enantiomers to repress colibactin biosynthesis was measured via transcription of the *clbB* gene through a *pclbB*-GFP reporter-assay in various types of growth media. The most inhibitory AAs were validated by RT-qPCR and were selected for further analyses in clinical isolates. D-Serine and D-Tyrosine emerged as the most repressive AAs with colibactin-induced DNA crosslinking reduced significantly by 51% and 70% in the presence of D-Serine and D-Tyrosine respectively. Furthermore, we observed that D-Serine significantly reduces by 41% the cytopathic responses observed during infection of HeLa cells with colibactin-producing strains. *In vivo* work conducted in C57BL/6 mice showed that the colibactin-producing strain, EcN, elicited its well-recognised probiotic activity only in the absence of D-Serine, implying modulation of colibactin by D-Serine *in vivo*. Our results contribute to the understanding of colibactin regulation and demonstrate the potential of D-Serine as a preventative in colibactin-associated CRC.

Abstract ID: 702

Repurposing bacterial cell-to-cell communication system for quorum sensing-mediated control in Saccharomyces cerevisiae

Presenting author: Dr. Aafke C. A. Van Aalst, DTU, Kgs. Lyngby, Denmark

Co-authors: *Emil Damgaard Jensen, Michael Krogh Jensen*

Introduction: The acyl-homoserine lactone (AHL) quorum-sensing (QS) system is a widely studied cell-cell communication mechanism in Gram-negative bacteria, enabling population-dependent regulation of gene expression. This system relies on the synthesis, release, and detection of AHL molecules, which accumulate as bacterial populations grow, triggering coordinated transcriptional responses. Due to its simplicity, modularity, and tunability, the AHL QS system has been extensively engineered in bacterial hosts to create synthetic transcriptional regulatory circuits for applications in biotechnology and synthetic biology. Despite its success in bacterial systems, the AHL QS system has not yet been engineered in yeast. Introducing AHL QS into yeast could offer significant advantages, including the ability to construct scalable, orthogonal regulatory systems for precise control of gene expression, independent of endogenous pathways. Therefore, in this study we aimed to engineer a QS-system in *Saccharomyces cerevisiae* for delayed autonomous and orthogonal regulatory control.

Objective: In this study we aimed to engineer the bacterial AHL QS-system into *Saccharomyces cerevisiae*.

Method: Prokaryotic LuxR-LuxI-type regulatory system was functionalized for use in *Saccharomyces cerevisiae*. Functional implementation of LuxR-based biosensor was facilitated by the use of growth-based screening of a mutant library of transcription factors.

Findings: After metabolic engineering, *Saccharomyces cerevisiae* strains were developed that produced signalling molecules in the nM-range. To facilitate selection of more sensitive transcription factors, a growth-based (counter-)selection system was developed to allow enrichment for transcription factor with a low OFF-state and a high ON-state in the presence of ligand. Using this screening platform to enrich for improved transcription factors, three different genotypes were identified with improved selectivity. N86 was confirmed to play an important role in LuxR sensitivity to ligands, corroborating literature on the native system in bacteria. Combining the improved sensitive HSL-

biosensor with the HSL-production resulted in successful implementation of the AHL QS-system in yeast, demonstrated by delayed and autonomously induced expression of GFP.

Conclusion: This study shows a first proof-of-principle of the implementation of an orthogonal prokaryotic quorum-sensing system functionalized in yeast. This paves the way for novel enabling control systems within biotechnological applications, such as for dynamic metabolic pathway regulation, cell population coordination, and multicellular system design.

Abstract ID: 2721 / Poster Number: 202.16P

Repurposing of the old drug clofoctol as colistin adjuvant

Presenting author: Alessandra Fortuna, PhD, Roma Tre University, Rome, Italy

Co-authors: *Alessandra Guiducci, Luigi Cutarella, Martina Fiabane, Cecilia Pinna, Loana Musso, Sabrina Dallavalle, Francesco Imperi, Paolo Visca, Mattia Mori, Giordano Rampioni, Livia Leoni*

Introduction: Colistin, despite its known toxicity, is a last-resort antibiotic used to treat lung infections caused by multidrug-resistant Gram-negative pathogens. However, the emergence of colistin resistance in various clinical contexts worldwide underscores the urgent need for colistin adjuvants.

Recently, we showed that clofoctol, an FDA-approved synthetic antibiotic active against Gram-positive pathogens, restores colistin susceptibility in colistin-resistant clinical isolates of ESKAPE pulmonary pathogens, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Objective: The aim of this study is to investigate the mechanism of action of the colistin-clofoctol combination, using *P. aeruginosa* as a model organism.

Method: Microbiological and structural chemistry approaches were combined: *i*) selection and genome sequence analysis of *P. aeruginosa* mutants resistant to the colistin-clofoctol combination; *ii*) computational molecular dynamics simulations (CMDS) and spectrophotometric analyses.

Findings: Resistant mutants emerged at a very low frequency. Genome sequencing revealed mutations conferring increased resistance not only to the combination but also to colistin alone. Hence, resistance to the combination arises from the modification of colistin targets rather than clofoctol targets. Molecular interaction studies showed that clofoctol binds to colistin in a 1:1 ratio. According to CMDS, clofoctol binding locks the colistin into a conformation that facilitates its interaction with the outer membrane.

Conclusion: Our working hypothesis is that clofoctol could act as a molecular chaperone, stabilizing colistin in its active conformation, thereby enhancing its antibacterial activity. Experiments aimed at further validating this hypothesis and exploring clinical applications of the colistin-clofoctol combination are ongoing.

Abstract ID: 3180 / Poster Number: 272.15P

Rep-WH1 prions as tools for containment of the phytopathogen Xylella fastidiosa

Presenting author: Prof. Doc. Rafael Giraldo, National Centre for Biotechnology (CNB-CSIC), Madrid, Spain

Co-authors: *Leticia Lucero-Lopez*

Xylella fastidiosa is a bacterial phytopathogen that proliferates in the xylem of the infected plants to the point of occluding their vases, thus leading to the irreversible drying of plants.

We aim to explore novel approaches to combat *X. fastidiosa*, since there is no solution for the infected plants apart from uprooting.

The characterization of the Rep-WH1 prions was carried out through the integration of microbiological (both in *E. coli* and *X. fastidiosa*) and molecular biology methods, advanced optical microscopy, TEM, biophysics.

We have found orthologues of the Rep-WH1 prion domain of the *Pseudomonas* plasmid pPS10 (PLoS Pathog. 2024. 20:e1012253) in the replication proteins of plasmids in *X. fastidiosa*. The expression of two of these Rep-WH1 prions decreased the viability of *E. coli* and *X. fastidiosa* cultured *in vitro*. In *E. coli*, the pXF51 prion causes the conversion of the bacillary cells into cocci. Expression of the Rep-WH1 prions decreased the virulence of *X. fastidiosa* on experimental infection of *Nicotiana* plants, compared with the control bacteria, as revealed by net reductions in the symptoms of withering, in the bacterial load (qPCR) and by an anomalous senescent morphology of bacteria within the xylem vases (TEM of sections across plant petioles). In *E. coli*, by means of fluorescence recovery after photobleaching (FRAP), we have found that the pXF51 *X. fastidiosa* prion undergoes a phase transition from solubility to amyloid, modulated by a Hsp70 chaperone.

These results open a way to the possible exploitation of these prions for the control of this phytopathogen.

Abstract ID: 3648 / Poster Number: 093.15F

Reservoirs of Microbial Treasures: Discovering Novel Endophytic Bacteria from Resilient Plants and Their Potential Role in Sustainable Agriculture in Arid Regions

Presenting author: Salma Mouhib, PhD, University Mohammed VI Polytechnic (UM6P), BENGUERIR, Morocco

Co-authors: *Khadija Ait Si Mhand, Nabil Radouane, Khaoula Errafii, Issam Meftah Kadmiri, Derly Madeleiny Andrade-Molina, Juan Carlos Fernández-Cadena, Mohamed Hijri*

Plants and their associated microbes in arid regions exhibit remarkable resilience to extreme environmental conditions. However, bacterial endophytes diversity and functional roles in plants adapted to these harsh environments, particularly in Africa, remain largely unexplored. In this study, 65 endophytic bacteria were successfully isolated from the leaves and roots of vegetative plants in Morocco's semi-arid and arid regions, including *Peganum harmala* (L.) and *Citrullus colocynthis* (L.). Whole-genome sequencing and phenotyping of these isolates identified 14 novel bacterial species. The genomic analysis revealed these bacteria's critical functional traits and potential applications. These findings highlight the value of these microorganisms as sources of bioactive compounds, advancing our understanding of microbial diversity and its ecological role. This study provides important insights into plant–bacteria interactions. It underscores the potential for leveraging these endophytes in biotechnological applications to promote sustainable agriculture, enhance food security, and support climate change adaptation.

Abstract ID: 1813 / Poster Number: 002.17S

Resilient Antarctic soil bacteria sustain trace gas oxidation across wide temperature ranges

Presenting author: Dr. Tess Hutchinson, Securing Antarctica's Environmental Future, Melbourne, Australia; Monash University, Melbourne, Australia

Co-authors: *Sophie Holland, Francesco Ricci, David Clarke, Pok Man Leung, Thanavit Jirapanjawat, Melodie McGeoch, Chris Greening*

Polar desert soils host diverse and abundant microbial communities despite limited nutrients and frequent temperature and light fluctuations. Adapting to such oligotrophic conditions, most bacteria possess high-affinity hydrogenases and carbon monoxide dehydrogenases, allowing them to survive on atmospheric trace gases such as hydrogen (H_2) and carbon monoxide (CO) for energy generation and chemosynthesis. Despite the foundational importance in sustaining microbial diversity in a slowly warming climate, little is known about the thermal flexibility of these trace gas oxidation processes. Here we measured oxidation of H_2, CO and methane (CH_4) in Antarctic soils under 14 different temperatures, ranging from -20 °C to 80 °C. Using the rates determined, we then built a model enabling prediction of trace gas oxidation rates across the continent under different climate warming scenarios. Additionally, metagenomic analysis was performed to determine dominant community members and their metabolic capacity. Our results show H_2 oxidation in Antarctic soil is an incredibly thermally tolerant process, occurring at fast rates from -20 °C to 75 °C. In combination with community analysis which shows these soils are abundant in Group I [NiFe] hydrogenases, these results infer the remarkable resilience of hydrogenotrophic microbes in these communities. CO oxidation is also observed at temperatures ranging from -20 °C to 42 °C, while CH_4 oxidation is much slower and occurs under a narrower temperature range, operating between 4 °C and 30 °C. Largely, rates increased with temperature and optimal activity ranged from approximately 10 °C to 30 °C. Modelling this, we show the activity of soil communities and the oxidation of climate-relevant trace gases will increase with future warming temperatures on the Antarctic continent. As polar regions cover approximately 5 x 10^6 km^2 of the Earth, this work highlights the important role these soils could play in predicting and mitigating greenhouse gas emissions.

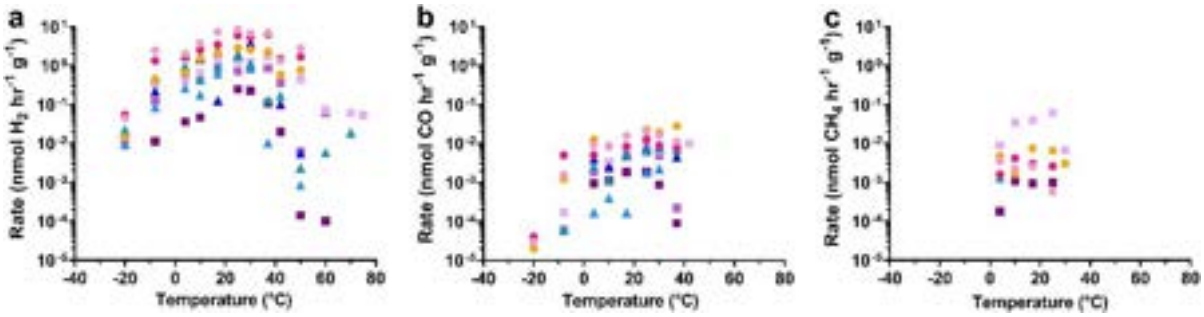


Figure 1: Rates of (a) H_2, (b) CO and (c) CH_4 oxidation (nmol hr^{-1} g^{-1}) by Antarctic soils across a wide range of temperatures ranging from -20 to 75 °C.

Abstract ID: 1692 / Poster Number: 098.17P

Resistome and mobilome analysis in microbiomes from five different meat industries using Oxford Nanopore Technology

Presenting author: Mrs. Elena Fernández-Trapote, Universidad de León, León, Spain

Co-authors: *Lauris Rojas-Rodríguez, Marcia Oliveira, Victoria Crespo-Torbado, Alba Puente, Rebeca Cordero-García, Miguel Prieto, Avelino Álvarez-Ordóñez, José Francisco Cobo-Díaz*

Antimicrobial resistance is considered a global pandemic. One issue is the potential transmission of antimicrobial resistant microorganisms and antimicrobial resistance genes (ARGs) through meat consumption.

The aim of this study was to characterise the resistome and mobilome of five meat industries, comprising both the slaughterhouse and the associated cutting plant, from different types of meat (pork, beef and chicken). To this end, a total of 200 samples were collected, from food contact surfaces, non-food contact surfaces, drains, the carcasses and the final product.

Extracted DNA was sequenced through Oxford Nanopore Technology (ONT). Reads were quality filtered (Porechop and Chopper) and ARGs were screened by blastn software against Resfinder database. Sample de novo metagenomic assembly was performed (Flye) and obtained contigs were taxonomically assigned (Kraken2). ARGs screening in contigs was performed as previously described at read-level and only ARGs-carried-contigs were used to the mobilome analyses performed (Platon, PlasmidFinder, IntegronFinder and MGEfinder).

The resistome analysis revealed a greater number of ARGs at the slaughterhouse than in the cutting plant. Read and contig-level analyses showed that the most prevalent ARGs were associated with resistance to tetracyclines, lincosamides, aminoglycosides and beta-lactams, with the genera *Acinetobacter*, *Aeromonas* and *Psychrobacter* being the main genera associated with these ARGs. Furthermore, some of the most abundant ARGs associated with these genera were predicted in plasmids, transposons and integrons, among other mobile genetic elements.

In conclusion, the present study provides a deep description of the resistome and mobilome in diverse meat industries using ONT sequencing.

Abstract ID: 3695 / Poster Number: 045.17P

Resistome dynamics in soil exposed to the herbicide S-metolachlor

Presenting author: Ivana Galic, INSTITUTE OF MOLECULAR GENETICS AND GENETIC ENGINEERING University of Belgrade, Belgrade, Serbia

Co-authors: *Nada Stankovic, Sasa Todorovic*

Soil microbiota is essential for maintaining soil health. Pesticides that disrupt microbial activities impair soil quality and harm the environment. Bacteria play a significant role in metabolizing chloroacetanilide pesticides. However, herbicide exposure induces environmental stress, triggering adaptive mechanisms such as horizontal gene transfer, which accelerate the establishment and spread of resistance. Surviving in pesticide-contaminated fields, bacteria become highly tolerant to pesticides/heavy metals and simultaneously resistant to antibiotics.

The goal of this study was to determine real-life impact of chloroacetanilide herbicide S-metolachlor on antibiotic resistance gene (ARG) abundance in herbicide-treated and untreated soils.

The soil from experimental fields sown with maize was sampled 1 week before and 4 weeks after spring spraying. The herbicide content in the soil was determined by LC-MS. Total metagenomic DNA was isolated from the treated and control samples and short-read shotgun sequencing was performed on the DNBSEQ-G400 platform (MGI). After the bioinformatics processing of sequences assembled contigs were checked against the CARD database with ResFinder/RGI.

Preliminary analyses suggest notable differences in ARGs distribution before and after herbicide treatment, as well as between treated and control plots. These differences likely reflect the mobility and persistence of the herbicide under real-life agricultural conditions.

Our study highlights the potential of herbicide to influence soil microbiome by disrupting microbial community's structure and potentially promoting antibiotic resistance and selecting resistant genes. This interaction raises concerns about the widespread use of herbicides in agriculture, as it may undermine antibiotic effectiveness, posing a threat to public health and global efforts to combat resistance.

Abstract ID: 2762 / Poster Number: 013.17S

Revealing the mechanism of insertion of Legionella's integral membrane effector proteins into host cell membranes

Presenting author: Ms. Sarah Trenz, University of Tübingen, Tübingen, Germany

Co-authors: *Sophie Schminke, Samuel Wagner*

During infection, *Legionella pneumophila* translocates more than 300 effector proteins into host cells using the specialized Dot/Icm type IV secretion system (T4SS). 20% of *Legionella*'s effector proteins contain a hydrophobic transmembrane domain (TMD) and are found at distinct host cell membrane compartments enabling a more specific range of functions. However, the mechanisms T4-secreted TMD-effectors (TMEs) use to target and insert into the correct membranes of the eukaryotic host remain to be elucidated.

As a potential model for the integration of TME proteins after T4 injection, we suggest a cytoplasmic TME intermediate that interacts with host chaperones to prevent aggregation prior to insertion into the target membrane. We hypothesize that this process might require interaction with host proteins involved in the post-translational biogenesis of membrane proteins in eukaryotic cells.

Initially, the subcellular localization of TMEs in host cell membranes was investigated by fluorescence microscopy. The analysis revealed that ectopically expressed *Legionella* TMEs mainly co-localize with the membranes of the endoplasmic reticulum (ER), while some TMEs are trafficked further to the plasma membrane. During infection, T4-secreted TMEs were predominantly observed in the membrane of the *Legionella*-containing vacuole (LCV) and the tightly associated ER, surrounding the LCV. To understand the relevance of host cell proteins involved in membrane insertion, the interacting proteome of bacterial TMEs was assessed. The intracellular environment of four *Legionella* TMEs, differing in hydrophobicity and position of their TMD, was characterized using TurboID-mediated proximity-dependent biotinylation and subsequent mass spectrometry. Proteomic analysis revealed that T4-secreted TMEs interact with host proteins that are involved in the biogenesis of membrane protein at the ER. Interestingly, TMEs with their TMD located towards the C-terminus exhibit interaction with several proteins involved in signal recognition particle (SRP)-dependent ER targeting and post-translational Sec61-mediated ER insertion.

To get a deeper insight into the potential role of the Sec61 translocon or co-ordinated insertases, such as the ER membrane complex (EMC), in the membrane integration of *Legionella* TMEs, we are currently employing siRNA-mediated knock-down and small molecule inhibition on these proteins.

In conclusion, these preliminary observations suggest that T4-secreted TMEs, depending on their TMD position, might utilize the Sec61 translocon, and its associated proteins, for a post-translational insertion into the rough-ER-like LCV membrane or the tightly associated ER membrane.

Abstract ID: 2689 / Poster Number: 137.17P

Reviving GE23077: Investigating Biosynthetic Pathways and Antimicrobial Potential of a Promising RNAP Inhibitor

Presenting author: Mrs. Letizia Bartolone, University of Insubria, Varese, Italy

Co-authors: *Flavia Marinelli, David Frasson, Joël F. Pothier, Andrew W. Truman, Martin Sievers, Francesca Berini*

Discovered in the 1990s as a product of the metabolism of the so-called 'rare' filamentous actinomycete *Actinomadura lepetitiana* DSM 10909, GE23077 is a non-ribosomally synthesised cyclic heptapeptide antibiotic that inhibits a wide range of bacterial RNA polymerases (RNAP) in vitro, while not affecting mammalian enzymes. After its discovery, the molecule was later overlooked, due to its limited *in vivo* antimicrobial activity, mainly exerted against *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, and *Mycobacterium smegmatis* isolates. However, with antimicrobial resistance spreading, including towards the major approved RNAP inhibitors (fidaxomicin and rifamycin), the development of new drugs to fight bacterial infections is essential. In this context, GE23077 might prove promising, as its target includes functionally critical residues of the RNAP that are unique.

Our project aims to better understand GE23077 production using multiple approaches as an essential step for its further development.

High-quality genome sequence of *A. lepetitiana* was recently obtained using Illumina MiSeq and MinION, followed by the identification and annotation of a putative biosynthetic gene cluster (BGC) for GE23077 production. Following the development of new methods for the genetic manipulation of this recalcitrant actinomycete, BGC attribution was confirmed by the knock-out of key genes. Overexpression studies of putative regulatory genes are ongoing. In parallel, *in vitro* studies are being performed on purified *A. lepetitiana* RNAP to evaluate its susceptibility to GE23077.

Elucidating the mechanisms behind the synthesis of this promising RNAP inhibitor may also open the way to combinatorial biochemistry approaches to modify the molecule and potentially improve its in vivo antimicrobial activity.

Abstract ID: 1921

Revolutionizing Low-pH Survival: Unveiling a New Ammonification Mechanism Coupled with NO Detox in Acididesulfobacillus acetoxydans

Presenting author: Dr. Irene Sanchez Andrea

Co-authors: *Reinier Egas, Cornelia Welte*

Introduction: Biological nitrate reduction shapes the bioavailability of nitrogen in ecosystems. Depending on the pathway—denitrification or dissimilatory nitrate reduction to ammonium (DNRA)—nitrogen is either lost as gas or retained in the system as ammonium. The acidophilic sulfate-reducing bacterium *Acididesulfobacillus acetoxydans* performs DNRA without canonical nitrite reductases, prompting questions about the enzymes underpinning this process. Furthermore, low-pH environments present unique challenges, including nitrosative stress generated from nitric oxide (NO). Understanding how *A. acetoxydans* copes with these conditions provides deeper insights into both nitrogen cycling and acidophilic microbial physiology.

Objective(s): This study aimed to (i) elucidate the enzymatic mechanisms behind nitrate-to-ammonium conversion in *A. acetoxydans*; (ii) identify and characterize potential alternative nitrite reductases involved in DNRA; and (iii) explore the strategies employed to detoxify NO and related reactive nitrogen intermediates under acidic conditions.

Method: Cultures of *A. acetoxydans* were grown under DNRA-promoting conditions. Concentrations of nitrate, nitrite, nitric oxide, hydroxylamine, and ammonium were monitored to pinpoint intermediate steps. Resting cell experiments were performed to confirm the conversion of nitrate, nitrite, and hydroxylamine into ammonium. Comparative transcriptomics and proteomics were conducted under nitrate- and sulfate-reducing conditions to highlight key enzymes. A potential pathway for nitric oxide detoxification was investigated by examining the roles of hybrid cluster protein (Hcp) and nitrous oxide reductase (NosZ).

Findings: The bacterium encodes a Nar-type nitrate reductase, although it lacks recognizable nitrite reductase genes. Transcriptomic and proteomic data revealed that nitrite is likely reduced to ammonia by the NADH-linked sulfite reductase AsrABC or a putative ferredoxin-dependent homolog of nitrite reductase NirA (DEACI_1836). The generation of nitric oxide as a by-product was observed both extracellularly (through nitrite disproportionation) and intracellularly (as a side reaction of NarG). *A. acetoxydans* mitigates nitrosative stress via Hcp, which converts NO to nitrous oxide, subsequently

reduced to dinitrogen gas by NosZ (in concert with NosFYD and PetABC). This detoxification pathway not only protects cells but also diverts reactive intermediates away from ammonium formation.

Conclusion: These findings broaden the understanding of DNRA pathways revealing novel nitrite reduction routes that maintain nitrogen within ecosystems. Moreover, the study highlights a unique low-pH nitrosative stress response present -at least- in acidophilic bacteria, where *A. acetoxydans* converts toxic NO into benign dinitrogen gas. This dual strategy has potential implications for interpreting (meta)genomic data and for managing nitrogen cycling in low-pH environments.

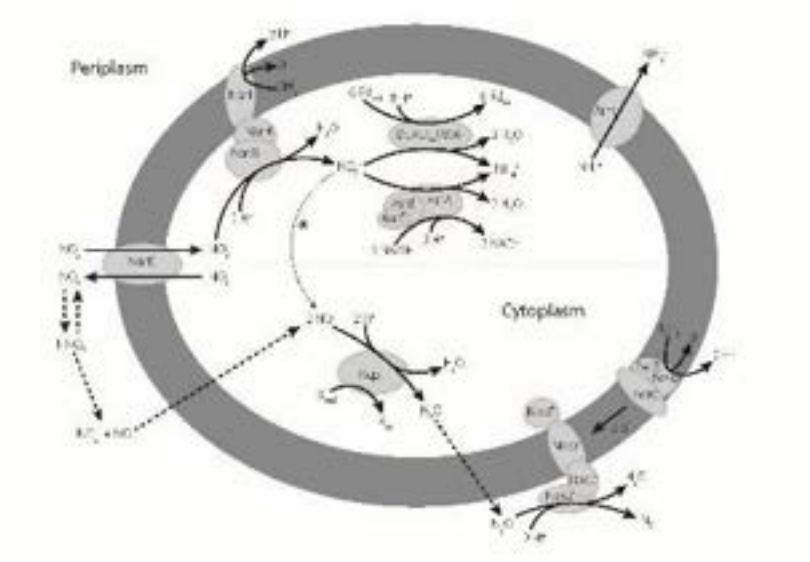


Figure 1: *Overview of the DNRA (top) and denitrification(bottom) pathways employed by A. acetoxydans.*

Abstract ID: 1353 / Poster Number: 090.17P

Risk Factors in diagnosing *Clostridioides difficile* Infections

Presenting author: Dr. Alexandra Kalacheva, Tokuda hospital, Sofia, Bulgaria

Introduction: Toxin-producing strains of *Clostridioides difficile (CD)* are the most commonly identified cause of healthcare-associated infection in the elderly. *C. difficile infection* (CDI) is a potentially fatal illness with an increasing incidence worldwide.

Objective: To present data on the risk factors in current practices for clinical diagnoses used in managing CDI.

Materials and Methods: We studied 116 clinical stool specimens from inpatients with acute diarrhoea suspected of nosocomial or antibiotic-associated diarrhoea (AAD) due to *C. difficile* from two general hospitals. Enzyme Immunoassay card tests (EIA) for detecting *C. difficile* toxins A (cdtA) and B (cdtB) from stool, automated CLIA assay for the detection of *C. diff*. GDH antigen and qualitative determination of cdtA and B in human faeces, and anaerobic stool culture were applied for CDI laboratory diagnostic. MALDI-TOF (Bruker) identified the suspected anaerobic bacterial colonies. The following methods were used as differential diagnostics LAMP method for evidence of *Salmonella spp*. and simultaneous detection of *Campylobacter jejuni* and *C. coli*, *E. coli Typing* RT-PCR detection kit (ETEC, EHEC, STEC, EPEC, EIEC), API 20E, and aerobic stool culture methods.

Results: Forty toxigenic *C. difficile* strains were isolated from all 116 diarrhoeal stool specimens tested, of which 38 (38/40) produced toxin B and two strains (2/40) were positive for both cdtA and cdtB. Of the faecal samples positive for cdtA (6/50) and/or cdtB (44/50) by EIA, 33 samples were *C. diff* culture negative, but positive for the following diarrhoea agents: 1/33 – *Salmonella enterica subsp. Arizonae* (LAMP, culture, API 20E); 2/33 *C. jejuni (LAMP, culture, MALDI TOF);* 1/33 – ETEC O142 and 2/33 - STEC O145 and O138 (*E. coli* Typing RT-PCR detection kit, culture); 2/33 *C. perfringens* (anaerobic culture, MALDI TOF); 2/33 – hyper-mycotic enterotoxigenic *K. pneumonia* and 2**/**33– enterotoxigenic *P. mirabilis (culture; PCR LT-toxin-encoding)*. Two of the 66 cdtB-positive samples (2/66) exhibited a similar misdiagnosis when analysed using the CLIA method. However, the PCR analysis indicated that they were cdtB-negative. In contrast, the LAMP method identified a positive result for *C. jejuni* in one sample and another one was STEC positive (stx1+/stx2+) by RT-PCR. Furthermore, there is an additional discrepancy in the CDI testing results. EPEC O86 (RT-PCR eae+) was isolated from a faecal sample that was

positive for GHA enzyme (CLIA) and negative for cdt A and cdtB (CLIA and PCR assay). However, the *C. diff* culture was negative.

Discussion: The findings support the hypothesis that certain human bacterial pathogens that produce enterotoxins other than *C. difficile*, as well as intestinal commensal microorganisms, including *Klebsiella sp.* and *Proteus sp.,* contribute to false-positive EAI card tests for *C. difficile* A and B toxins, which are the most widely used in the laboratory diagnosis of CDI.

In conclusion, *C. difficile* infection represents a significant challenge to clinical practice in terms of management and prevention of recurrence. It is recommended that toxin-only EAI tests should not be used as a stand-alone diagnostic tool for CDI. Their use should be limited to detecting toxins A and B. Accurate diagnosis of CDI requires a combination of clinical evaluation, diagnostic methods, and management practices.

Keywords: *Clostridioides (Clostridium) difficile;cdtA; cdtB, managing CDI, laboratory diagnostic*.

Abstract ID: 1187 / Poster Number: 196.15P

RNA-Seq Analysis Reveals Key Genes for Cs^+ Resistance in E. coli Strain ZX-1

Presenting author: Mr. Daiki Kojima, Toyo university, Asaka, Saitama, Japan; Toyo university Bio-resilience Research Project, Asaka, Saitama, Japan

Co-authors: *Masahiro Ito*

Since the Fukushima Daiichi Nuclear Power Plant accident in 2011, radioactive cesium (Cs) has been a significant environmental pollutant, making its removal a critical challenge. In our laboratory, a highly Cs^+-resistant Escherichia coli strain, ZX-1, was isolated. While the parental strain, Mach1, experiences growth inhibition at 300 mM CsCl, ZX-1 is capable of growing in conditions exceeding 700 mM CsCl (1).

This study aimed to identify genes associated with Cs^+ resistance through RNA-seq analysis of ZX-1 and its parental strain, Mach1. RNA-seq analysis under Cs^+-free conditions revealed that the transcription of the sodium-glutamate symporter (gltS) was constitutively upregulated in ZX-1. Under conditions of 700 mM Cs^+, the transcription of the potassium (K^+)-selective transport protein Kdp was significantly upregulated in ZX-1.

These findings suggest that the uptake of glutamate and K^+ may contribute to Cs^+ resistance by facilitating the competitive uptake of K^+, thereby reducing intracellular Cs^+ accumulation.

This study sheds light on the genetic mechanisms underlying Cs^+ resistance in the E. coli strain ZX-1, providing valuable insights into its molecular basis. With ongoing functional analyses of the identified genes, we are steadily advancing toward a comprehensive understanding of Cs^+ resistance. These findings are expected to contribute to the development of effective bioremediation technologies for Cs^+-contaminated environments.

Abstract ID: 4139

RND efflux pumps in Acinetobacter baumannii: integrating experimental and computational studies of promoters

Presenting author: Mireia López Siles, Universitat de Girona, Spain

Co-authors: *Sonia Prieto Martin Gil, Ana Tajuelo, Antonio J. Martín-Galiano, Michael J. McConnell*

Introduction: Resistance-Nodulation-Division (RND) efflux pumps, which can expel a wide range of antimicrobial compounds, are involved in multidrug resistance in *Acinetobacter baumannii*. Two recent studies led by our group [1,2] have addressed this issue from complementary perspectives: one through a genomic analysis of regulatory alterations in upstream regions of RND operon promoters, and the other through experimental evaluation of subinhibitory concentrations of antibiotics and disinfectants on RND gene promoter activity.

Objective: The aim of this talk is to present an integrated overview of the study of upstream regions of promoters and how they influence RND efflux pumps expression in *A. baumannii*.

Methods: In the first study, genomes of 4,130 *A. baumannii* clinical isolates were analysed to identify genetic events in upstream regulatory regions of RND operons promoters, including insertion sequences, small indels, and single nucleotide polymorphisms. A computational pipeline was developed to link these regulatory changes to potential increases in minimum inhibitory concentration (MIC) for multiple antibiotics and compared to current resistome prediction tools.

In the second study, luciferase reporter systems were used to quantify promoter activity of the *adeABC*, *adeFGH*, *adeIJK* operons in three *A. baumannii* strains. Exposure to subinhibitory concentrations of clinically relevant antibiotics and disinfectants were tested to detect changes in expression depending on growth phase.

Findings: A total of 487 non-redundant genetic events were identified, affecting functional elements such as promoter boxes, operators, and Shine-Dalgarno sequence, as well as DNA curvature and mRNA secondary structures. The computational pipeline developed was able to predict MIC increases often missed by conventional resistome predictors.

Subinhibitory concentrations of the compounds tested had variable effects on promoter activity that were highly dependent on the *A. baumannii* strain, the antimicrobial tested

and the growth phase. *adeABC* promoter activity showed the highest range of fold-changes whereas *adeIJK* promoter activity showed the lowest.

Conclusion: Together, these studies highlight the complexity of efflux pump regulation in *A. baumannii* and its impact on antimicrobial resistance. The integration of experimental and computational approaches offers a promising strategy for a more comprehensive prediction of antibiotic resistance. This strategy is expected to go beyond traditional resistome- and phenotype-based analysis of resistance. Altogether, it is expected that these tools help implement antimicrobial therapies in the clinical practice more effectively.

References:

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Abstract ID: 3331 / Poster Number: 176.17P

Role of anticancer drugs on the emergence of antimicrobial resistance in bacterial pathogens

Presenting author: Assoc. Prof. Giordano Rampioni, University Roma Tre, Rome, Italy; IRCCS Fondazione Santa Lucia, Rome, Italy

Co-authors: *Marta Mellini, Claudia Ridolfi, Giovanni Serratore, Livia Leoni, Francesco Imperi,*

Introduction: Infections caused by multidrug-resistant (MDR) bacteria represent a serious threat to cancer patients. Cancer patients face a higher risk of dying from bacterial infections compared to other hospitalized individuals. Chemotherapy may worsen infection outcomes by immunosuppressing patients, but also by directly impacting bacteria. In fact, certain anticancer drugs exhibit antimicrobial activity, disrupting the microbiota and enabling pathogenic strains to expand. Additionally, it was demonstrated that some anticancer drugs can increase bacterial mutation rates, promoting antibiotic resistance.

Objective: We hypothesised that treatment with anticancer drugs displaying antimicrobial activity could select for anticancer-resistant bacterial strains, that in turn could exhibit cross-resistance to antibiotics.

Method: To investigate this issue, we performed a screening campaign to find anticancer drugs with antimicrobial activity against relevant bacterial pathogens. Spontaneous anticancer-resistant mutants were selected and evaluated for eventual cross-resistance to antibiotics. Genome sequencing of parental and resistant strains is ongoing to identify mutations potentially linked to the resistance phenotypes.

Findings: Some anticancer drugs showed antibacterial activity against bacterial pathogens. Spontaneous anticancer-resistant mutants exhibited increased resistance to various antibiotics compared to parental strains. Genome sequencing will allow identifying genes involved in the resistance to anticancer drugs and antibiotics.

Conclusion: These preliminary data suggest that chemotherapy could directly contribute to the emergence of MDR strains. Identifying the role of specific anticancer drugs in inducing resistance will enable the development of guidelines to properly combine chemotherapies with antibiotics, possibly limiting the emergence of resistance in cancer patients.

Infections caused by multidrug-resistant bacterial pathogens represent a serious threat to cancer patients. Here we show that multiple anticancer drugs are endowed with antimicrobial activity against bacterial pathogens, and that spontaneous anticancer-resistant mutants exhibit increased cross-resistance to different antibiotics. Unravelling these mechanisms could improve infection management in cancer patients.

Abstract ID: 1507 / Poster Number: 460.15P

Role of Calcium-conducting viroporins of Human Cytomegalovirus in viral replication and pathogenesis

Presenting author: Prof. Anna Luganini, Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

Co-authors: *Giorgio Gribaudo*

Introduction: The US12 gene family of human cytomegalovirus (HCMV) encodes 10 contiguous proteins (US12-US21) with seven transmembrane domains (7TMDs). Although non-essential for *in vitro* growth, this proteins play significant roles *in vivo* infection to contribute to the CMV's success in its natural hosts by acting as viral regulatory proteins that can interfere with many biochemical and host cell physiological pathways. Among them, US12 and US21 resemble cellular Transmembrane Bax-Inhibitor 1 motif (TMBIM)[1], such as Bax inhibitor-1 and Golgi anti-apoptotic protein, acting as viroporins that alter intracellular Ca^{2+} homeostasis, just like TMBIM proteins[2].

Objective(s): Given that Ca^{2+} is a universal messanger, whose depletion from cellular stores has many cytobiological consequences, this study aims whether pUS12- and pUS21-mediated Ca^{2+} release impact some crucial cellular processes.

Method: The cellular Tetracycline (Tet)-Regulated Expression system was exploited to obtain Tet-inducible expression of pUS12-HA and pUS21-HA in not-infected cells. Cytosolic Ca^{2+} levels were measured by ratiometric approach using the Fura-2 AM probe for pUS21, while Golgi Ca^{2+} concentrations were assessed via FRET-based calcium imaging for pUS12. BAC-Recombineering technology was employed to generate recombinant viruses on the HCMV TR background, including: viruses encoding tagged versions of the US12 and US21 ORFs, or with point-mutated ORFs, and viruses in which the entire US12 or US21 ORFs were deleted. The TMD topology was predicted *in silico* using five different algorithms, and then experimentally verified by immunofluorescence assay with selective accessibility to epitopes. Expression and localization of pUS12 and pUS21 in infected cells were validated by Western blotting and immunofluorescence, as well as representative viral proteins IE, E, and L. For pUS21, apoptosis, chemotactic and random migration assays were conducted using a luminescence assay, a transwell system and time-lapse microscopy, respectively. Calpain activity was evaluated through a fluorometric assay measuring calpain-substrate cleavage. Adhesion assay was performed with DAPI-stained cell nuclei, and focal adesions were quantified by immunofluorescence staining against paxillin. Mass spectrometry was used for

interactions between pUS21 and cellular proteins, confirmed by co-immunoprecipitation and immunofluorescence.

Findings and conclusion: Using different recombinant TR-HCMV, we observed that both proteins are 7TMD proteins (with the N-terminal cytosolic and C-terminal luminal orientation) expressed with a late kinetic. pUS12 localizes with Golgi-derived membranes in cytoplasmic Virion Assembly Complex, whereas pUS21 associates with ER-derived vesicles. Recombinants TR-HCMV, in which the US12 or US21 ORF was removed or inactivated, exhibited growth defects in endothelial and epithelial cells, in fibroblasts for US21, indicating that pUS12 and pUS21 contributes to HCMV replication in specific cell types. These mutants displayed impaired expression of representative IE, E, and L viral proteins, despite normal viral entry, as indicated by nuclear delivery of virion pp65; even if US12-null virus expressed lower levels of pp71 compared with TRwt. Calcium imaging revealed that pUS12 and pUS21 reduce intracellular Ca^{2+} content, thus indicating that both HCMV proteins acts as a Ca^{2}-conducting viroporins. The pUS21-mediated Ca^{2+} content reduction protects the cells form pro-apoptotic stimuli[2]. Inducible cell lines expressing pUS21 demonstrated increased migration and focal adhesion turnover through calpain 2 activation, driven by US21-mediated Ca^{2+} alteration[3]. Moreover, the functional relationship between pUS21 and calpain 2 was suggested by the observation that talin 1, a known calpain proteolytic substrate, interact with pUS21 as observed by mass spectrometry and co-immunoprecipitation. Overall, these findings highlight two HCMV proteins able to hijack cell's Ca^{2+} homeostasis. While US21 is known to inhibit apoptosis and enhance cell adhesion and motility, pUS12 seems to be involved in the regulation of virion formation, and ongoing studies on virion proteomics will aim to clarify the cytobiological effects of pUS12-mediated Ca^{2+} modulation.

Abstract ID: 3368 / Poster Number: 319.15P

Role of different acetate permeases for tellurite (TEO32-) uptaker and resistancein cells of the facultative phototroph rhodobacter capsulatus

Presenting author: Prof. Stefano Fedi, Dept of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

Co-authors: *Matteo Ciaciavicchia*

Introduction: Tellurium (Te) is a metalloid of the periodic table group XVI that is found in the Earth's crust minerals. Metallic tellurium has found ever-increasing applications indifferent industrial applications, its widespread use poses concerns about its potential negative effects on the environment and human health. Tellurite exerts its toxic action in the cytoplasmic compartment mostly by triggering an increase in the generation of reactive oxygen species (ROS)

Objective: In *Rhodobacter capsulatus* strain B100, it has been shown that a mutant impaired in tellurite uptake became highly resistant to the oxyanion and the uptake defect was restored by the acetate permease actP2 gene, supporting an earlier observation indicating that acetate inhibits tellurite uptake in R. capsulatus cells, as both anions compete for the same permease

Methods: Since three different regions coding for acetate permeases were found in the genome of *Rhodobacter capsulatus* B100, deletion mutants for the different acetate permeases were constructed in this work in order to verify their specific involvement in the uptake of tellurite. The mutants were obtained by suicide plasmid homologous recombination technology

Findings: Internalisation analyses with the deletion mutants of the acetate permeases indicate that only one of them (ActP2-type) appears to be functional for the uptake of tellurite

Conclusion: Studies with mutants of acetate permease knockout genes have revealed the role of a specific acetate permease in the uptake of tellurite in *R. capsulatus* A distinctive structural feature is the presence of an extra 15 amino acid stretch in ActP2-type permeases.

Abstract ID: 3924 / Poster Number: 271.16S

Role of spring and urban waters in the spread of vancomycin-resistant enterococci (VRE) and other critical resistances: preliminary findings

Presenting author: Dr. Elisa Massella, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Piacenza, Italy

Co-authors: *Simone Russo, Sara Castaldo, Letizia Cirasella, Roberta Taddei, Silva Rubini, Camilla Torreggiani, Matteo Ricchi, Andrea Luppi*

Introduction: Enterococci represent primary agents of nosocomial infections and are often associated with resistance to Critically Important Antimicrobials (CIAs), including vancomycin. Microbial water quality is a pressing global concern, since the ingestion of water contaminated with microorganisms poses significant risks to human health.

Objective: This study investigates drinking water (DW), well water (WW) and spring water (SW) as potential sources of enterococci resistant to vancomycin (VRE) and/or to other CIAs.

Method: Between February 2023 and October 2024, 1062 water samples (DW, 681; WW, 333; SW, 48) were analysed. Enterococci were isolated using the membrane filtration method (ISO 7899-2:2000) and identified by MALDI-TOF. Antimicrobial resistance (AMR) profiles were assessed using the broth microdilution method. Minimal Inhibitory Concentration (MIC) results were interpreted according to CLSI or EUCAST breakpoints. Strains phenotypically resistant to vancomycin were screened for van genes presence using a multiplex PCR.

Findings: Thirty-seven *Enterococcus* isolates were detected in 30/1062 (2.82%) samples, predominantly in WW (17/333, 5.12%). Seven *Enterococcus* species were identified, predominantly *E. casseliflavus* (15/30, 50.00%) and *E. mundtii* (10/30, 33.33%). Interestingly, 4/30 (13.33%) samples were associated with multiple species. Notably, 16/37 (43.24%) strains were multidrug-resistant (MDR) from 3 to 6 different antimicrobial agents. The highest resistance frequencies were to quinupristin/dalfopristin (30, 81.08%), linezolid (15, 40.54%), erythromycin (12, 32.43%), ciprofloxacin/tetracycline/chloramphenicol (10, 27.02% all) and daptomycin/tigecycline (2, 5.41% both). Resistance to ampicillin, high-dosage gentamycin and teicoplanin was not detected. Vancomycin resistance, linked to *vanC* gene presence, was identified in 10 MDR *E. casseliflavus* strains from 6 WW, 2 DW and 1 SW.

Conclusion: These preliminary results suggest the aquatic environment doesn't seem involved in the diffusion of vancomycin resistance genes of clinical importance (i.e. *vanA* and *vanB*). However, 10/30 samples were associated with MDR *vanC* enterococci, already linked to human disease. The detection of resistance to different CIAs, even in DW, underlines the role of water as a potential vector of AMR in the community. Finally, our findings highlight enterococci as valuable indicators for monitoring waterborne AMR.

Abstract ID: 3586

Role of the bacterial regulator ppGpp on the host-pathogen interaction in organoid-derived monolayers infected with Adherent-Invasive Escherichia coli

Presenting author: Llorenç Fernández-Coll, PhD, Universitat de Girona, Girona, Spain

Co-authors: *Maria Nuria Ramos-Corominas, Isabella Dotti, Azucena Salas, Margarita Martínez-Medina*

Introduction: Adherent-invasives *Escherichia coli* (AIEC) are a group of strains associated with Inflammatory Bowel Diseases (IBD), such as Crohn's Disease or ulcerative colitis. AIEC can adhere to and invade intestinal epithelial cells, as well as survive and replicate inside of macrophages. Their mechanisms of pathogenicity are not fully understood, but it has been suggested that AIEC may have a differential transcriptional profile compared to non-invasive strains of *E. coli*. The bacterial regulator ppGpp has been shown to affect the expression of different virulence factors and may be important for this pathotype and its interaction with the host.

Objectives: To assess the role of ppGpp modulating pathogenic mechanisms and host's response during infection of AIEC compared to a non-AIEC strain.

Methods: Mutants unable to produce ppGpp ($ppGpp^0$) were obtained from the commensal strain MG1655 and the AIEC strain LF82. Differentiated organoid-derived monolayers (d-ODMs) from human colonic samples were infected with our set of strains (MG1655, LF82 and their $ppGpp^0$ mutants) and their adhesion and invasion capabilities were assessed. RNA from bacteria adhered to the d-ODMs was isolated 6 hours after infection and changes in the transcriptional profiles were determined through Dual RNA sequencing.

Findings: The absence of ppGpp reduced significantly the ability of LF82 to adhere to and invade d-ODMs. The transcriptomic study showed that a vast number of genes were regulated by ppGpp in the AIEC strain LF82 and the commensal strain MG1655 (Figure 1), but with different trends between strains. In LF82, several genes found to be activated by ppGpp are related to AIEC's pathogenicity. The changes promoted by the absence of ppGpp in LF82 prompted changes in the gene expression of the host, but not in the cells infected with the commensal strain MG1655. Several of the genes found upregulated in the cells infected with the LF82 $ppGpp^0$ strain were related to the host response to pathogens, increasing the production of interleukins and TNF-α.

Conclusion: Altogether, our data highlights the role of ppGpp in AIEC's pathogenicity that could explain the differences observed between AIEC and non-AIEC and provide evidence that its modulation can be target as possible therapeutic strategies against the pathotype.

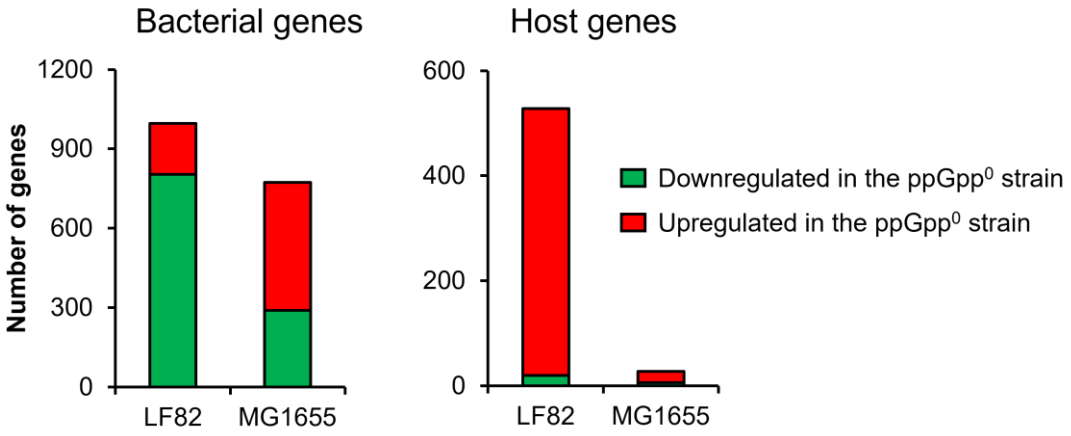


Figure 1. Number of differentially expressed genes (bacterial and host's) during the infection of d-ODM's with LF82 or MG1655 strains comparing between the $ppGpp^0$ and the WT strain.

Abstract ID: 2769 / Poster Number: 200.16P

Role of the gut microbiota in sarcopenia: gene expression patterns

Presenting author: Mrs. Susana Ruiz-Ruiz, FISABIO-Public Health, Valencia, Spain

Co-authors: *Ana Barberá, Vicente Pérez-Brocal, Rosario Ortolá, Fernando Rodriguez-Artalejo, Andrés Moya*

Sarcopenia, a state that precedes disability, is a disease associated with aging (in elders vary from 9.9 to 40.4%), characterized by the accelerated loss of skeletal muscle and impoverishment of muscle quality increased risk of falls and fractures. Human gut microbiota can influence physiology by regulating multiple processes, and it is also very relevant to skeletal muscle function and mass. Based on prevention strategies, the incorporation of interventions in the gut-muscle axis through the microbiome could have the potential to reverse the sarcopenic phenotype. Many studies show that the composition of the microbiota is significantly different between the elderly with and without sarcopenia. However, metatranscriptomics offers dynamic insights into gene expression patterns, metabolic activities, and host-microbiome interactions. To elucidate the role of the gut microbiota in the gut-muscle axis, we profiled metatranscriptomics of 216 fecal samples from the Seniors-ENRICA-2 cohort, categorized into sarcopenic (SCP) and non-sarcopenic (NSCP) groups. Our results showed statistically significant differences in 70 KOs (KEGG ORTHOLOGY) when comparing SCP and NSCP. After performing an enrichment analysis, a total of 31 KOs, grouped into 19 metabolic pathways, showed statistically significant differences between groups. We detected that SCP downregulated the paths related to purine and pyrimidine metabolism, which is associated with ATP metabolism, uric acid synthesis, and inflammation regulation. Some studies demonstrate that elevations in serum uric acid are related to sarcopenia status and the presence of reduced skeletal muscle mass. For that, to assess the exact role that the microbiota plays, it is necessary to conduct more analysis.

Abstract ID: 2727 / Poster Number: 367.15F

Role of the stringent response in the interaction of Pseudomonas aeruginosa with airway epithelial cells

Presenting author: Ms. Alessandra Guiducci, Roma Tre University, Rome, Italy

Co-authors: *Alessandra Fortuna, Veronica Felici, Andrea Sabatini, Serena Ciolfi, Paolo Visca, Elisabetta Affabris, Alessandra Sacchi, Giordano Rampioni, Livia Leoni*

Introduction: *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen known for causing persistent, life-threatening infections and exhibiting antibiotic resistance. Traditionally considered as an extracellular pathogen, it can invade and survive within professional and non- professional phagocytic cells, a strategy that contributes to evasion from immune responses and antibiotics.

Objective: Investigation of the role of the stringent response (SR), a global regulatory system involving the second messenger (p)ppGpp and the DksA protein, in *P. aeruginosa* survival within human carcinoma airway epithelial cells (A549).

Method: *P. aeruginosa* mutants inactivated in the SR (unable to synthesize (p)ppGpp and/or lacking DksA) were generated and phenotypically characterized. The intracellular survival of wild-type strain and SR mutants within A549 cells was investigated by CFU counts and confocal microscopy analyses, also in presence of bafilomycin A1 (a vacuolar acidification inhibitor). The impact of SR on the Type III Secretion System (T3SS) expression during the infection was investigated by transcriptional fusions.

Findings: *P. aeruginosa* survives inside A549 cells for up to 24 hours. SR mutants displayed significantly reduced intracellular survival relative to wild-type strain, with impaired evasion of vacuolar acidification. Reporter assays indicated that SR positively regulates the T3SS, with SR mutants showing decreased T3SS effector expression relative to the wild-type strain.

Conclusion: The SR, through regulation of T3SS, is crucial for *P. aeruginosa* survival within host cells. These findings highlight the SR as a potential therapeutic target for tackling *P. aeruginosa* chronic infections.

Abstract ID: 3039

Role of the yajC-secDF operon in Burkholderia

Presenting author: Elena Perrin, PhD, University of Florence, Sesto Fiorentino, Italy

Co-authors: *Iacopo Passeri, Marina Rinaldi, Miguel Valvano, Marco Fondi, Alessio Mengoni*

Introduction: SecDF proteins, included in the Resistance Nodulation Cell-Division Superfamily (RND) of efflux pumps, are present both in Bacteria and Archaea. Some roles have been suggested for this system in different microorganisms, mainly in Gram-positive bacteria, ranging from protein translocation across or into the inner membrane to antibiotic resistance. However, its function in bacterial cells remains elusive. Although this efflux system proved to be non-essential for cells viability in all the microorganisms in which it has been studied to date, recently, the use of the transposon mutagenesis with next-generation sequencing (TnSeq) revealed that the genes encoding SecDF are essential in various bacteria, including *Burkholderia* spp.

Objective(s): Since essential genes are considered excellent targets for new antimicrobial molecules, our aim is to understand the role of the SecDF system in *Burkholderia* species to evaluate its possible use as new antibiotics target.

Method: The two proteins are encoded by an operon that also contains the *yajC* gene. A *yajC-secD-secF* rhamnose-inducible conditional mutant of *Burkholderia cenocepacia* K56-2 has been constructed, allowing us to confirm the essentiality of this operon in this strain. This mutant has been used also to assess some phenotypes associated with SecDF in other microorganisms and to perform transcriptomic experiments in presence of different rhamnose concentrations.

Findings and Conclusion: Preliminary results indicate that the absence of this system determines:

- A down-regulation of protein synthesis and of flagellar assembly and chemotaxis;
- An up-regulation of alternative sigma factors associated with stress (RpoE and RpoH) and of some amino acid degradation pathways.

We are also trying to get suppressor mutants to elucidate the role of this operon. For the moment we didn't get any results, and this led us to hypothesize the presence of an internal promoter in this operon.

All the data that we are collecting will allow us to formulate some hypotheses, which will then be validated, on the possible role of this system in *Burkholderia*.

Abstract ID: 1151 / Poster Number: 194.15P

Role of UeMsb2 in Filamentous Growth and Pathogenicity of Ustilago esculenta

Presenting author: Prof. Zihong Ye, China Jiliang University, Hangzhou, China

Co-authors: *Jiang Wanlong, Hu Yinli, Zhang Yafen*

Ustilago esculenta is a dimorphic fungus that specifically infects *Zizania latifolia*, causing stem swelling and the formation of an edible fleshy stem known as jiaobai. The pathogenicity of *U. esculenta* is closely associated with the development of jiaobai and phenotypic differentiation. Msb2 acts as a key upstream sensor in the MAPK (mitogen-activated protein kinase) signaling pathway, playing critical roles in fungal hyphal growth, osmotic regulation, maintenance of cell wall integrity, temperature adaptation, and pathogenicity. In this study, we cloned the *UeMsb2* gene from *U. esculenta* (GenBank No. MW768949). The open reading frame of *UeMsb2* is 3015 bp in length, lacks introns, encodes a 1004-amino-acid protein with a conserved serine-rich domain, and is localized to the vacuole. Expression analysis revealed that *UeMsb2* is inducibly expressed during both hyphal growth and infection processes. Deletion of *UeMsb2* did not affect haploid morphology or growth rate in vitro but significantly impaired the strain's mating ability, suppressed filamentous growth, slowed host infection progression, and downregulated the expression of b signaling pathway genes associated with pathogenicity. Notably, the deletion of *UeMsb2* did not influence the in vitro growth of *U. esculenta* under hyperosmotic, thermal, or oxidative stress conditions. These findings underscore the critical role of *UeMsb2* in regulating the pathogenicity of *U. esculenta*. This study provides insights into the interaction between *U. esculenta* and *Z. latifolia*, particularly the mechanisms that drive host stem swelling.

Abstract ID: 3941 / Poster Number: 242.16P

Roles of O-glycans in the stability and immunogenicity of Cryptococcus neoformans Cda1 and MP88 mannoproteins

Presenting author: Dr. Eun Jung Thak, DONGDUK WOMENS UNIVERSITY, SEOUL, South Korea

Introduction: *Cryptococcus neoformans* is an opportunistic fungal pathogen causing life-threatening disease in immunocompromised individuals. *Cryptococcus neoformans* assembles two types of *O*-glycans on its proteins: major *O*-glycans without xylose and minor *O*-glycans containing xylose, which are mediated independently by Ktr3 and Cap6 mannosyltransferases.

Objective: In this study, we investigated the effect of *O*-glycan structures of the GPI-anchored mannoproteins MP88 and chitin deacetylase 1 (Cda1) on proteolytic cleavage and host-cell interactions.

Method: To investigate the effect of glycan structure on the immunogenicity of mannoproteins, purified MP88 and Cda1 were co-incubated with bone marrow-derived dendritic cells, and the immune response of host cells was analyzed via cytokine secretion. After co-incubation with mannoproteins for 12 and 24 h, the production of the proinflammatory cytokine tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) from BMDCs was quantified.

Findings: The absence of extended major *O*-glycans caused aberrant cleavage of the Cda1 protein, resulting in the loss of its glycosylphosphatidylinositol-anchor. Host cells incubated with mannoproteins from the CAP6-deleted strain showed reduced IL-6 secretion compared to those exposed to mannoproteins from the wild type and *ktr3*Δ strains. Moreover, mice immunized with Cda1 protein produced by the *ktr3*Δ strain showed higher anti-Cda1 antibody production compared to those immunized with wild type-Cda1, consistent with the enhanced IL-6 secretion.

Conclusion: These results highlight the contribution of xylose residues in *O*-glycans to the induced host immune response. Understanding the structure and function of *O*-glycans assembled on immunogenic proteins, particularly their roles in protein stability and host-cell interactions, would be useful in designing vaccines against fungal infections.

Abstract ID: 3202 / Poster Number: 287.15P

Rotating disk reactor microbial community composition and activity contributes to carbon and nitrogen removal efficiency in wastewater treatment

Presenting author: Mr. Hermes Hernan Bolivar-Torres, ISWA, University of Stuttgart, Stuttgart, Germany

Co-authors: *Peter Maurer, Esteban Gongora, Sara Kleindienst, Katharine Thompson*

Rotating disk reactors (RDRs) are a wastewater technology that facilitates biofilm formation on three consecutive rotating structures. RDRs remove organic carbon (OC) and nitrogen (N) but lack capacity to treat large volumes of sewage, which makes this technology less attractive than treatment via aeration tanks (ATs). RDRs achieve greater OC and N removal efficiency than ATs, despite processing wastewater once instead of recirculating ATs, and probably, contrasting microbial communities contribute to this. Here, we investigate the microbial community composition and activity of key genes involved in OC and N removal from RDRs and ATs. Sampling, DNA extraction, 16S rRNA gene and rRNA transcript amplification, amplicon sequencing, qPCR, and bioinformatics were performed. Our findings reveal that while microbial diversity on each RDRs varies, the community in the ATs is unchanged. Relative abundance analysis identified 152 unique genera on the RDRs and only 74 genera in the ATs. Additionally, our results show relevant variations in abundance of some genera involved in nitrification, such as Nitrosomonas and Nitrospira, with values from 0% on the first RDR to ~5% and ~7% on the last RDR, respectively. Conversely, these genera had relative abundance values of <2% in the ATs. Furthermore, 16S rRNA transcript abundances indicated more active microorganisms on the RDRs than in the ATs. Our results suggest that RDR biofilm formation supports variations in microbial community composition and activity that could enhance OC and N removal efficiency. Further studies are essential to confirm these observations and understand their implications for optimizing wastewater treatment processes.

Abstract ID: 1657 / Poster Number: 422.16F

Safeguarding Art and Health: Rosemary Extract as a Non-Toxic, Sustainable Alternative to Traditional Biocides for Canvas Conservation

Presenting author: Dr. Luca Di Stasio, University of Salerno, Fisciano, Italy

Co-authors: *Gianmaria Oliva, Giovanna Climaco, Paola Fiore, Giovanni Vigliotta*

The biodeterioration of paint pigments on canvas, caused by microorganisms interacting with the organic/inorganic compounds of paintings, poses a major challenge for preserving cultural heritage. Disinfection is essential to prevent degradation, but techniques must be carefully considered to avoid damage.

Traditional methods employ chemical antimicrobials (*e.g.*, Rocima 103, Biotin), which are effective against various microorganisms but toxic to the operators and environment, making them unsustainable. Our study focuses on developing a sustainable, effective and low-cost antimicrobial alternative able to preserve characteristics of artwork and reduce the health risk. Our research considered an 18th-century painting with microbial contamination on the reverse canvas. Seventeen bacterial strains were isolated using the contact-plate method, and the antimicrobial activity of a rosemary extract, made by homogenizing 10 g of fresh rosemary leaves in 20 mL of a 1:1 water-ethanol solution, was tested. In culture medium, the extract inhibited the growth of 74% of bacteria. It was then tested directly on canvas samples against microbial community present on it. Two methods were considered: spraying the extract onto the surface or using Evolon® tissue soaked in the extract. The spray reduced microbial growth by 100% after 24 hours and by ~75% after 48 hours, while fungi were eliminated in seven days. Likewise, Evolon® tissue reduced the abundance and size of bacterial colonies and eliminated 100% of the fungi. These results suggest that rosemary extract performs comparably to traditional biocides, offering a sustainable alternative that minimizes environmental impact and health risks without compromising the treatment efficacy.

Abstract ID: 3272 / Poster Number: 463.15P

Safety Assessment for Microbial Biostimulants: Merging Genomic Analyses with Literature Evidence for Safer Agricultural Applications

Presenting author: Dr. Gabriele Bellotti, Università Cattolica del Sacro Cuore, Piacenza, Italy

Co-authors: *Claudia Cortimiglia, Maria Elena Antinori, Pier Sandro Cocconcelli, Edoardo Puglisi*

Microbial biostimulants (MBs) have emerged as sustainable alternatives to synthetic fertilizers, offering eco-friendly solutions for boosting crop productivity. Despite their growing use, thorough safety evaluations are essential, especially for taxa not granted the Qualified Presumption of Safety (QPS). This study underscores the importance of integrating Whole Genome Sequencing with Extensive Literature Search (WGS-ELS) methods to ensure safe applications of MBs in agriculture. The main objective was to comprehensively assess the safety of QPS and non-QPS taxa commonly used in MB formulations. The study aimed to identify potential antimicrobial resistance (AMR) and virulence factor (VF) genes, thereby informing risk assessments and guiding regulatory considerations.

A total of 1,465 bacterial genomes were analysed: 1,314 QPS strains (including *Bacillus velezensis* and *Priestia megaterium*) and 151 non-QPS strains (*Azotobacter*, *Azospirillum*, and *Herbaspirillum* spp.). Public genomic data were screened for VF- and AMR-related genes using standard thresholds, which were subsequently refined based on ELS outcomes. MIC (Minimum Inhibitory Concentration) assays validated the genomic findings, while literature reviews contextualized the observed genes within potential health and environmental risks.

As expected QPS strains displayed minimal risk. Among non-QPS taxa, *Azotobacter* exhibited multiple VF hits, predominantly involving alginate, LPS, pili, and secretion systems that enhance environmental resilience rather than pathogenicity. Conversely, *Azospirillum* and *Herbaspirillum* demonstrated notable AMR profiles. Genome analysis and MIC tests confirmed chloramphenicol resistance genes (e.g., *ceoB*, *floR*, *catB*), alongside additional β-lactamases (Figure 1). Moreover, MIC assays revealed unexpected resistance to clindamycin and erythromycin, highlighting gaps in purely genomic predictions.

This integrated WGS-ELS workflow offers a robust framework for assessing the safety of MBs. By identifying specific AMR and VF genes in non-QPS taxa, the study provides essential insights for refining regulatory guidelines and promoting responsible MB usage, aligning with the broader vision of sustainable agriculture and environmental health protection.

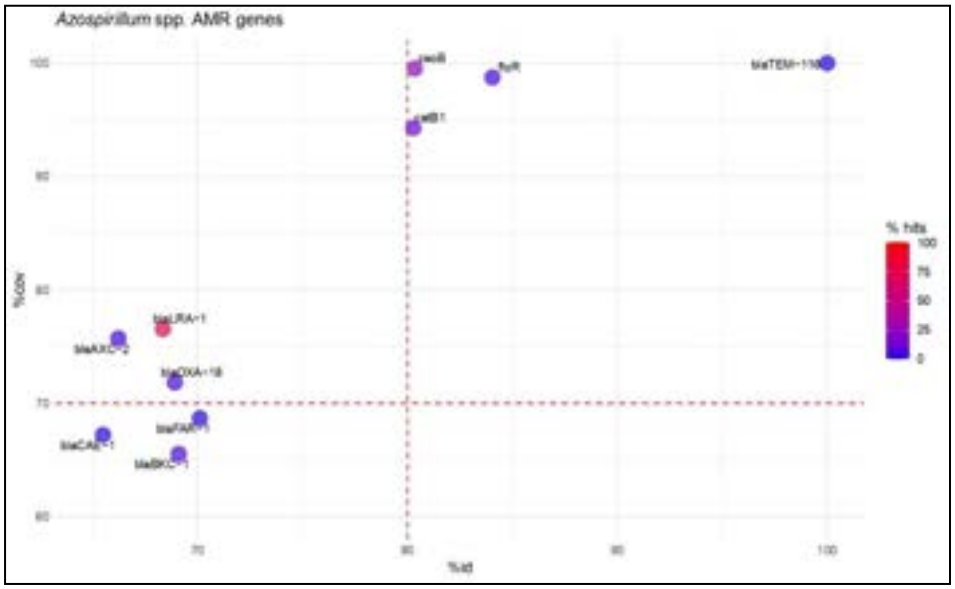


Figure 1: Bubble plot representing AMR hits in Azospirillum spp. The X-axis indicates the percentage identity, and the Y-axis shows the percentage coverage. Bubbles are coloured along a red-to-blue gradient based on the distribution of hits in the genus. Red dashed lines indicate the recommended thresholds at 80% identity and 70% coverage.

Abstract ID: 665 / Poster Number: 010.16P

Safety assessment of cow milk lactobacilli

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Mastitis is considered as the costliest disease on dairy farms. As treatment of mastitis relies heavily on antibiotics, there are increasing interest to find antibiotic-free approaches – such as beneficial lactobacilli strains.

A total of 350 cow milk samples were collected and cultured on selective Rogosa medium for lactobacilli. The isolates identified using by MALDI ToF-MS as lactobacilli were examined for antibiotic resistance and enzymatic activity in order to exclude isolates with unsuitable properties. Minimum inhibitory concentrations (MICs) of selected antibiotics were determined using the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST) and ISO 10932/IDF 223 standards. Enzyme activities of lactobacilli strains were tested using the apiZYM system (BioMérieux, France).

About 35% of isolates had an increased MIC value to kanamycin followed by chloramphenicol (15%) and streptomycin (7.5%). Sporadic strains were resistant to tetracycline (5%). Although the phenotype cannot confirm the presence or absence of transferable resistance genes, some of the observed levels are compatible with common transferable determinants. Almost 23% showed undesirable enzymatic activities such as β-glucuronidase, β-glucosidase and N-acetyl-β-glucosaminidase activities, which are associated with harmful effects in the colon by releasing aglycones and deconjugating glucuronic acid-conjugated carcinogens and based on that, these strains were excluded from further investigation.

Initial study (for now) selected 20 strains that will be subjected to whole genome sequencing (WGS) for their further suitability.

Abstract ID: 2924 / Poster Number: 284.15P

Salinization of a freshwater peatland increases methane production in quaking bog peat

Presenting author: Ms. Naomi Boerma, Radboud University, Nijmegen, Netherlands

Co-authors: *Annabel Legierse, Thomas Gremmen, Gijs Van Dijk, Martyna Glodowska*

Rising sea levels will increase salinization of freshwater wetland ecosystems, affecting coastal peatlands and below-sea-level regions such as the Netherlands. Over 30% of the world's soil organic carbon is stored in peatlands and this carbon is vulnerable to decomposition, releasing greenhouse gases like carbon dioxide (CO_2) and methane (CH_4). Therefore, the formation of new peat as quaking bogs is key to carbon sequestration in these systems. A knowledge gap remains on how salinization affects microbial activity, biogeochemical processes, peat formation, and carbon accumulation in quaking bogs. Moreover, recent evidence suggests CH_4 release from quaking bogs can be increased under salinization. In this field and laboratory-based study we aimed to identify key conditions driving microbial CH_4 cycling in quaking bogs. This involved geochemical analysis of the sediment and porewater, qPCR analysis of pmoA (aerobic methanotrophy) and mcrA (anaerobic methanotrophy and methanogenesis) as well as 16S amplicon sequencing. Additionally, activity of methanotrophs and methanogens was studied via incubations. High abundance of carbon and organic matter in the quaking bog porewater likely stimulate microbial activity, supported by increased methanogenesis observed in brackish quaking bog incubations, up to 2,5 times higher than the freshwater. Futrhermore, there was no indication of aerobic and anaerobic methanotrophy being influenced by salinization in quaking bogs. For the latter, this is likely due to the absence of a suitable electron acceptor, which was confirmed by hydrochemical data. Ultimately, this suggests that the higher CH_4 release originates from increased methanogenesis rather than a decrease in methanotrophy in brackish quaking bogs.

Abstract ID: 447 / Poster Number: 144.16P

Salmonella Free Salads – Development of a Rapid, Robust and Reliable Testing

Presenting author: Mr. Lazaros Konstantinou, EMBIO Diagnostics Ltd., Nicosia, Cyprus

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The detection of Salmonella in salads is essential for preventing salmonellosis, a significant public health concern characterized by symptoms such as diarrhoea, fever, and abdominal cramps. The standard analyses currently used for bacteria detection have significant limitations with the most important being their long procedural time that can be crucial for foodborne outbreaks. Early, rapid and accurate detection methods are vital for identifying contamination and mitigating the risk of widespread outbreaks. Ensuring the microbiological safety of salads, which by nature are products that do not undergo extensive processing to ensure food safety, not only protects consumer health but also upholds food safety standards and minimizes economic losses associated with foodborne illnesses. This research introduced a novel biosensor system for the rapid and precise detection of Salmonella spp. in salad samples. Utilizing a portable potentiometer integrated with cell-based biosensor technology (BERA), the system was optimized to process and analyse the data from a 3-minute analysis and immediately notify the end-user of the results via an Android or iOS device. The biosensors were developed with monoclonal mouse antibodies specific to Salmonella spp., evaluated in spiked salad matrices, and validated using the ISO 6579-1:2017 standard method. The system achieved an accuracy of 91.1% and performance characteristics ranging from 88.8% to 94.1%, managing to reliably differentiate contaminated from non-contaminated samples. The detection limit was established at 1 CFU g^{-1} across various salad types, highlighting its potential for practical use in food safety monitoring.

Abstract ID: 1016 / Poster Number: 281.17P

Scale-up of the production of L-Malic acid in engineered Ogataea polymorpha using methanol as the sole carbon source

Presenting author: Mrs. Alessandra Mauri, RWTH Aachen, Aachen, Germany

Co-authors: *Miroslava Yovcheva, Hendrik Ballerstedt, Lars M. Blank*

With a relatively low atmospheric concentration but a half-life of 120 years, carbon dioxide (CO_2) is the first greenhouse gas (GHG) responsible for global warming. Recently, the possibility of capturing and converting CO_2 to methanol (CH_3OH) and the rise of methylotrophic yeasts, which can use CH_3OH as their sole carbon and energy source, have offered a sustainable way to produce fine chemicals.

Envisioning a circular bioeconomy, to end fossil dependence, a two-phase laboratory-scale fermentation was established to enhance the yield of L-Malic acid in *Ogataea polymorpha* significantly. The optimised strain harbours a reductive TCA cycle and a molecule-selective membrane transporter. In a bioreactor system, a batch phase of 10 g/L glycerol was followed by a fed-batch one using 80% v/v methanol solution as feed. The fermentation broth was thoroughly sampled to monitor substrate consumption and product formation via HPLC analysis. Biomass formation and substrate toxicity were assessed by monitoring OD_{600}. Finally, by implementing an automated pulse-feeding strategy, an L-Malic acid titer of 15 g/L was achieved, with a yield of 0.13 mole malic acid per mole methanol. This yield is 100% higher than the formerly published ones and represents 40% of the theoretical maximum yield of 0.25.

This result marks a successful milestone in scaling up malic acid production using C1 substrates in *Ogataea polymorpha*. Indeed, further scale-up efforts, along with process and feeding optimisation, are expected to increase yield and titer further, showcasing a promising approach as a contribution to reducing GHG emissions.

Abstract ID: 120 / Poster Number: 163.16P

Schizophyllum commune exopolysaccharides reduce Salmonella invasion to human gut epithelium and activate macrophage toward M1 polarization

Presenting author: Assoc. Prof. Parameth Thiennimitr, Faculty of Medicine, Chiang Mai University, Muang, Thailand

Co-authors: *Arishabhas Tantibhadrasapa, Pattarapon Boonpan, Songbo Li, Thanawut Chotmanee, Jaturong Kumla, Nakarin Suwannarach, Saisamorn Lumyong*

Introduction: The rise of multidrug-resistant Salmonella enterica raises the alarm about alternative treatment for acute non-typhoidal salmonellosis. The antibacterial potential of the edible mushroom Schizophyllum commune, belonging to the phylum Basidiomycota, has been reported. However, the role of S. commune exopolysaccharides (EPS), the vital component in its physiological process, on host gut immunity is still under investigation.

Objective: To explore the in vitro anti-bacterial and immunomodulatory roles of crude S. commune EPS on the human gut epithelium (T84 cells) and murine macrophage (RAW264.7 cells) against Salmonella infection.

Methods: Fourier transform-infrared spectroscopy (FT-IR) analysis was used to characterize the EPS composition. The cytotoxicity test of EPS was done using an MTT assay. The reduction in S. enterica serovar Typhimurium gut invasiveness and inflammation by the EPS were investigated using gentamicin protection and real-time PCR, respectively. Macrophage polarization was determined by fold change in the expressions of M1 and M2 cell surface markers, including their related cytokine genes.

Findings: FT-IR analysis confirmed the typical polysaccharide patterns of carbohydrate-containing EPS. There is no direct anti-Salmonella effect of EPS, and Salmonella can grow on S. commune EPS. However, pretreatment with S. commune EPS significantly reduced the invaded Salmonella numbers and proinflammatory cytokine gene expressions in T84 and RAW264.7 cells. S. commune EPS increased the expression of Cd11c, the M1 macrophage cell surface marker, without altering Cd206, Tlr2, and Tlr4 expressions.

Conclusion: The crude S. commune EPS modulates gut immunity against Salmonella infection in vitro.

Abstract ID: 2687 / Poster Number: 320.17P

Screening and identification of novel chitin deacetylases for the sustainable valorisation of chitin

Presenting author: Melissa Bisaccia, PhD, University of Insubria, Varese, Italy

Co-authors: *Ana Šimatović, Immacolata Serra, Elisa Margherita Maffioli, Gabriella Tedeschi, Flavia Marinelli, Francesca Berini*

Chitin is one of the most abundant organic molecules after lignocellulose, yearly produced in nature in the range of 10^{10}-10^{11} tons. However, its direct applications are mainly restricted to low value-added products (*e.g.,* animal feed and agricultural fertilizers) because of the high crystallinity and inflexibility of its structure. On the contrary, molecules such as chitosan, N-acetylglucosamine, and chitooligosaccharides, which derive from its deacetylation and/or hydrolysis, have attracted attention because of their water solubility and various favourable bioactive properties.

Consequently, there is much interest in the discovery of novel chitinases and chitin deacetylases (CDAs), which would allow to produce them in a more targeted and environmentally friendly way, as opposed to the currently prevalent chemical route. To this point, we are applying both in silico and functional screening approaches in order to identify novel CDAs from bacterial strains originating from diverse ecological niches, from European soils to the extreme Antarctic Ocean. Three putative CDAs have thus far been identified from a soil *Streptomyces* sp. MEL8 strain, a marine Antarctic *Acinetobacter* sp. C33 strain, and from a *Nonomuraea* sp. strain. The enzymes' biochemical characterization revealed promising features concerning their temperature activity profile and tolerance to various salts, solvents, and detergents. This will favour the development of sustainable bioprocesses for chitin valorisation, with applications in a wide range of industrial sectors, including polymer functionalization, enzyme immobilization, and biocatalysis, as well as packaging, biomedical, pharma, cosmetics, and food ones.

Abstract ID: 2072 / Poster Number: 156.15P

Screening of As-Resistant bacterial strains from the Bulk soil and the rhizosphere of mycorrhizal Pteris vitiate cultivated in an industrial multi-polluted site

Presenting author: Dr. Giorgia Novello, Università del Piemonte Orientale, Alessandria, Italy

Co-authors: *Elisa Gamalero, Cesaro Patrizia, Daniela Campana, Nadia Massa, Guido Lingua, Elisa Bona*

Arsenic (As) contamination poses significant environmental and health concerns globally, particularly in regions with high exposure levels due to anthropogenic activities. As phytoremediation, particularly through the hyperaccumulator fern *Pteris vittata*, offer a promising approach to mitigate arsenic pollution. Bacteria and mycorrhizal fungi colonizing *P. vittata* roots are involved in As metabolism and resistance and plant growth promotion under stressful conditions.

A total of forty-five bacterial strains were isolated from bulk soil and the rhizosphere of mycorrhizal *P. vittata* growing in an industrial As-polluted site. Bacteria were characterized by their plant-beneficial traits, tolerance to sodium arsenate and arsenite, and the occurrence of As-genes. This study highlights differences between the culturable fraction of the microbiota associated with the rhizosphere of mycorrhizal *P. vittata* plants and the bulk soil. Moreover, several strains showing arsenate tolerance up to 600 mM were isolated. All the bacterial strains possessed *arsC* genes, and about 70% of them showed *arrA* genes involved in the anaerobic arsenate respiration pathway.

The possible exploitation of such bacterial strains in strategies devoted to the assisted phytoremediation of arsenic highlights the importance of such a study in order to develop effective in situ phytoremediation strategies.

Abstract ID: 2531 / Poster Number: 127.17P

Screening of new chemical compound that suppresses the activity of LER, a master transcriptional regulator for virulence genes in Enteropathogenic Escherichia coli

Presenting author: Ms. Samia Akter, Kyungpook national university, Daegu, South Korea

Co-authors: *Seunghyeok Bang, Jeongah Kim, Seung Ji Kang, Kyung hwa Park, Minsang Shin*

Enteropathogenic *Escherichia coli* (EPEC) is a major cause of severe diarrhoea in infants, especially in developing countries, contributing to significant morbidity and mortality. Its pathogenicity is driven by the locus of enterocyte effacement (LEE), which encodes a Type III secretion system (T3SS) responsible for injecting bacterial effectors into host cells. This leads to attaching and effacing (A/E) lesions, disrupting intestinal function and inducing inflammation. Ler (LEE-encoded regulator) is a key activator of LEE operons, overcoming the silencing effects of H-NS to drive the expression of critical virulence factors. Due to its central role in EPEC pathogenesis, Ler is an ideal target for therapeutic intervention. With the rise of antibiotic resistance, alternative strategies targeting virulence mechanisms rather than bacterial survival are urgently needed. This study aims to identify small-molecule inhibitors of Ler to suppress LEE-mediated virulence. High-throughput screening, and transcriptional analysis will be employed to discover and validate potential inhibitors. By targeting Ler, this approach seeks to disarm EPEC without promoting resistance, providing a novel strategy to mitigate its pathogenicity. Developing anti-virulence therapies targeting regulatory pathways could revolutionize the treatment of bacterial infections, offering a sustainable alternative to traditional antibiotics and reducing the global burden of EPEC-related diseases.

Abstract ID: 3296 / Poster Number: 216.17P

Screening of repurposed chemicals and Insights from Thermal Proteome Profiling: compound B13 as a promising adjuvant for the resensitization of MRSA to β-lactam

Presenting author: Mr. Alexandre Mahé, Université de Caen Normandie – UR 4313 CBSA, Caen, France

Introduction: In recent decades, multi-drug resistant (MDR) strains have emerged among Gram-positive bacteria. To combat the spreading of antimicrobial resistance, one strategy is to identify new druggable targets that will restore antibiotic efficiency when hit. Many studies highlight the teichoic acid (TA) D-alanylation pathway – a cell wall modification mechanism involving 5 proteins encoded by the *dltXABCD* operon – as a promising target. In this context, Pasquina *et al*. described in 2016 amsacrine as an inhibitor of DltB, a membrane protein involved in translocating D-alanyl moieties onto TAs. Despite its promising ability to resensitize methicillin-resistant *Staphylococcus aureus* (MRSA) to several antibiotics, the molecule inhibits eukaryotic topoisomerase, making amsacrine clinically irrelevant.

Objectives: To bypass this issue, this study aims to screen a collection of chemicals derived from amsacrine or identified *in silico* as potential DltB inhibitors, and better characterize the resulting hits.

Methods: The chemicals were screened for their ability to resensitize a clinical MRSA isolate to imipenem (IPM), belonging to the β-lactam family. The toxicity of effective chemicals was then assessed in HaCaT cells using a LDH cytotoxicity assay, and on the animal model *Galleria mellonella*. Their ability to inhibit TAs D-alanyation was also checked. In parallel, a proteomic approach called Thermal Proteome Profiling (TPP) was set up. This method identifies the target of a molecule by comparing the thermal stability of proteins in treated versus untreated samples, as target binding alters the protein's melting behavior. Here, a previously described inhibitor of DltA (May *et al.,* 2005) was used to validate the method.

Findings: Of the 60 tested chemicals, 2 resensitized the MRSA clinical isolate to IPM, and 2 showed an antibacterial activity alone at 25 µM. Among these 4 hits of interest, none was able to inhibit the TAs D-alanylation pathway. Three of them induced either lethality in *G. mellonella,* or cytotoxicity on HaCaT cells and were therefore excluded from the study. Taken together, these results highlight the last hit, named B13, as a promising imipenem IPM adjuvant with unknown target(s). Finally, TPP performed on *S. aureus* raw protein extracts demonstrated a significant thermal shift regarding DltA (Figure 1).

Conclusion: As expected, TPP analysis identified DltA as the major target candidate of the DltA inhibitor. In addition, the method provided information on the thermal stabilization of proteins known to interact with DltA, suggesting that TPP can detect whole interaction pathways. Taken together, these results validate the TPP protocol as a new approach to identify the unknown target(s) of B13.

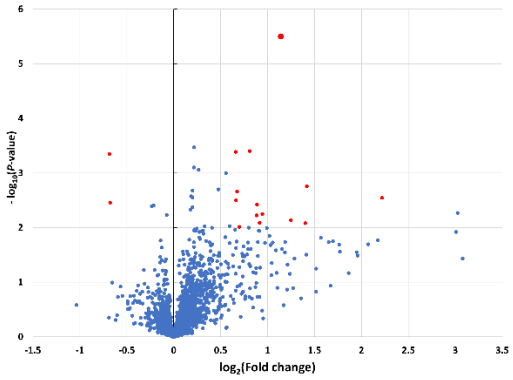


Figure 1: Statistical comparison (t-test) of soluble fractions of each identified protein (n = 2048) between Mock and DltA inhibitor (20 µM) conditions at 53.3°C. Red and blue dots correspond to significant and non-significant differences, respectively. Log_2(Fold change) and $-log_{10}$(*P*-value) thresholds are set to 0.6 and 2, respectively.

Abstract ID: 1065 / Poster Number: 417.15P

Screening sugar transport proteins from the termite gut microbiome for improved lignocellulosic biomass conversion using microbial cell factories

Presenting author: Mr. Laurens Lambrecht, BioPort Group, Centre for Synthetic Biology, Ghent University, Ghent, Belgium

Co-authors: *Magdalena Calusinska, Inge Van Bogaert*

Background: Biotechnological production processes, based on microbial cell factories to convert lignocellulosic biomass and other waste streams into high-quality products, are increasingly replacing less sustainable and labour-intensive chemical production processes. These microbial cell factories often require in-depth pathway engineering and fine-tuning to bring microbial production efficiency up to an industrial scale. An often neglected strategy is the optimization of the transport (e.g. pentose sugar import) across the biological membrane.

Objectives: The objective consists of optimizing the import process of xylose, arabinose and cellobiose in *E. coli* by discovering and characterizing novel and improved transport proteins. To this end, candidate import proteins are screened from a metagenomic dataset created from the termite gut microbiome, one of the most important lignocellulosic degrading natural systems found in nature.

Methods: Transport proteins are selected based on functional sequence analysis and a computational pre-screening consisting of substrate docking and molecular dynamics simulations. Subsequently, characterization experiments are conducted to establish the transporter sugar specificity, kinetic parameters (such as the uptake rate and sugar affinity) and to what extend their functioning can alleviate carbon catabolite repression when glucose is present.

Results: Import proteins were selected and are being characterized experimentally. In addition, the computational pre-screening is being optimized in combination with experimental validation to further improve the selection of promising transport proteins from omics data.

Abstract ID: 2926 / Poster Number: 154.17P

Seagrass symbionts as a source of antibiotics: from estuaries to innovation

Presenting author: Dr. Grant January, School of Biomedical Sciences, Faculty of Health, University of Plymouth, Plymouth, United Kingdom

Co-authors: *Kathryn Burdon, Zsófia Tauber, Jarmo-Charles Kalinski, Rutendo C. Mupfumi, Sean Kelly, Rosemary Dorrington, Mathew Upton*

Antimicrobial resistance has become a global public health concern and new anti-infective agents are needed to overcome this "silent" pandemic[1]. Natural products (NPs) are crucial antimicrobial agents and offer the most promising source for drug discovery compared to synthetic compounds[2]. In this study, seagrass from the River Yealm estuary, Devon, United Kingdom, and their microbial symbionts were investigated for antimicrobial activity.

A bacterial collection produced from local-UK seagrass was screened for antimicrobial activity using phenotypic assays against ESKAPE pathogens. Isolates with promising bioactivities were triaged for further characterisation including "One Strain Many Compounds (OSMAC)" fermentations, *in vitro* bioassays, metabolomics, whole genome sequencing, and *in silico* mining for biosynthetic gene clusters (BGCs).

One promising isolate, *Paenibacillus peoriae*, or SG1, displayed preliminary antibacterial and antibiofilm activities against Gram-positive and -negative pathogens. OSMAC experiments were conducted, and crude extracts fractionated using reverse-phase chromatography, with active fractions currently undergoing bioactivity screening. Untargeted metabolomics and molecular networking by HR-LC-MS/MS will be used to chemically dereplicate the compounds of interest. The genome was sequenced and antiSMASH prediction revealed BGCs, which could putatively produce antimicrobial compounds with broad and narrow-spectrum antibacterial, antiviral, and antifungal activity. The BGCs with potential novelty will be investigated in more detail – in an attempt to link the 'omic' datasets and elucidate the active NPs.

In this study, we demonstrate the value of investigating marine environments and their inhabitants for specialised metabolites with potentially new bioactivities towards drug-resistant pathogens, by integrating classical microbiology and modern '-omics' tools.

Abstract ID: 1228 / Poster Number: 197.15P

Seasonal and vertical dynamics in the microbial communities in a flooded and former mica mine (Quebec, Canada)

Presenting author: Elise Lhoste, PhD, University of Quebec in Montreal, Montréal, Canada; Interuniversity Research Group in Limnology, Montréal, Canada

Co-authors: *Elisa Pastier, Violaine Ponsin, Maikel Rosabal, Cassandre Lazar*

Artificial lakes formed from former mining activities represent unique and underexplored environments, with diverse microbial communities, but their understanding still lacking. For example, the Blackburn Mine (Quebec, Canada), is an interesting site due to its mining history and microbial ecology, remains unknown. This study aimed to understand the effects of seasonal variation and depth on bacterial, archaeal, and micro-eukaryotic communities in a stratified water column and biofilms. Samples were collected by technical divers from the surface to 52 m depth during spring, summer, and autumn of 2021–2022, and analyzed using 16S/18S rRNA gene sequencing. The results revealed that while seasonal changes did not significantly impact environmental variables such as dissolved oxygen, dissolved carbon or NH_4/NH_3, they strongly influenced microbial community composition, along with depth gradients. The relative abundance of archaeal genera displayed stability across depths compared to bacterial and micro-eukaryotic communities. The mine was stratified into three distinct zones based on oxygen concentration. In the oxic zone, the bacterial phylum Actinobacteria, a core member of freshwater ecosystems, dominated, along with the methanogenic archaeon *Methanosarcina*. The transition zone was characterized by a high abundance of the bacterial genus *Chlorobium*, with methanogens such as *Methanospirillum*, *Methanosaeta* also prevalent. The anoxic zone hosted sulfur-reducing bacteria (*Desulfomonile*, *Desulfobacca*), sulfur-oxidizing bacteria (*Sulfuricurvum*), and methanogenic/methanotrophic archaea. Eukaryotic communities exhibited a notable presence of algae, particularly from the Chrysophyceae class, and protists. These findings suggest that microbial communities in the mine are integral to sulfur and carbon cycling, emphasizing the ecological significance of mining-associated aquatic systems.

Abstract ID: 3708 / Poster Number: 193.17P

Seasonal distribution of antibiotic resistance genes and core bacteria within swine farms from a one-health perspective

Presenting author: Mr. Gihan Lee, Korea Maritime and Ocean University, Busan, South Korea

Co-authors: *Keunje Yoo*

Antimicrobial resistance (AMR) presents a significant effect on the human, animals, and the environment. Especially, swine farms are recognized as one of the hot spots of antibiotic resistance genes(ARGs) and antibiotic resistant bacteria. However, the seasonal distribution of ARGs and microbial communities in swine farms remains insufficiently understood. This study aimed to comprehensively uncover the ARGs and core bacteria of feces, surfaces, bioaerosols, and human nasals on swine farms. In this study, 300 samples were collected from three different types of pigs from four swine farms during the summer and winter seasons. We used 16S rRNA gene sequencing and shotgun metagenomic sequencing to analyze the microbial composition and ARGs. Beta-diversity analysis showed distinctions depending on season ($p < 0.05$). At the genus level, *Staphylococcus* spp. and *Corynebacteria* spp. were significantly more prevalent in bioaerosols and surface during the summer season. Notably, ARGs revealed a similar trend depending on the seasons, and the abundance was higher in summer compared to winter. *Staphylococcus* and *Corynebacterium* have a positive correlation with major antibiotic resistance genes, showing the potential for multi-drug resistance in the two main genera regardless of the season. These findings highlight that potential pathogens and ARGs pose a higher risk to human health in summer than in winter. Our results confirm that ARGs were notably affected in summer, and the risk of spreading ARGs to the environmental dissemination.

Abstract ID: 3230 / Poster Number: 083.16P

Seaweed-Derived Bioactives: Developing a Novel Synbiotic to Support Immune Function

Presenting author: Ms. Holly Sedgwick, University of Reading, Reading, United Kingdom

Co-authors: *Anisha Wijeyesekera, Glenn Gibson, Jessica Adams*

Introduction: Seaweed-derived bioactives, particularly complex polysaccharides, are fermented by the gut microbiota and may have prebiotic potential. In prior *in vitro* work, alginate, laminarin, and fucoidan, have been shown to modify the gut microbiota and its metabolites, such as short chain fatty acids (SCFA). Synbiotics are emerging as a promising dietary-driven approach to improve health through targeting of the gut microbiota.

Objective: To understand further the influence of seaweeds on the gut microbiota, in order to develop an optimised synbiotic food product to support immune health.

Methods: Using dynamic pH anaerobic batch fermentations, pure complex polysaccharides from brown seaweeds and whole brown seaweeds were screened for prebiotic potential (in triplicate, using three independent stool donors). Fermentation samples were collected at 0, 8, 24, and 48 hours. Changes in microbial ecology were assessed using 16S sequencing paired with Fluorescence *In Situ* Hybridisation with Flow Cytometry. SCFA production was determined through Gas Chromatography-Flame Ionisation Detection. ^{1}H-NMR Spectroscopic Analysis was used to identify further relevant metabolite production including gamma-aminobutyric acid.

Findings: SCFA analysis showed that alginate, laminarin, as well as whole brown seaweed *Ascophyllum nodosum* significantly increased acetate production during fermentation compared to the negative control. Furthermore, laminarin additionally significantly increased propionate production compared to the negative control. This analysis indicates that alginate, laminarin, and *Ascophyllum nodosum* are suitable candidates for further screening for prebiotic properties.

This *in vitro* work will support the development of a novel synbiotic product to later test *in vivo*.

Abstract ID: 3127 / Poster Number: 418.17P

Selection and Characterization of Microbial Communities Under the same Environmental Stress Conditions for Polyhydroxyalkanoates (PHA) Biosynthesis

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Co-authors: *Cristiana Torres, Juan Martínez-Bla Martínez-Bla, Rui Oliveira, Filomena Freitas, Maria Ascensão Reis*

Microorganisms in nature live in complex ecosystems known as microbiomes, where the collective capabilities of microbial communities often surpass those of individual species. Through interspecies communication mechanisms, including metabolite exchange and molecular signalling, microbial consortia establish a division of labour, which enhances their productivity and resilience to environmental fluctuations. This communal lifestyle contributes to the stability of microbial consortia, making them more adaptable to diverse environmental conditions. By harnessing these attributes, natural microbiomes from various ecosystems can be engineered to produce commercially valuable metabolites, such as biopolymers.

Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible polyesters synthesized by specific microbial species, offering a sustainable alternative to conventional plastics. The feast-and-famine (F/f) strategy is a method employed to enrich microbial consortia in PHA producers, involving alternating periods of carbon excess (feast) and carbon limitation (famine). This approach induces PHA-producing microbes to growth while non-PHA-storing organisms are eliminated from the system.

In this study, aimed at enriching microbiomes in PHA-producers, natural samples from diverse habitats across Portugal underwent F/f cycling (Figure 1). Over a period of one month, these microbiomes evolved into PHA-enriched mixed microbial consortia (MMCs), which were characterized by biomass quantification, nutrient profiling, PHA content and microbial composition analysis. While all microbiomes transitioned into PHA-enriched MMCs within 33 days, their initial compositions played a significant role in shaping the final consortia. Dominant phyla included Bacteroidetes, Proteobacteria, and Actinobacteria, with variations attributed to geographic and environmental factors. Notably, Proteobacteria predominated in most samples, while Actinobacteria became

the dominant group in specific environments. Microbiomes derived from Corroios Marshland and Moss collected from olive trees exhibited the highest PHA yields. This study provides valuable insights into the evolutionary dynamics of microbiomes under controlled conditions and offers guidance for engineering and predicting the development of PHA-producing consortia from natural sources, facilitating the sustainable production of biopolymers through microbiome manipulation.



Figure 1: Diverse sampling locations across Portugal where microbiomes were collected (Setúbal, Leiria and Aveiro

Abstract ID: 3322 / Poster Number: 254.16P

Selection of Pseudomonas aeruginosa phages and improvement of their lytic activity for a therapeutic approach in lung infection

Presenting author: Ms. Sara Bolognini, University of Pisa, Pisa, Italy; University of Siena, Department of Medical Biotechnologies, Siena, Italy

Co-authors: *Caterina Ferretti, Elisabetta Trovato, Noemi Poma, Laura Rindi, Arianna Tavanti, Mariagrazia Di Luca*

Pseudomonas aeruginosa is a high priority pathogen due to its ability to easily select drug-resistant clones[1]. Phage therapy represents a promising alternative to treat difficult infections both alone and/or in combination with antibiotics[2]. The aim of this study was to isolate and characterise new bacteriophages targeting *P. aeruginosa* and enhance their lytic activity for phage therapy application in a patient with lung infection.

Bacteriophages were isolated from environmental sources by enrichment method. Phages were genotypically (sequencing, taxonomy, genome annotation, functional predictions) and phenotypically characterised (TEM, replication cycle, temperature and pH stability) to identify therapeutic ones.
To treat a patient candidate for phage therapy with a lung infection from MDR *P. aeruginosa* PV, active phages from the collection were identified.
To enhance the lytic activity of phages, 5-days direct evolution was explored. After 15 rounds mutant phages were compared to the wild-type via 24-h bactericidal assays and growth kinetics. Mutations occurred in trained phages were assessed by sequencing.

Between the therapeutic-phage collection, phage 9 resulted active against patient isolate. Growth kinetics curves showed phage 9 was able to control the bacterial growth up to 2h. Therefore, it was trained against the patient strain to improve its lytic ability. After 15 evolution-rounds phages revealed enhanced performance compared to the wild-type one, showing a 3-log10 reduction in the bacterial load after 24h.

Trained phages sequenced showed mutations in tail protein, suggesting possible better phage-bacteria interaction, and also highlighting them as key genes during phage infection.

Therapeutic phages isolated from environment can develop enhanced lytic activity by direct evolution against a specific strain of interest.

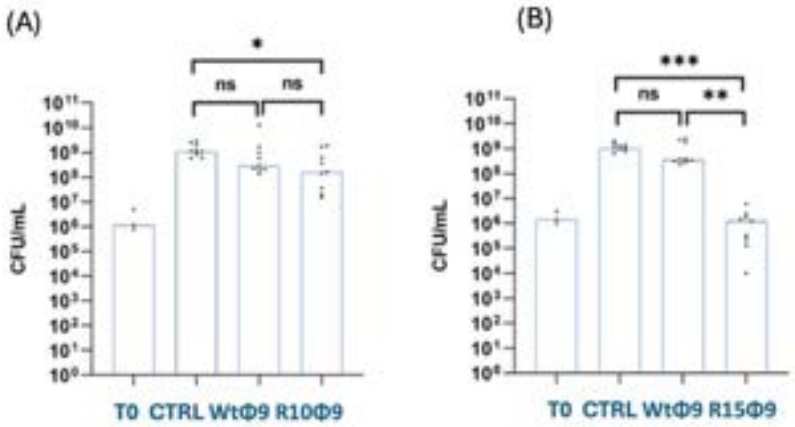


Figure 1: Bacterial load, expressed as CFU/mL, counted for each different condition after the 24-hour treatment with phage 9 and its evolved mutants (A) of round 10 and (B) of round 15. In addition, were represented T0, that was the bacterial load at starting time before incubation, and growth controls as reference. Data were obtained from three independent experiments and every condition was tested in triplicate. In the graph the median of points was represented. The Kruskal-Wallis test using Dunn correction was performed for multiple comparisons of all conditions with the other. *p≤0.05; **p≤0.01; ***p≤0.001; ns p>0.05. N=3

Abstract ID: 3919 / Poster Number: 241.16P

Selection of Recombinant Human Synthetic Antibody Fragments (Fab) targeting Shiga Toxins Stx1 and Stx2 produced by Shiga Toxin-Producing Escherichia coli

Presenting author: Bruna De Sousa Melo, PhD, Butantan Institute, São paulo, Brazil

Co-authors: *Gabriel Correia Lima, Roxane Maria Fontes Piazza, Daniela Luz*

Introduction: Among the diarrheagenic *Escherichia coli* pathotypes, Shiga toxin-producing *E. coli* (STEC), and its enterohemorrhagic subgroup (EHEC), stand out for their potential to cause severe diseases, such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), due to their main virulence factor, which is Shiga toxin (Stx) production, a potential target for detection, which is one botterneck for STEC infection survailance and containment. The Stx presents as two types and their variants. Currently, the diagnosis for STEC detection is routinely performed through molecular tests or commercial kits, which have disadvantages due to their selectivity only for the O157 serogroup and their high cost. Antibodies are excellent molecules that can be used as tools for detection in diagnostics. By using recombinant DNA technology and methods such as Phage Display, it is also possible to obtain recombinant antibody fragments (rAbs), such as Fab, with *in vitro* immune repertoires, without the need for immunization in living hosts.

Objective: Use phage display technology to isolate human Fab fragments (Fab) that can serve as a tool for Stx diagnosis and STEC monitoring.

Method: In this work, we used a human Fab fragments synthetic library to select high-affinity antibody fragments against the Stx1 and Stx2 toxins by Phage Display. The selection was successfull on generate high-affinity clones which were analyzed for specificty, affinity and paratope saturation by ELISA, showing promissing detection candidates. Also the CDRs of each clone were analyzed by Sanger sequencing.

Conclusion: Once further characterized, these promissing molecules could be a fast, cheap and efficient tools to be use on STEC diagnosis, for survanlance and outbreack containment.

Abstract ID: 3593

Selective activation of prokaryotic taxa in hyperarid desert soils in dependence on water availability

Presenting author: Dr. Anna Hakobyan, University of Bonn, Bonn, Germany

Co-authors: *Dorothee Kinkel, Wiebke Sickel, Ramona Mörchen, Maximilian Becker, Claudia Knief*

Introduction: The Atacama Desert constitutes one of the most extreme habitats on Earth, due to extremely low water availability. Despite that, microbial life has been detected in the hyperarid core, even at the driest locations of the Atacama Desert, using culture-dependent and -independent approaches. Microbial diversity and abundance decrease with increasing hyperaridity, especially with the transition from arid to hyperarid conditions.

Objectives: In the presented study, we aimed to investigate the intricate dynamics of microbial communities in soils from an elevation and aridity gradient near Paposo in the Atacama Desert. We clarified how these communities respond to the environmental gradients and to hydration events. Key aspects of our study were (I) to determine the threshold levels of water required to activate microbial metabolic activity and (II) to observe whether all bacterial taxa in hyperarid soils are triggered into activity with the same amount of water.

Methods: To evaluate the dependency of microbial activity on water availability, we conducted *ex-situ* irrigation experiments of desert soils collected along the aridity gradient within five elevations and two soil depths. We used different amounts of water to simulate precipitation events of different intensities. We determined microbial activity in dependence on water availability by measuring O_2 consumption and analyzed changes in community composition based on 16S rRNA gene amplicon sequencing. To identify specifically the taxa that become metabolically active following hydration, soil samples were incubated with a low (10% water holding capacity of the soil (WHC)) or high (50% WHC) amount of $H_2{}^{18}O$ for stable isotope probing (SIP) followed by community analysis.

Findings: The initial 16S rRNA gene amplicon sequencing analysis confirmed our expectations regarding prokaryotic diversity and community changes with increasing elevation and corresponding aridity. Here, a strong positive correlation (r (0-1 cm) = -0.789*** and r (1-10 cm) = -0.627***) to the decreasing bacterial alpha diversity was detected. Likewise, elevation/aridity was the main factor significantly affecting

prokaryotic community composition (PERMANOVA: $R^2 = 0.31$; $p = 0.001$), followed by soil depth (PERMANOVA: $R^2 = 0.04$; $p = 0.002$). Increasing water availability induced detectable respiratory activity only in the least arid coastal Paposo region, while microbial activity remained carbon-limited and thus inactive at the drier sites. $H_2{}^{18}O$ addition and subsequent DNA-SIP analysis revealed that, members of the *Actinobacteriota*, *Bacteroidota* and *Proteobacteria* were the most responsive bacterial taxa being activated. Under the highly limited conditions of 10% WHC, oligotrophic and halotolerant *Actinobacteriota* and *Proteobacteria* were the most responsive, whereas a shift towards more heterotrophic and copiotrophic *Proteobacteria* and *Bacteroidetes* was detected with increasing WHC to 50%.

Conclusions: Our findings indicate that the soil microbiota in arid and hyperarid desert soil is in general largely metabolically inactive. Soil wetting can trigger the activation of specific microbial taxa in the arid, but not in hyperarid regions of Atacama Desert due to carbon limitation. Low levels of available water do not yet reactivate the total microbial community but very selectively well-adapted taxa.

Abstract ID: 3258 / Poster Number: 407.17P

Selective bioelectrocatalytic synthesis of 25-hydroxyvitamin D3 employing steroid C25 dehydrogenase

Presenting author: Mr. Dennis Kosian, University of Freiburg, Freiburg im Breisgau, Germany

Co-authors: *Niklas Ortlieb, Max Willistein, Ralf Wessbecher, Johanna Schell, Anna Fischer, Matthias Boll*

Vitamin D_3 deficiency is a growing global health problem, affecting calcium and phosphate homeostasis, and is associated with numerous skeletal as well as acute and chronic diseases[1]. Studies have shown that the circulating 25-OH form of vitamin D_3 (calcidiol) is more efficient as a supplement compared to vitamin D_3 preparations[2]. However, currently available methods for chemical and enzymatic calcidiol synthesis suffer from low selectivities and formation of unwanted by-products[3], highlighting the need for novel synthesis strategies.

Here, we present studies on the molybdenum-dependent steroid C25 dehydrogenase 1 ($S25DH_1$) from denitrifying *Sterolibacterium denitrificans* Chol-1S that hydroxylates steroids at C25, including the secosteroid vitamin D_3, with water as co-substrate. Using the betaproteobacterium *Thauera aromatica* K172 as heterologous production host, we demonstrated the highly selective whole cell-based formation of calcidiol with product titers up to 1.85 g L^{-1} (4.6 mM) within 50 h[4]. In addition to $S25DH_1$-producing *T. aromatica* cells and vitamin D_3 as substrate, the reaction requires cyclodextrin, which acts as a solubiliser for the water-insoluble substrate, and an electron acceptor such as inorganic potassium ferricyanide. To further optimize this system, we opted to electrochemically regenerate the electron-accepting ferricyanide. By modifying the growth medium for the cultivation of $S25DH_1$-producing *T. aromatica* cells, we achieved biofilm growth directly on the electrode. Using these biofilms grown on electrodes, we showed highly efficient mediated electron transfer during $S25DH_1$-catalyzed 25-OH-vitamin D_3 synthesis, with up to 200 µA cm^{-2} current density output.

Abstract ID: 2452 / Poster Number: 138.15P

Selenium and Cysteine Synergy: Enhancing methane production rates in hyperthermophilic methanogens

Presenting author: Dr. Rubens P. Calegari, Aarhus University, Aarhus, Denmark; The Novo Nordisk Foundation CO2 Research Center – CORC, Aarhus, Denmark

Co-authors: *Merijn Schutgens, Lars D. M. Ottosen, Michael V. W. Kofoed*

The growing need to reduce greenhouse gas emissions has driven interest in CO_2-based fuels as a sustainable energy solution. Methane production through biomethanation represents a promising approach for converting CO_2 and H_2 into renewable methane, supporting carbon recycling efforts. Hyperthermophilic methanogens are particularly appealing for this process due to their fast kinetics and ability to thrive under extreme conditions, making them highly effective catalysts. This study optimised selenium and cysteine concentrations to enhance methane production in five species: *Methanocaldococcus lauensis*, *Methanothermus fervidus*, *Methanocaldococcus bathoardescens*, *Methanotorris igneus*, and *Methanocaldococcus jannaschii*. Incubations under selenium (0.46 nM to 61.8 µM) and cysteine (0.69 mM to 11.35 mM) were used to form kinetic models predicting methane production rate increases ranging from 50.9% to 131.1%, with optimal selenium concentrations of 329–452 nM and cysteine concentrations of 1.00–2.49 mM. Experimental validation demonstrated methane production rate improvements of 33.9% for *M. bathoardescens* (from 0.70 ± 0.02 to 0.94 ± 0.01 mmol CH_4 h^{-1}) and 19.3% for *M. jannaschii* (from 0.75 ± 0.02 to 0.90 ± 0.01 mmol CH_4 h^{-1}). Analysis showed no adverse effects from optimised media, with *M. jannaschii* achieving higher peak cell counts under model conditions (6.0×10^8 ml^{-1}) compared to DSMZ conditions (4.0×10^8 ml^{-1}), and *M. bathoardescens* showing comparable peak counts (~2.0×10^8 ml^{-1}). These findings underscore the importance of selenium and cysteine in regulating methanogenesis and demonstrate the potential of hyperthermophilic methanogens as efficient catalysts for CO_2-to-methane conversion.

Abstract ID: 601 / Poster Number: 071.17P

Self-administration of antibiotics among senior undergraduate students in faculties of pharmacy in Jordan: A cross-sectional study

Presenting author: Prof. Doc. Reham Saleem Aljalamdeh, Philadelphia University, Amman/jordan, Jordan

Co-authors: *Muna Barakat, Drear Balawi, Rami Musleh*

Introduction: The practice of self-medication with antibiotics is growing globally, particularly in developing countries. Such practice is associated with increasing health related problems, importantly, antibiotic resistance. This health issue has consequences that affect morbidity and mortality rate.

Objectives: A survey was conducted to investigate knowledge, attitude, and practice of self-medication with antibiotics among senior undergraduate pharmacy students.

Method: a cross-sectional study was performed using a validated questionnaire to collect data. The questionnaire was randomly distributed online to senior undergraduate pharmacy students from different universities. The knowledge, attitude, and practice of self-medication with antibiotics were assessed. Data analysis was performed using SPSS

Findings: 60.0% of senior undergraduate students reported self-medication with antibiotics, the most frequently used antibiotics for self-medication is amoxicillin 53.8%, ciprofloxacin 17.5%, and metronidazole 13.8% during the last 6 months. Respiratory health issues were the most common for self-medication with antibiotics with tonsilitis 38.8%, followed by common colds 26.3%. The decision for self-medication was based on previous prescription for similar disease and succeful treatment in the past.

Conclusion: The prevalence of self-medication with antibiotics among senior undergraduate pharmacy students is high. The practice of self-medication with antibiotics is alarming and increasing awareness of students is required to decrease health consequences of self-administration with antibiotics such as antibiotic resistance.

Abstract ID: 1328 / Poster Number: 298.16S

Self-produced biofertilizer for staple crops in Sri Lanka: rhizosphere transplant from the weed Panicum sp. promotes the growth and modulate chili and rice microbiome

Presenting author: Prof. Sara Borin, University of Milan, Department of Food, Environmental and Nutritional Sciences, Milano, Italy

Co-authors: *Lorenzo Vergani, Mekhala Chandrasekara, Chathurika Wanninayake, Francesca Mapelli, Sanath Hettiarachi*

Climate change and interlinked soil degradation impact crop yields and threaten food security, with major effects on smallholder farmers in the Global South, that urge the need for agricultural practices counteracting the fertilizer costs and maintaining human and ecosystem health. This work focused on the evaluation of a low-tech biofertilizer locally self-produced by rural farmers, based on rhizosphere transplant from the weed *Panicum* sp. (Guinea grass). The hypothesis is that the microbiome associated to this highly invasive species in Sri Lanka has the potential to confer resilience and stress tolerance to the host, benefits that could be transferred to crops cultivated in the same area, minimizing the limitations of inoculant establishment given by the change in soil type and environmental conditions.

This work aimed at evaluating in field conditions the efficacy of microbiome transplant from Guinea grass to staple crops, chili and two local rice varieties. Besides effects on plant productivity, we aimed at evaluating the impact of the transplanted microbial community, invaders of the crop microbiome, on the rhizosphere bacterial and fungal communities.

Root wash (RW) and arbuscular mycorrhizae inocula from *Panicum* sp. were locally prepared and supplied by farmers in several field trials to different crops such as chili and rice var. suwadel and var. kuruluthuda, with decreasing doses of chemical fertilizer. Crop productivity was recorded at the end of plant cycle and amplicon Illumina sequencing of bacterial 16S rRNA and fungal ITS was applied to monitor the effect of transplant on rhizosphere microbiome at different plant growth stages.

Both the biofertilization treatments significantly improved productivity on chili and one of the rice varieties, suwadel. Transplants supplied with 50% of the optimal fertilizer dose improved yields comparable to 100% chemical fertilization, indicating the potential for

this biofertilizer to halve the agrochemical input needed. The microbiome transplant modulated the overall rhizosphere microbial communities, which showed a different structure from the non-treated plants, especially at the flowering stage. Taxa present in RW and significantly differentially enriched in the treated plant rhizosphere were identified, demonstrating that the *Panicum* sp. microbiome was able at some extent to colonize crops.

Overall, this experimentation allowed to test in field conditions the feasibility of the rhizosphere transplant-based biofertilizer and described the impact of the inoculant on the crop rhizosphere community, together with raising awareness on the microbiome potential in sustainable agriculture intensification.

Abstract ID: 1246 / Poster Number: 198.15P

Semisynthetic microbial consortia for the study of bioleaching community assembly under controlled conditions

Presenting author: Camila Rojas-Villalobos, PhD, Universidad San Sebastián, Facultad de Ingeniería, Arquitectura y Diseño, Santiago, Chile; CCTE Ciencia y Vida, Fundación Ciencia y Vida, Santiago, Chile

Co-authors: *Fernando Díaz-González, Sofía Reyes-Impellizzeri, Abraham Zapata-Araya, Celia Larrea-Duharte, Catalina López-Kleim, Yasna Gallardo, Hector Carrasco, Matias Castro, Francisco Issotta, Simón Beard, Raquel Quatrini*

The Agrio River (RA) is a volcanic-glacial and acidic Andean watercourse exhibiting a strong physicochemical gradient that shapes distinct microbial communities of applied interest. To investigate community assembly processes relevant to biomining and bioremediation, and elucidate their rules and dynamics, we established semi-synthetic microbial consortia from two geographically separated sites along RA. Environmental water and sediment samples were used to inoculate a 2x2 factorial experiment, varying culture medium (cell-free RA water vs. saline basal medium) and supplementation (with vs. without RA sediments). Ferrous sulfate and elemental sulfur provided a mixed energy source, and pH and temperature were set at 2.5 and 25.

Over one year, periodic sampling enabled biomass quantification, physicochemical monitoring, and 16S rRNA amplicon profiling. While natural samples displayed high alpha diversity (Shannon: 7.9-10.8), experimental consortia exhibited lower diversity (3.3-4.2), indicative of community selection and stabilization. Beta diversity (Bray-Curtis) confirmed substantial differentiation from natural sites (0.84-0.99) and strong similarity among experimental conditions (0.08-0.17), despite differences in their setup. The experimental consortia exhibited a significant divergence from natural samples, converging into a stable microbial community dominated by *Acidiphilium* (35-41%), *Metallibacterium* (20-24%), *Acidocella* (10-16%), *Acidithiobacillus* (8-13%), and *Thiomonas* (0.7-1%)—taxa commonly involved in sulfur, iron and nitrogen cycling at low pH.

These findings demonstrate that controlled incubations can recreate key AMD-associated microbial consortia, offering a platform to study bioleaching dynamics. Future work will explore their responses to global and directed perturbations using native and synthetic vesicles, and ecological interventions, contributing to applied and fundamental research in microbial ecology and biomining.

Abstract ID: 1076

Sex-based differences in prenatal innate immunity: divergent foetal responses to Mycoplasma hominis infection between males and females

Presenting author: Valentina Margarita, PhD, University of Sassari, Sassari, Italy

Co-authors: *Valeria Lodde, Laura Doro, Paola Rappelli, Pier Luigi Fiori, Ilaria Campesi*

Introduction: The sex differences affect the inflammatory response in the adult, with more active

immune responses in women than in men linked with higher pro-inflammatory cytokines secretion [1]. Although these biological differences in susceptibility to infections are also reported in early life [2], there is limited data describing the sex-specific foetal immuno-response to intra-amniotic infections.

Objectives: This study aimed to investigate the response of cells from the amniotic fluid (AFC) of male and female foetuses in mid-pregnancy upon infection with *Mycoplasma hominis, a* pathogen involved in adverse pregnancy events [3].

Method: We designed an experimental model to investigate sex-specific innate immune responses by infecting male and female AFCs with a clinical isolate of M. hominis. In this model, we evaluated the gene and protein expression levels of IL-1β, IL-6, IL-8, and TNF-α, the key pro-inflammatory cytokines implicated in preterm labour. Additionally, we compared the gene and protein expression of TLR-2 and TLR-4, the production of nitrites, and the levels of selected miRNAs in male and female AFCs infected by mycoplasmas.

Findings: The gene expression of *IL-1β, IL-6, IL-8, TNF-α,* and *TLR2* were higher in infected females AFCs*,* while only *IL-6, IL-8* and *TLR4* expression were up-regulated in infected males. Protein levels of IL-6, TNF-α and TLR2 were up-regulated exclusively in infected females (Figure1). Furthermore, infected female AFCs produced higher levels of nitrites. miRNAs expression revealed an up-regulation of miR-29a-3p in infected females, and miR-223-3p in infected males, with miR-29b-3p showing up-regulation in both sexes upon infection.

Conclusion: Our results showed that female foetuses exhibited greater stimulated cytokines production during the mid-trimester of pregnancy than male foetuses, suggesting that differential inflammatory responses could affect maternal health and foetal development, predisposing to sex-specific foetal programming. The foetal sex

could help to diagnose risk predictions of pregnancy outcomes and prevent future disease in adulthood.

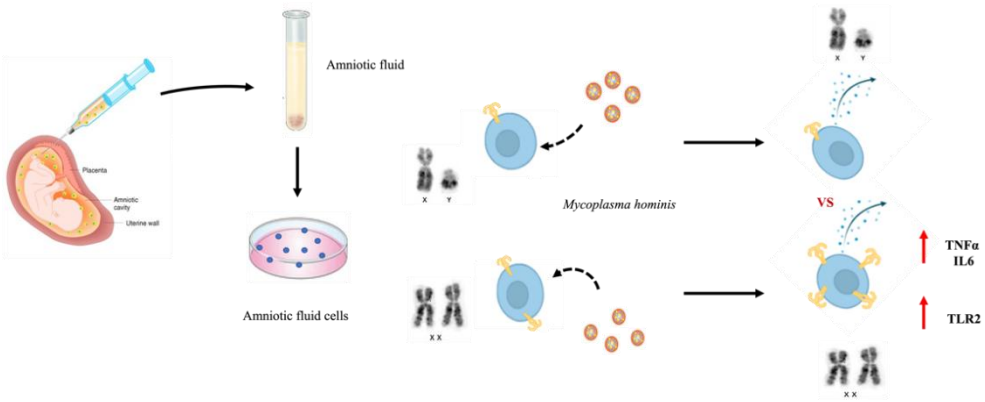


Figure 1: Male and female amniotic fluid cells were infected with a clinical isolate of *M. hominis* to explore the potential impact of sex-specific innate immune responses before birth.

Abstract ID: 2950 / Poster Number: 075.16P

Sex-specific signature in the gut microbiota of children with cow's milk allergy

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Introduction: Medical advances highlight the importance of considering sex/gender in the treatment of many diseases. Women typically exhibit a stronger immune response to vaccines and microbial infections. However, the extent to which immune response differences affect immune diseases is little explored, despite epidemiological data indicating differences in prevalence and prognostics among women and men. One example is cow's milk allergy (CMA), a disease caused by abnormal immune responses against proteins found in cow's milk. Changes in the gut microbiota of children with CMA have been observed, but whether these changes are the same in boys and girls remains unexplored.

Objectives: We looked for a sex-specific signature in the gut microbiota of CMA children, and asked whether a family history of allergies affects the children's microbiota differently according to sex.

Methods: 16S rRNA amplicon sequencing was applied to characterize the microbiome of 14 milk-allergic children, 18 children who developed oral tolerance to milk proteins, and 36 healthy controls. PERMANOVA and differential abundance analyses using ANCOM were employed to evaluate differences in overall microbiome composition and specific taxon abundances.

Findings: We detected significant differences in the gut microbiota between boys and girls with CMA. *Intestinimonas*, *Hungatella* and *Anaerotruncus* increased in abundance in girls with CMA but not in boys. Moreover, girls, but not boys, had a characteristic gut microbiota when they had a family history of allergies even if they were healthy.

Conclusions: These results open the window to developing personalized microbiome-targeted treatments for children with CMA based on their sex.

Abstract ID: 1289 / Poster Number: 427.16P

Shared Laboratory Platform to Drive Innovation in Biological Products Industries

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The Instituto Biológico (IB), with 97 years of history, is part of the Research Institutes network linked to the Secretariat of Agriculture of the State of São Paulo (Brazil). The IB stands out for its modern infrastructure and qualified team, conducting research on diseases and producing and supplying immunobiologicals for health programs, such as antigens for brucellosis and tuberculosis diagnosis. Brazil has approximately 100 veterinary industries, 30 of which are dedicated to vaccine production, mainly for cattle, making the country self-sufficient and the largest global producer. However, the United States and Europe lead in technological diversity and innovation. The global veterinary vaccine market, valued at USD 18.18 billion in 2024, is expected to reach USD 26.87 billion by 2029. To promote innovation and public-private partnerships, the IB created LABinov, a facility spanning nearly 500 m^2 with two biosafety level 3 laboratories (BSL-3). This space enables simultaneous projects with advanced infrastructure and a multidisciplinary team. In less than two years, research on bovine viral vaccines is already underway, and LABinov has a waiting list for its use. These partnerships benefit the market with high-quality solutions while generating resources for IB to maintain its structure, always focused on innovation.Despite its leadership in local production, Brazil still faces challenges compared to developed countries. This sustainable model reduces dependence on imported inputs, stimulates technological innovation, and offers more reliable products to the population.

Abstract ID: 1248 / Poster Number: 199.15P

Shedding light on the metabolic pathway of nitrate-reducing Fe(II)-oxidation in *Ferrigenium straubiae*

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A significant knowledge gap remains in the understanding of nitrate-reducing Fe(II)-oxidizing (NRFeOx) pathways. While heterotrophic, mixotrophic, and autotrophic NRFeOx bacteria have been described, the metabolic advantages and mechanisms of this process remain unclear. Here we present our most recent findings on autotrophic NRFeOX by investigating *Ferrigenium straubiae* the dominant (98%) member of a well-studied autotrophic NRFeOx culture (culture KS). We isolated *Ferrigenium straubiae*, allowing a detailed study of its physiology, which revealed that the pure culture could not sustain the capability of NRFeOx. Metagenomic and transcriptomic analyses comparing pure culture *F. straubiae* grown under microaerophilic Fe(II)-oxidizing conditions with in community grown *F. straubiae* in iron(II)-/nitrate-containing medium revealed previously overlooked proteins of unknown function encoded in the *F. straubiae* genome that may play a role in NRFeOx. Using in silico tools such as AlphaFold, HHpred, Foldseek, and ProFunc, we performed structural and functional predictions and placed these proteins into a novel NRFeOx pathway model.

By identifying *F. straubiae* genes that are highly expressed under nitrate-reducing Fe(II)-oxidizing conditions, this work provides a foundation for advancing the understanding of autotrophic NRFeOx. Functional studies of key *F. straubiae* proteins, particularly those involved in electron transfer from Fe(II), will further elucidate their roles in NRFeOx.

Abstract ID: 1157 / Poster Number: 217.15P

Shiga Toxin 2 Induces S-phase Cell Cycle Arrest and Aggravates Cell Death Independent of ER Stress under High Salt Conditions

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Shiga toxin 2 (Stx2), a main virulence factor produced by Shiga toxin-producing *Escherichia coli*, (STEC), is implicated in severe conditions such as hemolytic uremic syndrome (HUS) and acute renal failure. Given the kidney's unique environment, which involves exposure to high salt concentrations during filtration, the interplay between Stx2 and high salt conditions remains poorly understood. This study investigates the effects of Stx2 under hyperosmolar conditions using the human kidney epithelial cell line HK-2 treated with NaCl and Stx2. Our findings reveal that Stx2 induces greater cell death and inflammation under high NaCl conditions compared to normal media. Interestingly, ER stress markers were not significantly accumulated in the NaCl+Stx2 group relative to the PBS+Stx2 group. Furthermore, high salt conditions appeared to mitigate Stx2-induced mitochondrial damage and reactive oxygen species (ROS) generation. High NaCl exposure also upregulated heat shock protein 70 (HSP70), which suppressed Stx2-mediated ER stress. Pre-treatment with HSP70 inhibitors exacerbated ER stress and cell death in the NaCl+Stx2 group, highlighting HSP70's protective role under hyperosmolar conditions. Additionally, Stx2 treatment in high salt conditions induced caspase-independent cell death, as evidenced by reduced lactate dehydrogenase release upon pan-caspase inhibition, and aggravated S-phase cell cycle arrest. These suggest that Stx2's cytotoxicity under high salt conditions is driven by mechanisms distinct from ER stress. In conclusion, this study provides new insights into the pathogenesis of Stx2-mediated renal damage, emphasizing the role of high salt conditions in modulating toxin effects. Understanding the interaction between salt homeostasis and Stx2 may inform novel interventions for HUS patients.

Abstract ID: 2741 / Poster Number: 142.17P

Signature gene based targeted genome mining approach

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The Actinomycetota (or Actinobacteria) is a great source of natural products with diverse chemical structures and variety of biological activities. However, the classic (Waksman) approach used in antibiotics discovery has exhausted itself leading to frequent rediscovery of most abundant compounds. On the other hand, genes responsible for biosynthesis, regulation and transport of bacterial natural products are arranged into biosynthetic gene clusters (BGCs). These clusters often contain signature genes encoding for unique features like antibiotic resistance or unusual enzymatic reactions. These signature genes comprise a great possibility for preselection of natural products with specific features or activities.

In this study we applied a signature gene directed genome mining for screening for potentially valuable BGCs within the actinobacterial genomes. For this the antiSMASH-resulted BGC database was created using all publicly accessible actinobacterial genomes. This database when combined with the BLAST search engine (AntismashBLAST tool) gives a possibility to find BGCs with desired signature genes. When using DNA gyrase, isoleucyl tRNA synthetase and proteasome subunit Beta genes as queries, the NRPS BGC from Streptomyces sp. DSM 41529, the trans-AT PKS BGC from Nocardia puris DSM 44599 and the NRPS-T1PKS BGC from Streptomyces sparsogenes DSM 40356 were selected, respectively. These BGCs do not have neither compounds assigned nor known counterparts in Actinobacterial genomes.

The selected BGC were cloned with a transformation-associated recombination (TAR) approach applying K1 yeast killer toxin as a counterselectable marker. The cloned BGCs will be expressed in Streptomyces host strains and tested for production of compounds with specific biological activities and molecular targets predicted from the query gene used for the BGC selection.

Abstract ID: 1086 / Poster Number: 083.17P

Silent Carriers: How Medical Devices Harbor Methicillin-Resistant Staphylococcus aureus

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Introduction: Healthcare-associated infections (HAIs) are significant challenges in neonatal and pediatric units. Methicillin-resistant *Staphylococcus aureus* (MRSA), characterized by its resistance to β-lactam antibiotics and virulence factors like Panton-Valentine leukocidin (PVL), poses severe risks in these settings. Medical equipment, often shared among patients, can act as reservoirs for MRSA transmission.

Objective: To investigate the prevalence of MRSA in medical equipment from neonatal and pediatric units, assess resistance profiles, and identify biofilm production and virulence factors.

Method: Eighty bacterial samples were collected from stethoscopes, sphygmomanometers, and pulse oximeters in a hospital in São Paulo, Brazil. Genotypic and phenotypic analyses were performed to detect MRSA, SCC*mec* types, and PVL. Biofilm production was evaluated using the crystal violet method. Antimicrobial susceptibility was assessed via disk diffusion.

Findings: Out of 63 isolates, 19 (30.1%) were identified as *S. aureus*, and 17 (26.9%) were MRSA. PVL was detected in 57.8% of MRSA strains. SCCmec types IV and V, linked to community-associated strains, were prevalent, while hospital-associated types I–III comprised 29.4%. High resistance was observed for penicillin (94.7%) and doxycycline (89.5%), with linezolid showing 100% sensitivity. Biofilm production was non-adherent in 64% of isolates, weakly adherent in 25%, and strongly adherent in 11%.

Conclusion: The study highlights the role of medical equipment in MRSA transmission in neonatal and pediatric units, emphasizing the need for rigorous hygiene protocols, continuous resistance monitoring, and improved disinfection practices to mitigate HAIs.

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Abstract ID: 3607 / Poster Number: 296.15P

Silicon Bioavailability and Plant Growth Promotion by Rhizospheric Silicate Solubilizing Bacteria

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Silicate solubilizing bacteria (SSB) play a crucial role in enhancing plant growth and development, especially under challenging biotic and abiotic conditions, by facilitating the release of soluble silicon (Si) in form of silicic acid from insoluble silicate minerals in the soil. (Epstein 2009; Kang et al, 2017; Chopra, et al 2021).

This study was conducted to isolate, identify, and characterize SSB from the rice rhizosphere and identify their plant growth-promoting rhizobacteria (PGPR) activities. The isolated strains were screened for their ability to solubilize silicate minerals through qualitative and quantitative assays. Identification of the strains was carried out using MALDI-TOF and by determining the analytical profile index. The selected strains were further identified based on 16S rRNA gene sequencing and phylogenetic analysis. The strains were identified as R1 *Pseudomonas putida*, R14 *Enterobacter asburiae*, R17 *Klebsiella variicolla*, and R27 *Enterobacter cloacae*.

The isolates showed a high silica solubilization index. Wheat (*MACS6478*) plants grown from seeds in a hydroponic system treated with SSB strains displayed significantly higher germination rates, as well as significant differences in shoot length, root length, shoot girth, and chlorophyll content compared to non-treated control wheat plants. All selected isolates were able to successfully colonize wheat roots. The isolates were also capable of nitrogen fixation, starch solubilization, and indole acetic acid production. These bacterial inoculants, displaying multiple traits beneficial to host plants, can be perfect candidates in the development of secure and effective treatments as an alternative to chemical fertilizers.

Abstract ID: 2613 / Poster Number: 358.16P

Single-mutant variants of QrcABCD provide new insights into its energy conservation mechanism

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Co-authors: *Américo G. Duarte, Inês Cardoso Pereira*

Sulfate-reducing prokaryotes are found in a multitude of anoxic environments, where they play a pivotal role in both the carbon and sulfur cycles. These microorganisms can couple the oxidation of hydrogen or carbon compounds with the dissimilatory reduction of sulfate or sulfite, using highly conserved respiratory membrane complexes and enzymes. In *Nitratidesulfovibrio vulgaris* Hildenborough (*Dv*H), the Quinone-reductase complex (QrcABCD) is an electrogenic respiratory membrane complex that mediates electron transfer from periplasmic hydrogen and formate oxidation to the reduction of the menaquinone pool in the membrane, which will be further used for sulfate/sulfite reduction in the cytoplasm. To better understand the physiologic role and bioenergetic mechanism of QrcABCD in sulfate reducers, a *Nv*H knock-out strain lacking the qrcABCD genes was constructed and a homologous overexpression system for QrcABCD was developed. Phenotypic studies of the strain expressing recombinant QrcABCD and variants, under different growth conditions, show that QrcABCD is essential for hydrogen or formate oxidation under sulfate but not sulfite respiration, and that some of the single point mutations in conserved aminoacids totally impair growth. Additionally, kinetic experiments with QrcABCD and variants reconstituted in liposomes containing menaquinone-4, with its physiologic electron donor – Type I cytochrome c_3, confirm that some of the targeted aminoacids are crucial for quinone-binding/proton uptake. This work provides new insights in Qrc molecular mechanism and enables further understanding of its role in sulfate reduction, and of homologous complexes in different bioenergetic contexts.

Abstract ID: 619 / Poster Number: 269.17P

Single-particle quantification of SARS-CoV-2 virus-like particles using flow virometry

Presenting author: Dr. Yong-Tae Jung, Dankook University, Cheonan-si, South Korea

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SARS-CoV-2 virus-like particles (VLPs) were generated by co-transfecting HEK 293T cells with four plasmids, the spike (S) and nucleocapsid (N) plasmids, each engineered to encode a 3xHA tag at the C-terminus, in conjunction with membrane(M) and envelope (E) plasmids. Co-localization of the S and N proteins within the VLPs was confirmed by Western blot analysis using anti-HA antibodies. To enable fluorescent quantification of VLPs, the S protein's C-terminus was fused with enhanced green fluorescent protein (EGFP), and the N protein was tagged with EGFP at either the N- or C-terminus. Transient transfection of 293T cells with(S-GFP)NME, S(N-GFP)ME, or S(GFP-N)ME plasmids efficiently produced fluorescent VLPs, each demonstrating infectivity in A549-hACE2 cells. Additionally, a two-plasmid system was developed to simplify fluorescent VLP production by incorporating internal ribosomal entry site elements between N3xHA and S-GFP, and between the M and E genes. Transfecting 293T cells with these plasmids produced VLPs with a fourfold higher N protein concentration (800n/mL) compared to the four-plasmid system (200ng/mL), as measured by ELISA. Flow virometry analysis confirmed VLP production with an average diameter of 80 nm and a concentration of 1.68 x 10^8 particles/ml. This study demonstrates efficient fluorescent VLP production using a two-plasmid system, compared to the traditional four-plasmid approach. Additionally, we validated flow virometry as a robust method for single-particle analysis of fluorescent VLP size and concentration.

Abstract ID: 3243 / Poster Number: 173.17P

Six-Year Genomic Surveillance of carbapenem-resistant Klebsiella pneumoniae in a Tertiary Hospital

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Since 2005, our hospital has monitored multidrug-resistant organisms (MDROs) to track trends and detect nosocomial transmissions. Among MDROs, carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) has become a major public health concern. To investigate KP evolution, we sequenced 817 strains from 614 patients (2019–2024) using Whole Genome Sequencing (WGS) on the Illumina® platform. Ridom SeqSphere+ was used to construct transmission trees and identify strain characteristics, including Sequence Type (ST) and resistome, while virulome analysis was performed with Kleborate.

The predominant STs identified were ST307, ST101, ST512, ST147, ST395, and the emerging ST6668. ST6668, part of clonal complex 147 and associated with blaNDM-1, has become prevalent since 2022, causing 9 clusters with 67 patients. This increase coincided with a multi-hospital outbreak in Pavia (February–August 2023). Resistance analysis revealed KPC-3 (64%) as the dominant variant, followed by KPC-2 (34%). The prevalence of NDM-1 increased significantly between 2022 and 2024 (82% of NDM), driven by ST6668.

Virulence analysis ranked ST395, ST101 and ST147 as the most virulent, with scores of 3.7, 2.3 and 1.9, respectively. Interestingly, ST395 is characterized by 87% of its strains having a virulence score of 4, while the other STs display greater heterogeneity. Notably, 29% of ST147 strains exhibit a high virulence score of 4, which is absent in ST6668 strains.

WGS enables real-time monitoring of nosocomial strains, optimizing therapy and identifying emerging threats. Ongoing virulome profiling may further improve outbreak management and microbial control strategies.

Abstract ID: 3336 / Poster Number: 107.16P

Skin and water microbiome investigation to uncover the relation between a Midichloria-like organism and the disease red mark syndrome in rainbow trout, Oncorhynchus mykiss

Presenting author: Ms. Giulia Zarantonello, National Institute for Aquatic Resources, DTU Aqua, Copenhagen, Denmark

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Red mark syndrome (RMS) is an infectious disease affecting rainbow trout that manifests through hyperaemic skin lesions. The disease has been linked to a *Midichloria*-like organism (MLO) of which only the 16S rRNA gene sequence is known. All attempts to isolate and culture this microorganism have been unsuccessful. A clear association between MLO and RMS has thus not been established – at least not by traditional Koch's postulate standards. Here, we used a cohabitation infection model of RMS to follow the development in the microbial community of water and skin over the course of infection and development and healing of RMS lesions. Sampling days (day post-cohabitation, DPC) were set up to represent different phases of the disease: start of cohabitation (DPC 0), pre-clinical stage (DPC 35), early lesions (DPC 50), peak RMS (DPC 75), healing phase (DPC 95), and completely healed (DPC 115). On each sampling day, all fish were anaesthetized, and a gross RMS score was given to each fish. In addition, a subpopulation of cohabitants was euthanized and sampled. Lesion (if present) as well as non-lesion sites were sampled from the cohabitants. Short-read 16S rRNA sequences were recovered from skin and water samples and correlated with the RMS gross score. We found the microbiome of affected areas differed markedly from the unaffected skin samples, with MLO being among the dominating features. Importantly, the cohabitation model provided a temporal perspective to better understand the microbial dynamics underlying disease, from development to healing.

Abstract ID: 2218 / Poster Number: 162.15P

Sludge retention time impact during traditional activated sludge wastewater treatment on the fate of antibiotic-resistant bacteria and associated genes

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Conventional activated sludge (CAS) is the most widely used technology for secondary treatment of municipal wastewater. Although the CAS process reduces total bacterial abundance, high loads of antibiotic resistant bacteria and associated genes (ARB&ARGs) remain in the effluent. Sludge retention time (SRT) is one of the main operating variables of CAS that is expected to affect bacterial survival and proliferation. Therefore, this study aimed to understand how SRT may influence the bacterial community and associated ARB&ARGs dynamics during CAS wastewater treatment.

To achieve this, two laboratory CAS units were operated continuously for 85 days in an urban wastewater treatment plant (UWWTP), one with a SRT of 7 days and the other with a SRT of 30 days. All other operating conditions were the same in both units. The abundance and prevalence of ARGs and the composition of the bacterial community were examined weekly in the final treated effluent and sludge. Additionally, chemical and biological oxygen demand, total phosphorus, nitrogen and suspended solids were measured once a week.

It is hypothesized that prolonged SRT may facilitate the adsorption of ARB&ARGs onto sludge, resulting on their removal from the final treated effluent. However, prolonged SRT may also create additional opportunities for horizontal gene transfer events, possibly leading to the spread of ARB&ARGs, which may be enhanced by prolonged exposure to high concentrations of organic and inorganic compounds.

Abstract ID: 841 / Poster Number: 379.15P

Small Packages, Big Messages: Extracellular Vesicles in Host-Symbiont Crosstalk

Presenting author: David Saiz-Martínez, PhD, University of Valencia (UV), Valencia, Spain; Institute for Integrative Systems Biology (I2SysBio), Paterna, Spain

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Introduction: Mutualistic symbioses are essential for evolutionary success, enabling hosts to adapt to ecological niches through intricate interactions with their symbionts. In *Blattella germanica*, the dual symbiotic system comprising *Blattabacterium cuenoti* and a diverse gut microbiota plays a critical role in maintaining host homeostasis and facilitating metabolic exchanges. While antimicrobial peptides and small RNAs are established mediators of symbiotic interactions, the role of extracellular vesicles (EVs) in host-symbiont communication remains poorly understood.

Objective: This study aims to investigate the potential role of EVs in mediating molecular communication between *B. germanica*, *Blattabacterium cuenoti*, and the gut microbiota by characterizing their protein and RNA cargo.

Method: EVs were isolated from the hemolymph of *B. germanica* using size exclusion chromatography and analyzed via LC-MS/MS and RNA sequencing. Bioinformatics tools were employed to cluster proteins and RNAs, identifying their potential functions and relevance to host-symbiont interactions.

Findings: Preliminary analyses suggest that EVs carry RNA and protein molecules potentially involved in regulating symbiont populations, modulating host gene expression, and facilitating interspecies communication. Specific proteins have been identified as potential markers for distinct EV subpopulations, indicating functional specialization.

Conclusion: These findings highlight EVs as key players in the molecular dialogues of symbiotic systems, offering novel insights into host-symbiont communication. This work lays the groundwork for future studies exploring the mechanisms of EV-mediated signaling in insect-bacteria symbioses and other similar systems.

Abstract ID: 951 / Poster Number: 355.17P

Smart biofertilizers and bacterial community profiles: from maturation to impact in the field

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Plant-based compost mixtures have proven to be a sustainable solution for improving plant growth and soil fertility. Our aim was to determine the dynamics of bacterial shifts by amplicon sequencing during the composting process in a 14-day period, to identify the bacteriobiota in the final product and to test the different formulations in the maize cropping system. We analyzed the maturation of plant-based compost (T1), with the addition of biochar after (T2) and during the composting process (T3). In addition, the same formulations were coated with bacterial formulations (Priestia megaterium and Glutamicibacter sp., T4-T6) and their effects on maize growth were investigated. T3 showed significant differences compared to T1 and T2. The T3 formulation was dominated by the taxa Hymenobacter, Pedobacter, Pseudomonas, Rhizobiaceae, Sphingobacterium and Sphingomonas only on the first day, while Dyadobacter and Methylobacterium were abundant in T1. Differential analysis revealed that Bacillus, Cellvibrio and Geobacillus as well as Ureibacillus, Thermobacillus, Thermoactinomycetaceae and Pusillibacter were significantly more abundant only in mature T3, while Acinetobacter, Isoptericola and Sphingobacterium were characteristic of T1 and T2. T3 showed higher values for C/N ratio, P2O5 and K2O compared to the other varieties. The field results showed that T3 and T6 produced the tallest maize plants, while T5 and T6 had a higher grain weight. In terms of yield results, T8 was ahead, followed by T2 and T6. The addition of biochar in combination with bacterial formulations resulted in different bacterial communities and improved plant growth, underlining its potential as an effective agricultural supplement.

Abstract ID: 2020 / Poster Number: 436.15P

Snapshots of stress induced nucleoid dynamics in Helicobacter pylori thanks to 4f-SAMMY-seq, a hi-C alternative to map chromosome accessibility

Presenting author: Eva Maria Pinatel, PhD, National Research Council, Milan, Italy

Co-authors: *Elena Chiti, Ugo Maria Iannacchero, Valentina Rosti, Andrea Vannini, Chiara Lanzuolo, Davide Roncarati*

Introduction: Four fraction Sequential Analysis of MacroMolecules Accessibility (4f-SAMMY-seq) is a new genome-wide, sequencing-based technique to map chromosome accessibility that, in *eukaria*, proved to be powerful as Hi-C without requiring restriction enzymes or crosslinking. Its application to bacteria can improve cross-species comparisons and foster the study of chromosome conformation toward the still unexplored field of nucleoid dynamics.

Objective: This study aims to adopt 4f-SAMMY-seq to investigate chromosome conformation dynamics in *H. pylori* comparing exponential growth and stress conditions (i.e. stationary-phase or iron supplement). We will then investigate the correlation between these dynamics, gene expression regulation and functional alterations integrating them with multiple –omic data.

Method: 4f-SAMMY-seq relies on the biochemical separation of DNA into sequential fractions with decreasing accessibility. Fractions are sequenced and a contact-based matrix can be derived to define chromosome domains using standard Hi-C analysis tools, while DNA accessibility alterations across conditions can be measured comparing the relative ratios of fraction pairs.

Findings: We obtained a fine map of bacterial chromosome accessibility with just 1500000 reads per sample. We defined the dynamic alterations of nucleoid structure among those conditions, revealing that stress increases and reshapes mainly less accessible DNA signals, and we are correlating these data to gene expression and/or transcription factor binding site annotations to further interpret our results.

Conclusion: We applied for the first time to bacteria 4f-SAMMY-seq technique, which is now available to investigate different prokaryotes species, and proved its ability to provide high-resolution chromosome accessibility maps to capture nucleoid dynamics avoiding major Hi-C bias.

Abstract ID: 2003 / Poster Number: 082.15P

Soil microbial communities responses along afforestation dynamic in two protected areas in North-East Italy

Presenting author: Speranza Claudia Panico, PhD, University of Udine, Udine, Italy

Co-authors: *Giorgio Alberti, Giovanni Luca Sciabbarrasi, Guido Incerti*

In this study, we examine the ecological dynamics in the topsoil following the natural afforestation of abandoned agricultural land, a spontaneous process that has been occurring for decades at both national and European levels. The main objectives are: i) to clarify how physico-chemical properties of the topsoil change along the successional gradient, ii) to provide an overview of the response of soil microbial communities along the same gradient, and iii) assess causal relationships between soil predictors and microbial responses, including community composition and diversity, as well as abundance of bacterial and fungal taxonomic groups. The study areas were located in the Foreste Casentinesi National Park and in the Julian Prealps (Italy). In both areas, we identified five successional stages, based on historical orthophotos (1954-2020), replicated across four chronosequences: grassland-pasture (G), shrubland (S), early (E), intermediate (I), and late afforestation (L). Replicated topsoil samples (0–10 cm) were analysed for pH, bulk density (BD), and organic carbon (OC), and total nitrogen (N) contents. Microbial communities were assessed from environmental DNA extracted by the fine soil fractions followed by DNA metabarcoding using ITS and 16S markers for fungi and bacteria, respectively. Results showed that as the succession progresses, soil acidification and a reduction in bulk density occur, coupled with an increase in soil organic matter at later stages in mature soils. However, such trends are quantitatively affected by site-specific variability. Bacterial and fungal communities responded differently to forest regrowth after grassland abandonment: fungi, mainly Ascomycota and Basidiomycota, exhibited greater specialisation in mature successional stages, while bacteria, dominated by Proteobacteria and Verrucomicrobiota, showed more site-specific traits. Comparisons between the two study areas highlighted a lower variability in microbial diversity in the Foreste Casentinesi National Park, likely due to its more homogeneous environmental conditions, including plant cover. Our study underlines the functional importance of soil biota in promoting and sustaining carbon storage in forest soils undergoing natural afforestation. On a broader scale, the study highlights the value of nature-based solutions such as rewilding for climate neutrality and biodiversity conservation.

Abstract ID: 3006 / Poster Number: 064.15P

Soil Microbial Communities responses to combined Chemical and abiotic environmental stressors

Presenting author: Dr. Gonzalo Tortella, UNIVERSIDAD DE LA FRONTERA, TEMUCO, Chile

Co-authors: *Ivette Fernandez, PAOLA FINCHEIRA, Sebastian Leiva, OLGA RUBILAR, Martin Fernandez-Baldo, MAURICIO SCHOEBITZ,*

Extreme environmental conditions triggered by global climate change, such as severe drought and salinity, combined with chemical contaminants, create complex scenarios that disrupt the composition and functionality of soil microbial communities. This study aimed to evaluate the combined effects of salinity drought and chemical contaminants such as copper nanoparticles and pesticides on the functionality and structure of soil microbial communities. Therefore, agricultural soil samples were subjected to salinity (low, medium, and high), severe drought conditions, and CuNPs at 1, 10, 100 mg/kg and carbendazim at 6 mg/kg during 90 d. During this period were evaluated acid phosphatase, β-glucosidase, dehydrogenase (DHA), FDA hydrolysis, urease, potential nitrification rates, amoA and amoB abundance genes and microbial structure were also evaluated by metagenomic analysis of the 16S rRNA gene. The results showed that acid phosphatase and FDA hydrolysis significantly decreased under severe drought and high salinity, particularly at 100 mg/kg CuNPs. β-glucosidase remained resilient under moderate salinity with low CuNP concentrations, suggesting cooperative microbial interactions; dehydrogenase activity (DHA) recovered at low CuNP concentrations. qPCR analysis revealed that AOA communities maintained ammonium oxidation activity under severe stress, whereas AOB abundance significantly declined. We concluded that the impacts of drought and salinity were more pronounced than those of chemical contaminants, indicating that climatic factors dominate microbial dynamics under stress conditions. This highlights the need to consider the synergistic effects of emerging contaminants and climatic stressors on soil ecosystems.

Abstract ID: 3219 / Poster Number: 028.15P

Soil microbial dynamics and greenhouse gas exchange in Greenlandic glacial outwash plains

Presenting author: Ms. Grace Marsh, École Polytechnique Fédérale de Lausanne, Sion, Switzerland

Co-authors: *Ianina Altshuler*

Accelerated glacial retreat in Greenland drives increased development of glacial outwash plains, the climatic impact of which remains largely unknown. Receding glaciers uncover outwash plains of nutrient poor sediment where succession occurs spatially, creating a deglaciated chronosequence. These chronosequences are useful for studying soil development by microbes, which generate organic carbon and nitrogen pools that are essential for the growth of higher plants. Importantly, the effects of microbial colonisation, taxonomic diversity, succession, and functional potential of active taxa on greenhouse gas (GHG) fluxes in outwash plains is not well understood.

We investigated soil GHG fluxes and the associated microbiome at two glacial outwash plains in Greenland, Russell Glacier (Kangerlussuaq) and Kiattuut Sermiat (Narsarsuaq). Soil samples for genetic and physicochemical analysis were collected at vegetated, non-vegetated and water submerged sites across both outwash plains. Microbial community structure and functional potential were characterised through metagenomic analysis of over 100 samples. Further, paired GHG flux measurements were recorded at each site using a static flux chamber with carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) gas analysers. We report a general trend of CH_4 uptake (-2.54 to 0.44 nmol CH_4 m^{-2} s^{-1}) and CO_2 production (-0.19 to 3.30 µmol CO_2 m^{-2} s^{-1}) across this environment at both studied locations. While we identified minimal N_2O flux (-0.05 to 0.03 nmol N_2O m^{-2} s^{-1}).

Overall, we characterise the role of microbial communities in the biogeochemical processes of Greenlandic glacial outwash plains and describe the greater climatic impact of these rapidly developing environments.

Abstract ID: 2224 / Poster Number: 013.15P

Spatiotemporal dynamics of ammonia- and nitrite-oxidizing prokaryotes in stratified freshwater lakes

Presenting author: Dr. Albin Alfreider, University of Innsbruck, Innsbruck, Austria

Introduction: Nitrifying prokaryotes play a critical role in the nitrogen cycle of aquatic ecosystems, however, in freshwater lakes our understanding of the environmental factors shaping their diversity and distribution is still limited.

Objectives: This study investigates the depth-dependent and temporal distribution of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and anaerobic ammonium-oxidizing bacteria (AnAOB) in stratified lakes, with a focus on the interactions among these groups and identifying the environmental factors shaping their ecological niches.

Method: Water samples were collected along depth profiles in stratified lakes and analyzed by sequencing rRNA and functional genes using DNA/RNA extracts. Droplet digital PCR was used to quantify selected taxonomic groups of nitrifiers and AnAOB. The investigation strategy included the determination of a wide array of environmental parameters to characterize variations between samples and study sites.

Findings: Distinct depth-related patterns in nitrifying communities were observed, with *Ca*. Nitrosopumilus and *Nitrospira* dominating the oxygenated hypolimnion of large lakes. *Nitrosospira* and *Ca*. Nitrotoga were prevalent in smaller lakes. Comammox *Nitrospira* were found in low abundance in the water column of lakes. However, their numbers were significantly higher at the sediment-water interface, indicating a preference for a particle-attached lifestyle. AnAOB were primarily detected in the deep hypolimnion, their occurrence closely correlating with ammonium and oxygen gradients. In conclusion, these findings provide new insights into the ecological roles and niche differentiation of ammonia- and nitrite-oxidizing prokaryotes in freshwater lakes.

Abstract ID: 2904

Specific community compositional 'fingerprints' accurately predict soil microbial Biodiversity Ecosystem Function relationships

Presenting author: Dr. Damien Finn, Thünen Institut für Biodiversität, Braunschweig, Germany

Co-authors: *Julia Schroeder, Christopher Poeplau, Christoph Tebbe*

Introduction: The theory underlying microbial Biodiversity Ecosystem Function (BEF) relationships seeks to link the ecology of microorganisms with their functionality. Two major issues with BEF studies are: 1) how to logically consider biodiversity, e.g., as alpha-diversity or composition; and 2) robustly demonstrating whether a BEF exists, in statistical terms of low predictive error.

Objectives: This study sought to demonstrate BEF relationships between soil microbial communities and functions involved in both organic matter mineralisation and incorporation into biomass, i.e., growth. Twelve different aspects of biodiversity were systematically tested and their predictive error for BEFs compared. These aspects included taxonomy and protein-encoding functional genes, as either univariate alpha-diversity or multivariate composition.

Method: Soils were sampled across eight distinct growth stages of winter wheat, covering pre-, active and post-rhizosphere development. The activity of three extracellular enzymes involved in organic carbon, nitrogen and phosphorus mineralisation were measured. Microbial metabolic quotient, mass specific growth rate and carbon use efficiency were analysed by ^{18}O-DNA labelling. This provided three mineralisation functions and three growth-associated functions for statistical modelling. Shotgun metagenomics was performed on soil DNA with the Illumina NovaSeq 6000 platform and processed with the MetaWrap pipeline. Statistical modelling of BEFs was conducted by randomised cross-validation (50% training, 50% testing) with 500 replications for univariate (alpha-diversity) and boosted regression tree (composition) biodiversity data derived from metagenome sequencing.

Findings: Alpha-diversity indices failed to predict all mineralisation and growth-associated BEFs (21 – 36% error). The composition of functional genes also had a wide error range (5 – 26%). Composition-based taxonomic models demonstrated the best predictive capacity e.g., 5% error for cellobiohydrolase (CBH) at Class level, Fig 1. This was consistent for monophosphatase activity and microbial metabolic quotient (5 and

11% error, respectively). The remaining functions were less reliably predicted (15 – 26% error).

Conclusion: Ecosystem functions for organic matter mineralisation and growth of soil microbial communities were most strongly associated with compositional 'fingerprints' of taxa. Statistical models of the fingerprints show that, when a community is comprised of certain proportions of certain taxa, their collective functionality can be predicted within 5% error. An added benefit to composition-based BEF modelling is that microbial ecologists can make logical connections between taxonomic groups and functions (e.g., Fig 1). In contrast, alpha-diversity is a poor predictor of BEFs and cannot provide direct explanations for why a community has a certain function. Finally, due to their quantitative nature, such fingerprints provide a basis for incorporating microbial biodiversity information into process-based models of ecosystem function.

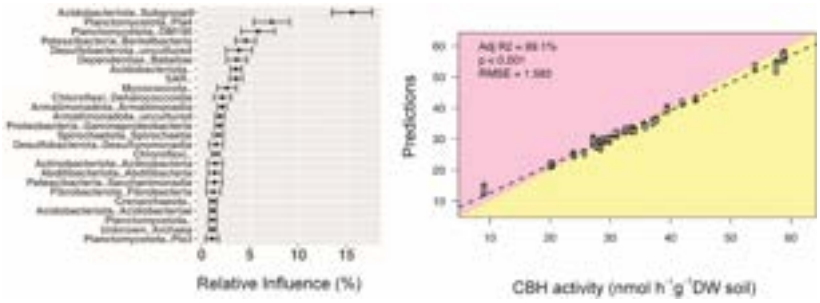


Figure 1: Based upon the compositional 'fingerprint' of certain taxa identified as relatively influential for cellobiohydrolase (CBH) activity in a soil microbial community (left), their collective function in terms of CBH activity can be reliably predicted (right), demonstrating the CBH-BEF.

Abstract ID: 3021

Sphingomonas as key drivers of microbiome-mediated nitrous oxide flux hotspots in winter rye cover cropping on a sandy soil in northeast Germany

Presenting author: Dr. Simon Thierry Okiobe, The Leibniz Institute for Agricultural Engineering and Bioeconomy, Postdam, Germany

Co-authors: *Susanne Theuerl, Annette Prochnow, Christiane Herrmann, Katharina Schulz, Sophie Schuster-Hankel*

Agricultural soil microbiomes are critical drivers of biogeochemical processes and represent significant, yet underexplored, contributors to global greenhouse gas emissions. In agricultural soils, microbiome-driven nitrous oxide (N_2O) flux rates increase exponentially with rising nitrogen (N) inputs. However, the responses of the soil microbiome and hence the development of knowledge-based N management strategies across spatial and temporal scales remain poorly understood. Therefore, the identification of microbial species as indicators of N_2O flux hotspots constitute a critical research gap. Here, we investigated the dynamics of soil microbiomes in winter rye cover cropping on a sandy soil in northeast Germany in response to innovative N management practices, including the application of calcium cyanamide-treated cattle manure, across multiple temporal sampling points during the winter season. The results showed pronounced temporal shifts in microbial diversity, taxonomic composition, and key functional N-cycling genes with *nirK*-carrying microbial communities peaking after fertilization. Temporal dimensionality played a dominant role in shaping microbiome dynamics compared to spatial variability across N management strategies. To elucidate the role of indicative microbial taxa for N_2O emissions, we linked shifts in microbial taxa to *in situ* N_2O fluxes, categorizing the fluxes into low and high rates. Advanced random forest modeling pinpointed for the first time, *Sphingomonas* particularly *Sphingomonas daechungensis*, as a key indicator of high N_2O flux hotspots. While calcium cyanamide-treated cattle manure altered the relative abundances of certain microbial species, *Sphingomonas daechungensis* was left unaffected. Fertilizer application-induced temporal alterations in soil nitrate levels emerged as the main substrates for microbial-derived N_2O fluxes, overshadowing the influence of functional N-cycling genes. These findings enhance the agro-ecological significance of our study by linking microbiome diversity and microbial species composition dynamics to *in situ* N_2O moments in winter rye cover cropping on a sandy soil. By integrating microbiome-informed N management

strategies, we are opening new windows to mitigate N_2O emissions and enhance the sustainability of agricultural systems.

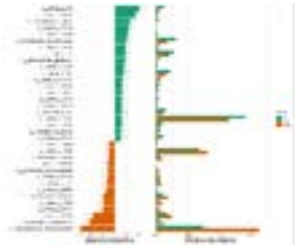


Figure 1: Key indicative taxa associated with N_2O flux rates. Random Forest Model Mean Decrease Gini (MDG) metrics represent the relative importance of taxa, with higher values indicating greater importance. We deployed the all-in-one full-length long-read Oxford Nanopore platform for sequencing. To elucidate the role of indicative microbial taxa in N_2O emissions, we linked shifts in microbial taxa to in situ N_2O fluxes, categorizing these fluxes into low (green) and high rates (Orange).across the season.

Abstract ID: 2338 / Poster Number: 122.17P

Spread of antibiotic resistance in pathogens from Swiss raw meat products

Presenting author: Dr. Lisa Thönen, Food Microbial Systems, Agroscope, Schwarzenburgstrasse 161, Bern, Switzerland

Antimicrobial resistance is a major public health concern. The increasing demand for meat is driving intensive livestock farming that relies on antibiotics, contributing to the spread of antibiotic resistance in food-producing animals. Animal-derived products can serve as a pathway for the transfer of resistant bacteria from animals to humans. The aim was to evaluate the presence of antibiotic-resistant bacteria in ready-to-eat meat products from Swiss butcheries. Resistant pathogens were isolated through selective plating. Antibiotic resistance phenotypes were determined by broth dilution using a semi-automated system. By short- and long-read whole genome sequencing the presence of antibiotic and metal and biocide resistance genes was profiled. We identified several carbapenem- and third-generation cephalosporin-resistant Enterobacterales, two vancomycin-resistant Enterococci and one methicillin-resistant Staphylococcus aureus. Whole genome sequencing confirmed most antibiotic resistance phenotypes with corresponding genotypes. In addition to antibiotic resistance genes, this analysis revealed diverse and abundant metal and biocide resistance genes, although their impact on resistance phenotypes requires further investigation. The presence of metal resistance genes suggests potential co-selection for antibiotic resistance. Long-read sequencing revealed that while antibiotic resistance genes are primarily located on chromosomes, metal resistance genes are often found on plasmids and are therefore susceptible to transfer. Overall, this study highlights ready-to-eat meat products as a reservoir for the transfer of both antibiotic and stress resistance genes from animals to humans.

Abstract ID: 1886

Stability of Aspergillus niger Genomes: Mutation rates in Evolved and Industrial Citric Acid Production Strains

Presenting author: Ms. Adéla Schandl, Technische Universität Wien, Vienna, Austria

Co-authors: *Matthias Steiger*

Introduction: *Aspergillus niger* is a filamentous fungi widely used in the biotechnological industry for citric acid production, a key component in food, pharmaceutical, and cosmetic industries. With the increasing demand for citric acid, there is a continuous need to enhance production efficiency through strain development.

Objective: For this aim, an industrial strain of *A. niger* was developed by a partner company. This industrial strain was used as the parental strain for further evolutionary experiments, resulting in three evolved strains obtained through 14 passages in a selection pipeline of single conidia cultivation.

Method: Whole genome sequencing of the industrial strain and the evolved strains was performed using the Illumina platform to assess mutation rates and genomic stability. Sequence data were aligned to the reference *A. niger* genome of approximately 34 Mbp, and genomic variants were identified through bioinformatic analysis.

Findings: Our findings revealed that the industrial strain exhibited 884 variants compared to the reference genome. The evolved strains, derived from the industrial strain, exhibited between 119 and 147 variants. Notably, the number of variants in the evolved strains was consistently low, further highlighting the high genomic stability during the evolutionary process.

Conclusion: In conclusion, the results indicate that *A. niger* maintains genomic stability throughout evolutionary cultivation. The mutation rates observed in industrial and evolved strains underscore the suitability for industrial applications where genetic stability is critical for consistent citric acid production. However, this evolutionary approach should be modified for higher mutation rates to potentially further improve citric acid production.

Abstract ID: 1510 / Poster Number: 187.16P

Staphylococcus aureus in Melanoma: Development of Combined Therapies for Chronic Intracellular Infections

Presenting author: Mr. Sergio Fernández Martínez, University of Leon, Leon, Spain

Co-authors: *Helena Álvarez Ferrero, Blanca Lorente Torres, Pablo Castañera, Jesús Llano Verdeja, Farzaneh Javadi Marand, Álvaro Mourenza, Luis M. Mateos, Michal Letek Polberg*

Staphylococcus aureus is an opportunistic pathogen capable of infecting a wide range of tissues. A key aspect of its pathogenicity lies in its ability to invade and persist within host cells, adopting a facultative intracellular lifestyle that poses significant challenges for treatment and immune clearance. Intracellular infections by *S. aureus* have recently been implicated in the onset and progression of skin cancer, contributing to cellular transformation and tumor development.

This study aims to develop novel antimicrobial therapies targeting intracellular *S. aureus* by combining repurposed drugs with other antimicrobial agents. Using a melanoma infection model, we tested a range of anticancer drugs identified from a previous high-throughput screening of 6,995 repurposing candidates. These compounds were first evaluated for their antimicrobial activity against *S. aureus* and subsequently tested *in vitro* in combination with host- and pathogen-targeted anti-infective agents.

Preliminary findings from cellular models reveal that certain anticancer drugs demonstrate notable antimicrobial activity against intracellular *S. aureus*. When combined with other therapeutic agents, these drugs show synergistic effects, significantly enhancing bacterial eradication. These results suggest that combinatorial therapies targeting both the host cell and the pathogen offer a promising approach to treating *S. aureus* infections in melanoma. Future work will focus on optimizing these strategies and validating their clinical potential, paving the way for innovative treatments that address the dual challenge of intracellular infections and cancer progression.

Abstract ID: 2936 / Poster Number: 157.17P

Staphylococcus aureus mRNA degradation pattern response to antibiotic treatment

Presenting author: Mr. Razmik Sargsyan, Armenian Bioinformatics Institute, Yerevan, Armenia

Co-authors: *Lilit Nersisyan, Davit Hakobyan*

Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) infections are a growing challenge in healthcare. Better understanding of MRSA responses to antibiotics and strain-specific variations may aid in developing new treatment strategies.

Objective: We aimed at exploring rapid bacterial responses to various classes of antibiotics in different clinical MRSA strains, based on sequencing 5' monophosphorylated (5'P) mRNA degradation co-translational intermediates through a technique called 5PSeq, which informs about ribosome position, indicating disruptions in the process of translation by ribosome-targeting antibiotics or as indirect indicators of stress response to antibiotics of other classes.

Methods: We have compared the response of four MRSA strains supplemented with 10 antibiotics targeting the processes of translation, replication, transcription and cell wall synthesis. We sequenced 5'P mRNA after 20 min of treatment. We correlated mRNA 5' endpoint patterns with growth assay based antibiotic sensitivity and developed a statistical framework for rapid, automated assessment of antibiotic sensitivity and response mechanisms.

Results: The new statistical framework distinguished sensitive and resistant MRSA strains for ribosome-targeting antibiotics. For tetracycline, it revealed altered translation frame preference, likely from targeting the ribosome A-site. The framework partially indicated indirect effects of other antibiotics, though with less precision and high strain specificity.

Conclusion: We characterized rapid antibiotic responses of clinical strains of *S. aureus* to different antibiotics, developed a new approach to analysis of fast response patterns occurring on the RNA decay level. Combined with further genetic determinants of antibiotic response, this work may advance understanding of resistance mechanisms and treatment strategies.

Abstract ID: 3854

Strain-Specific Effects of Vaginal Probiotics in formulation of Functional Fermented Milk designed for Women's Well-Being

Presenting author: Davide Gottardi, PhD, Department of Agricultural and Food Sciences, University of Bologna, Cesena, Italy

Co-authors: *Margherita D'Alessandro, Maria Alessia Schout, Silvia Malavolta, Beatrice Vitali, Carola Parolin, Santina Romani, Maria De Angelis, Giuseppe Celano, Rosalba Lanciotti, Francesca Patrignani*

Fermented milks are widely recognized as excellent carriers for probiotic bacteria, and growing evidence highlight the potential of functional foods to influence human health. It is already well established that probiotic strains exhibit strain-specific behaviors. This characteristic is crucial not only for selecting strains to achieve desired health effects but also for optimizing food formulations, as each strain may positively or negatively impact the final product's quality. This study, conducted as part of the FUNPRO-L project, aims to develop a functional fermented milk using skim milk, the starter culture ST-440 (Sacco) (a mix of *Streptococcus thermophilus* strains producing exopolysaccharides), and probiotics of vaginal origin with potential benefits for women's well-being. Five probiotic strains – *Lactobacillus crispatus* BC1, BC4, BC5 and *Lactobacillus gasseri* BC9, BC12 – at different concentrations (8, and 7 log CFU/ml) were initially tested for their survival by plate counting samples upon fermentation and after 28 days under refrigerated conditions (5°C). Subsequent evaluations focused on the three most promising strains (*L. crispatus* BC1, BC4, and BC5) at 8 log CFU/ml. Parameters assessed during 28 days of storage at 5 °C included pH, microbial viability (on M17 and MRS agar at 45°C and 37°C for 48 hours, for *S.*

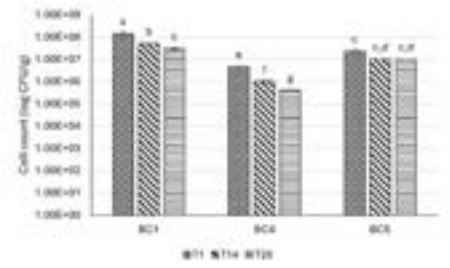


Figure 1: Cell load viability (log CFU/g) of the vaginal strains (L. crispatus BC1, BC4 and BC5) in fermented milk during the storage in refrigerated conditions (after 1, 14 and 28 days from production).

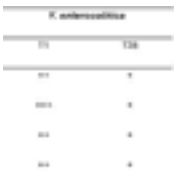


Table 1. Evaluation of the antagonistic activity of the fermented milks against enterotoxigenic Escherichia coli H10407, Salmonella Typimurium, and Yersinia enterocolitica over refrigerated storage.

thermophilus and *L. crispatus*, respectively), water-holding capacity (WHC, measured gravimetrically), rheological properties (using a Rheometer), amino acid profiles, volatile molecule profiles (via SME-GC/MS), and antimicrobial activity against enteric and urogenital pathogens (using overlay assays). Among the tested strains, BC1 demonstrated the highest survival rate (around 0.5 log reduction), followed by BC5 (less than 1 log), while BC4 exhibited a reduction of more than 1 log during the 28 days of storage (Fig. 1). From a technological perspective, after 28 days of storage, fermented milk with BC5 maintained a higher value of WHC (23.3 vs 16.7 %) and had a significant greater yield stress value (1.16 Pa) and consistency index (3.30 Pa x sn) with respect to the other samples and control (0.17 Pa and 0.16 Pa x sn, respectively, for control). Presence of probiotic significantly enhanced the release of free amino acids, with BC5 contributing the most on the post-fermentation and BC4 during storage. Strain-specific effects of the strains were observed in volatile molecule profiles, with an increase in acetic acid and other short and medium chain fatty acids already after fermentation. In the antimicrobial assay, BC1 and BC5 exhibited the strongest and most sustained activity against *Escherichia coli* H10407 (ETEC), *Yersinia enterocolitica*, and *Salmonella* Typhimurium up to 28 days (Tab. 2). These findings position BC1 and BC5 as the most promising strains for the development of functional fermented milk targeting women's well-being. This work highlights the need to balance three key aspects—probiotic functionality and viability, technological characteristics, and sensory properties—to achieve an optimal formulation. The results will be applied to industrial-scale production and subsequent clinical trials to validate the product's health benefits for women.

Abstract ID: 2169 / Poster Number: 114.16P

Strain-specific galactose utilization by commensal E. coli mitigates Salmonella establishment in the gut

Presenting author: Dr. Christopher Schubert, ETH Zurich, Zurich, Switzerland

Salmonella enterica serovar Typhimurium (*S*. Tm) is a major cause of gastrointestinal diseases worldwide. To date, options for prevention or curative therapy remain limited. The gut microbiota plays a protective role against enteric diseases, particularly in preventing establishment and proliferation of *S*. Tm. While most research has focused on microbiota-mediated pathogen exclusion during the later, inflammation-dominated stages of infection, little is known about how microbiota members mitigate *S*. Tm early gut colonization. To address this gap, we conducted *in vivo* competitive experiments using *S*. Tm and different commensal *E. coli* strains. We observed a significant reduction in pathogen load, which was strain-specific and particularly evident with *E. coli* 8178. To investigate the underlying molecular mechanisms, we conducted an *in vivo* screen using a *S*. Tm mutant library, which includes a wide range of carbohydrate utilization mutants, both in the absence and presence of *E. coli* strains. Our findings revealed that *E. coli* 8178-mediated *S*. Tm competition was driven by the exploitation of galactose during the early stage of infection. Identifying galactose as a key metabolite in pathogen exclusion by gut microbiota members enhances our mechanistic understanding of microbiota-mediated protection and opens new avenues for developing microbiota- and dietary-based strategies to better control intestinal infections.

Abstract ID: 2938 / Poster Number: 134.16S

Strain-specific impacts of Desulfovibrio on neurodegeneration, lifespan, and food preference in a C. elegans Parkinson's disease model

Presenting author: Dr. Khosrow Mohammadi, University of Helsinki, Helsinki, Finland

Co-authors: *Dongming Zhang, Per Saris*

Introduction: Gut microbiota has been increasingly linked to neurodegenerative diseases such as Parkinson's disease, with *Desulfovibrio* (DSV) species implicated in α-synuclein aggregation.

Objectives: This study aimed to elucidate the pathogenic effects of various DSV species in human neurodegeneration. Using a transgenic *Caenorhabditis elegans* NL5901 model organism, we examined the strain-specific impacts of DSV on human α-synuclein aggregation, lifespan, and food preference.

Methods: Six DSV strains were used in this study, two human isolates (*D. desulfuricans* DSS1, Parkinson's patient-derived, and *D. piger* DSM 749), two animal isolates (*D. piger* DSM 106036 and *D. desulfuricans* DSM 6949), and two environmental isolates (*D. desulfuricans* DSM 12129 and *D. vulgaris* DSM 644). Gene expression analysis was performed using RT-qPCR to evaluate genes associated with proteostasis and apoptosis regulation (*hsf-1, hsp-16.1, hsp-16.2, egl-1*), oxidative stress response (*skn-1, gst-4, sod-3*), and lifespan regulation and dopamine synthesis (*daf-16, cat-2*). Oxidative stress and Reactive Oxygen Species (ROS) formation was determined using DCFH-DA, and α-synuclein aggregation was quantified using fluorescence microscopy.

Findings: *1. Neurodegeneration:* DSM 6949 and DSS1 induced the highest α-syn aggregation levels ($p < 0.05$), correlating with the strongest downregulation of *cat-2* (0.13–0.51-fold) and proteostasis-related genes (*hsp-16.2*: 0.05–0.43-fold). DSM 644-fed worms exhibited minimal aggregation, supported by robust upregulation of *hsp-16.1* (2.01-fold) and *daf-16* (1.55-fold).

2. ROS Formation: ROS levels were significantly higher in all DSV-fed groups than in the control (*E. coli* OP50), with the highest levels observed in DSM 6949-fed worms (3.42-fold change). This was associated with impaired antioxidant defenses due to downregulation of *sod-3* (0.22-fold) and *skn-1* (0.46-fold). DSM 644-fed worms had comparatively lower ROS levels (1.56-fold change), reflecting relatively high *sod-3* expression (0.74-fold) and

moderate upregulation of *gst-4* (1.75-fold) and *skn-1* (1.95-fold), though still above the control.

3. Lifespan: DSM 644-fed worms exhibited the most significant lifespan extension, with a median survival time of 28 days, compared to 20 days in the control group (*E. coli* OP50) ($p < 0.05$). This aligns with the efficient activation of stress response pathways, as evidenced by the upregulation of *daf-16* (1.55-fold). Conversely, DSS1- and DSM 6949-fed worms showed shorter lifespan extensions, with median survival times of 24 days and 26 days, respectively.

4. Food Preference: At both 6 and 24 hours, *C. elegans* displayed distinct food preferences, strongly favoring environmental strain DSM 644 while showing a strong dislike to the human-isolated strain DSM 749.These findings suggest strain-specific bacterial differences detectable by nematodes.

Conclusion: This study highlights the strain-specific effects of DSV on host physiology. The environmental strain DSM 644 demonstrated a more balanced impact on neurodegeneration, lifespan extension, and food preference, albeit with elevated ROS levels. In contrast, human- and animal-isolated strains such as DSS1 and DSM 6949 showed pathogenic potential, reflected in increased α-synuclein aggregation and ROS formation. These findings underscore the importance of microbiota composition in modulating neurodegeneration, oxidative stress, and aging processes in *C. elegans* NL5901 as a model organism.

Abstract ID: 2406 / Poster Number: 124.17P

Streptococcus agalactiae in pregnant women colonizing the genital tract and their sensitivity to antibacterial drugs

Presenting author: Viktoriia Potochylova, PhD, Kyiv City Maternity Hospital № 2, Kyiv, Ukraine

Co-authors: *Kateryna Rudnieva, Elena Moshynets, Olga Iungin, Viktoriia Sobetska, Danylo Puzikov*

Introduction: Intrauterine infection of the fetus is an urgent problem in obstetrics, since in the absence of adequate treatment or progression of the disease, complications of pregnancy, childbirth and the postpartum period are possible. According to R. Nandyal, 15-45% of pregnant women have a positive screening result for Streptococcus agalactiae. The aim of this study was to compare the resistance of strains isolated from pregnant women in Kyiv region.

Methods: We analyzed 372 isolated microorganisms for 2023 and 10 months of 2024. Primary inoculation was performed on chromogenic medium for group B Streptococcus. Identification and sensitivity determination were performed using the microbiological analyzer “VITEK 2 compact 15”, the disc diffusion method (DDM) or the method of determining minimum inhibitory concentrations (MIC). For the analysis, a 0.5 McFarland suspension was used with incubation for 18-20 h at 5 % CO_2 at 35 °C. Statistical calculations were performed by the WHONET 5.6 program.

Results: In 2023, 198 strains were isolated, among which 68.01 % were resistant to benzylpenicillin, 80.8 % to erythromycin, 8 % to clindamycin, 0 % to linezolid, 21.2 % to norfloxacin, and 11.6 % to vancomycin. According to EUCAST, the susceptibility of streptococci of groups A, B, C, G to β-lactams is determined on the basis of the index to benzylpenicillin. At the same time, the SLSI provides for the determination of growth retardation zones for cefepime, cefotaxime, and ampicillin. The following indicators were obtained: ampicillin – 34.14 %, cefepime – 20.96 %, cefotaxime – 22.58 %.

Conclusions: Increased resistance to the screening benzylpenicillin and at the same time sensitivity to ampicillin, cefepime and cefotaxime indicate the natural presence of penicillinase in the pathogen.

Abstract ID: 3622

Streptomycete extracellular membrane vesicles affect growth and differentiation of bacterial strains and tomato plants

Presenting author: Assoc. Prof. Giuseppe Gallo, University of Palermo, Palermo, Italy; NBFC, National Biodiversity Future Center, Palermo, Italy

Co-authors: *Teresa Faddetta, Flavia Cannizzaro, Noemi Sannasardo, Guglielmo Puccio, Ciro Caldiero, Valentina Ricciardi, Loredana Abbate, Sara Amata, Carla Rizzo, Giorgio Nasillo, Antonio Palumbo Piccionello, Francesco Mercati,*

Streptomycetes are renowned for their morpho-physiological differentiation program, bioactive compound production, and plant-growth promoting (PGP) effects (Faddetta *et al*., 2023). Recent studies highlighted the roles of extracellular membrane vesicles (MVs) in mediating intercellular communication and in transporting a complex array of biomolecules (Sangiorgio et al., 2024). Thus, this study aims to characterize MVs produced by *Streptomyces coelicolor* and *Streptomyces violaceoruber*, focusing on their cargo and functional roles.

The MVs were isolated from the two strains – cultivated in a liquid minimal medium for six days – using an ultracentrifugation protocol (Faddetta *et al*. 2022). MV were characterized by scanning transmission electron microscope (STEM) and dynamic light scattering. STEM was also used for streptomycete strain morphology. Molecular profiling carried out on MVs, streptomycete strains and tomato (*Solanum lycopersicum*) seedlings involved omics investigations, such as whole genome shotgun and RNAseq analyses by Next-Generation Sequencing, and High-Performance Liquid Chromatography and Gas Chromatography coupled with Mass Spectrometry analyses. MV functional assays were performed to evaluate: antibacterial activity against the Gram-positive *Kocuria rhizophila* and the Gram-negative *Escherichia coli* tester strains; *in vitro* PGP effects using *S. lycopersicum* tomato seedlings; horizontal gene transfer using the *S. coelicolor bldA* mutant, unable to perform morpho-physiological differentiation.

Streptomycete MVs, showing an average size of 100-200 nm, have a cargo comprising the whole genomic DNA, a set of mRNA and sRNA which were bioinformatically analyzed for their putative functional roles, besides proteins and metabolites. Notably, both strains' MVs exerted a PGP effect on *S. lycopersicum*, with the *S. coelicolor* MV-exposed tomato seedlings being characterized for their transcriptome in comparison to the unexposed

controls. Additionally, *S. violaceoruber* MVs exhibited antimicrobial properties against the Gram-positive tester strain. Intriguingly, colonies of *bldA* mutant strains, obtained after an exposure to *S. coelicolor* MVs, showed distinct morphological characteristics in comparison to the colonies of unexposed *bldA* strains obtained in parallel control experiments. Among the MV-exposed strains, two isolates were selected for further morphological and molecular investigations. One out of them showed a stable capability to perform morphological differentiation (Figure 1). Furthermore, both selected strains showed a peculiar production of volatile organic compounds compared to those produced by the parental *bldA* mutant. These two MV-exposed *bldA* strains have been also investigated for their genome sequences, revealing specific mutations, and transcriptome profiles in comparison to the parental *bldA* mutant.

Altogether, these findings highlight the ability of streptomycete MVs to influence growth and differentiation of bacterial strains and tomato plants. Additionally, the MV ability to mediate intra- and inter-kingdom interactions underscores their potential as biotechnological tools.

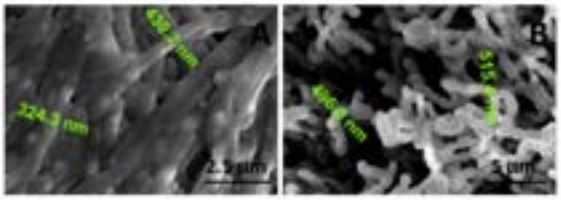


Figure 1: STEM images of the mycelia of parental (A) and MV-exposed (B) *bldA* strains grown on agar-medium plates. The MV-exposed strain clearly shows the presence of typical streptomycete spore chains, contrary to what is observed for the parental *bldA* strain.

Abstract ID: 3151 / Poster Number: 343.15S

Stronger at the seams: Antibiotic stress enhances amino acid leakage and cross-feeding

Presenting author: Mr. Constantinos Xenophontos, IRCCS Humanitas, Milan, Italy

Co-authors: *Abhishek Vaidyanathan, Gabriele Micali*

Microbial communities of heterotrophic bacteria play vital functions in ecosystems, from contributions in the carbon cycle to digestion in the human gut. Such broad metabolic diversity of heterotrophic bacteria results in metabolic redundancy and complementarity in communities. Redundancies lead to the evolution of gene loss, as described in the Black Queen Hypothesis. The outcome of gene losses in amino acid biosynthesis is the emergence of auxotrophic bacteria, as opposed to prototrophic bacteria that can synthesise all necessary amino acids. In the last decades, widespread use of antibiotics has been disrupting these communities and promoting the emergence of antibiotic resistance. Due to the complexity of natural microbial communities, our understanding of how antibiotics specifically impact species interactions and growth dynamics in these communities is still limited.

We used prototrophic *E. coli* and single-gene-knockout mutants to create different auxotrophic strains for single amino acids. Using microfluidic devices, we studied how prototroph-auxotroph cocultures grow and exchange amino acids (cross-feeding) (Fig. 1). We challenged these experimental cocultures with different levels of antibiotics that inhibit protein synthesis, to create perturbations in the internal amino acid homeostasis.

We expected that this disruption would impact the leakage and exchange of amino acids between the strains. In turn, a disrupted exchange of amino acids should reflect on the growth dynamics between coculture strains. We verified that prototroph-auxotroph cocultures can coexist, both with (Fig. 1 left) and without (Fig. 1 middle) amino acid supplementation, with the exchanged amino acids acting as a necessary public good in the latter case. However, the introduction of antibiotics revealed unexpected dynamics — Sublethal concentrations of antibiotics can strengthen cross-feeding interactions, enhancing the growth of auxotrophs (Fig. 1 right). We believe this is because the reduction in the prototrophs' protein synthesis increases internal amino acid pools and therefore increases leakage and exchange between cells.

An increase in exchanged amino acids can therefore enhance cross-feeding interactions in communities, such as between host microbiome species and pathogens. These

results can help us understand how antibiotics and the spread of antibiotic resistance influence the growth dynamics of microbial communities.

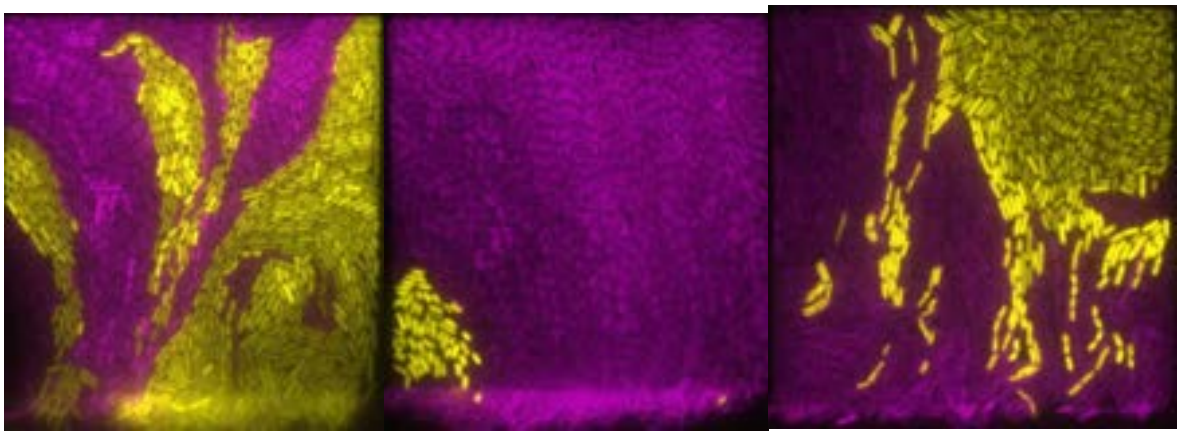


Figure 1: Cocultures of *E. coli* cells growing in a microfluidic device. Magenta cells are a prototrophic WT strain and yellow cells are auxotrophic knockout mutants. Left panel shows both strains growing in supplemented media without antibiotics. Middle panel shows the interaction of the strains in amino acid unsupplemented media, without antibiotics. Right panel shows the effects of unsupplemented media with antibiotics, that enhance the growth of the auxotrophic cells.

Abstract ID: 2906 / Poster Number: 264.15P

Structure, composition and function of outer membrane vesicles produced by Vibrio cholerae: Role in microbiota interactions and host infection

Presenting author: Mr. Matheus Luchetta da Fonseca, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; Sorbonne Université IMPMC, Paris, France

Cholera, a disease caused by the bacterium *Vibrio cholerae*, poses a global health challenge, primarily spread through the ingestion of contaminated water or food. Increases in Vibrio spp infections worldwide are linked to rising sea temperatures and natural disasters fuelled by climate change. Besides the toxin, other virulence factors are crucial for the bacterium's pathogenicity, including outer membrane vesicles (OMVs). Due to their small size (20-300 nm), visualization of extracellular vesicles requires electron microscopy for their morphological characterization. However, preparing samples for transmission microscopy alters the structure of the vesicles through drying, leading to the production of artifacts that make precise characterization of the structures difficult. The use of Cryo-electron tomography allows native structures visualization and allows the identification of proteins, lipids and other components present in these vesicles. The aim of this study is to investigate *Vibrio cholerae* membrane vesicles structures, specifically determining morphological changes in OMVs under Pi limitation during bacterial growth. The analyzed vesicles showed two populations – one between 20-40 nm and another between 120-150 nm. The characteristics between these two populations are also quite different, with the smaller vesicles being extremely decorated with membrane proteins and the larger vesicles having an electron-dense internal content. Further studies investigating bacterial vesicles composition, production, and mechanisms in pathogenic and commensal bacteria from various environments can offer insights into microorganism-host/environment interactions, facilitating the development of new therapeutic and biotechnological tools.

Abstract ID: 3159 / Poster Number: 215.17P

Structure-function relationship of AsmA-like proteins involved in outer membrane biogenesis

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Co-authors: *Camilla Pederzoli, Marianna Bufano, Antonio Coluccia, Francesco Imperi*

Background: The outer membrane (OM) is an essential structure of diderm bacteria composed of phospholipids, lipopolysaccharide, integral proteins and lipoproteins (1). The mechanism mediating transport of phospholipids from the cytoplasmic membrane to the OM has remained elusive for decades until recent studies performed in *Escherichia coli* and *Pseudomonas aeruginosa* proposed proteins belonging to the AsmA-like protein family as responsible for this process. In both bacteria, YhdP is the most important AsmA-like protein for cell viability and OM homeostasis (2-4).

Objectives and methods: In this study we aimed at investigating the structure-function relationship of selected *P. aeruginosa* AsmA-like proteins that are essential but redundant for bacterial cell viability. Bioinformatic analysis revealed the presence of a hydrophobic region in the YhdP C-terminal domain (here named CHR), that is absent from other AsmA-like proteins. Plasmids that express YhdP mutant variants and YdbH-YhdP fusion proteins were generated and their effect on growth, cell envelope integrity, and antibiotic susceptibility of *P. aeruginosa* and *E. coli* mutants deficient in essential AsmA-like proteins was assessed, in order to verify the importance of the YhdP CHR for AsmA-like protein functioning. In frame deletion mutagenesis was performed to confirm the results of the ectopic expression assays and to verify if the OM lipoprotein YnbE is required for YdbH activity in *P. aeruginosa*, as recently found in *E. coli* (5).

Findings: Differently from the wild-type protein, expression of YhdP without the entire C-terminal domain or lacking only the CHR did not restore the growth of a conditional mutant (here named Δ4C) which lacks the four *P. aeruginosa* essential AsmA-like proteins. The in-frame deletion of the sequence encoding the CHR in the chromosomal *yhdP* gene caused growth and OM stability defects almost comparable to the deletion of entire *yhdP* gene, confirming that the hydrophobic region in the C-terminal domain of YhdP is critical for its function. Expression of a YdbH-YhdP fusion protein, in which the C-teminal region of YhdP was fused to YdbH to reproduce the length of YhdP (named YdbH-YhdP long), fully restored the growth of the Δ4C mutant, whereas a shorter YdbH-YhdP fusion protein, where YdbH was fused to the YhdP C-terminal domain only (named YdbH-YhdP short), did not. YdbH-YhdP long was also more effective than the wild-type protein

YdbH in restoring vancomycin resistance and cell envelope integrity in AsmA-like protein deficient mutants. In addition, the expression of *P. aeruginosa* YhdP and YdbH-YhdP long completely rescued the growth defect and vancomycin ipersusceptibility of an *E. coli* mutant deleted in selected *asmA*-like genes, demonstrating that they can also work in a heterologous host. By generating Δ*ynbE* mutants we confirmed that YnbE is crucial for YdbH activity also in *P. aeruginosa*, but the expression of YdbH-YhdP long was found to promote growth even in YnbE-deficient cells.

Conclusions: In this study we demonstrated that the CHR of *P. aeruginosa* YhdP is crucial for the functionality of this AsmA-like protein, and that *P. aeruginosa* YdbH works better when fused to the C-terminal domain of YhdP, but only if the length of the fusion protein is similar to that of YhdP. Moreover, the fusion of YdbH with the C-terminal region of YhdP likely prevents the interaction between YdbH and its protein partner YnbE but allows YdbH to carry out its function even without YnbE. These results, combined with the finding that *P. aeruginosa* wild-type or chimera proteins carrying the YhdP CHR also work in *E. coli*, corroborate the hypothesis that the YhdP CHR might mediate insertion of phospholipids into the OM without OM protein partners.

Abstract ID: 3900 / Poster Number: 197.17P

Study of biofilm degrading ability of bacteriophages on antibiotic-resistant and biofilm-forming S. aureus strains isolated in Georgia

Presenting author: Mr. Davit Lazviashvili, George Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia; Ilia State University, Tbilisi, Georgia

Co-authors: *Nino Grdzelishvili, Veriko Berulava, Tamar Tatrishvili, Giorgi Chelidze, Nina Chanishvili, Nata Bakuradze*

Bacterial infections have resulted in millions of deaths, with Staphylococcus aureus infections being the second most common cause. The most severe cases involve methicillin-resistant strains (MRSA) showing resistance to multiple classes of antibiotics. The formation of biofilms enhances bacterial defense, making treatments less effective. This situation highlights the urgent need for alternative therapies, such as bacteriophage-based treatments.

This study aimed to compare the biofilm activity of the sequenced phage vB_SaS_GE1 (NCBI:txid2914449) and the commercially available phage SB-1 (NCBI:txid1007127) against antibiotic-resistant and biofilm-forming *S. aureus* isolates.

Bacterial species were identified using bacteriological and biochemical tests. Antibiotic resistance was assessed through the Kirby-Bauer disk diffusion method and PCR. Biofilm formation was analyzed using 96-well polystyrene plates, and PCR targeting the icaA gene. Phage susceptibility was evaluated using the streak method and measuring the efficiency of plating (EOP).

Out of 25 isolates from clinics in Tbilisi, 20 were confirmed as *Staphylococcus aureus*, including eight multi-resistant strains and two cases of MRSA. Seven strains, including the MRSA, were identified as biofilm-forming. Phage susceptibility tests showed that 18 strains were sensitive to vB_SaS_GE1, and 15 to SB-1. Efficiency of plating (EOP) analysis performed on the 10 most sensitive strains indicated high efficiency in six biofilm-forming *Staphylococcus aureus* strains and moderate efficiency in two strains.

Abstract ID: 3970 / Poster Number: 403.15P

Study of the composition of nasal microbiome in patients with AERD and ability of isolates to form biofilms

Presenting author: Olha Bondarenko, PhD, University of Parma, Parma, Italy

Co-authors: *Maryna Mishyna, Yuliya Mozgova, Alessandra Marchese, Roberta Pisi, Rocco Accogli, Annalisa Frizzelli, Marina Aiello, Alfredo Chetta*

Background: Aspirin-exacerbated respiratory disease (AERD) is a chronic respiratory condition characterized by a triad of symptoms: asthma, chronic rhinosinusitis with nasal polyps and an allergic reaction to aspirin. Dysbiosis of the nasal cavity microbiome has not been studied in the cohort of patients with AERD.

Aim: determin of nasal microbiome and the ability of isolates to form biofilm.

Methods: taking swabs from the middle nasal passage and subsequent identification of some isolates using MICRO-LA-TEST kits. Laser scanning confocal microscopy was used to visualize biofilm and determine optical density.

Results: nasal microbiome was represented by the following species: *H.parainfluenza* 10.2%, *M.catarrhalis* 8.8%, *S.aureus* 8.6%, *Corynebacterium spp.* 7.08%, *P.aeruginosa* 4.3%. In steroid-resistant patients, gram-negative microflora prevailed: *H. parainfluenza, M. catarrhalis* and *P. aeruginosa*. 12 clinical strains of *M. catarrhalis* were isolated from patients with AERD. Adhesion of bacterial cells was observed after 2 hours, which contained cluster of cells with high luminescence. The optical density of biofilms inoculated with *M. catarrhalis* isolates was 3.28±0.18 units of optical density.

Conclusions: in nasal microbiome, there is an increased content of gram-negative pathogenic microflora, which can form dense biofilms. It is not a characteristic indicator of the causative agents of AERD in this group of patients. Further research is needed to identify factors contributing to the increased incidence of gram-negative pathogens and to understand the implications of steroid therapy.

Abstract ID: 3262 / Poster Number: 102.15F

Study of the endothelial response to SARS-CoV-2 in vitro infection of human lung microvascular endothelial cells

Presenting author: Lucia Signorini, PhD, University of Milan, Milan, Italy

Co-authors: *Federica Novazzi, Sara Boutahar, Sarah D'Alessandro, Nicoletta Basilico, Federica Perego, Maria Dolci, Francesca Drago, Nicasio Mancini, Serena Delbue*

Infectious agents can damage vascular endothelium causing vascular diseases. SARS-CoV-2 infection has been strongly associated with overproduction of proinflammatory mediators, causing critical illness. The mechanism underling this link remains unclear. The aim of the project was to investigate the correlation between SARS-CoV-2 infection and endothelial disfunction in an *in vitro* model of human lung microvascular endothelial cells (HULEC-5a).

HULEC-5a cell line was infected with three different SARS-CoV-2 lineages (B.1, B.1.617.2 and B.1.1.529, BA.1). Cells and their medium were collected at different timepoints post-infection (p.i.) and analyzed by the means of quantitative real time PCR and plaque assays to define the kinetic of infection. A panel of cellular inflammatory transcripts was analyzed by the means of real time PCR. Nitric oxide pathway was studied by analyzing transcriptional and proteomic expression of endothelial nitric oxide synthases.

HULEC-5a was to susceptible to SARS-CoV-2 infection showing the presence of the virus in both cells and medium collected at all the timepoints p.i. Inflammatory transcripts analysis showed a significant increase in the transcript levels of pro-inflammatory and anti-inflammatory transcript at different conditions p.i. The NO pathway was post transcriptional regulated.

To our knowledge, this is the first demonstration of the capability of lung endothelial cells to be directly infected with SARS-CoV-2 different lineages. The increase of pro-inflammatory transcripts after infection might be linked to cytokine overproduction, as experienced with infections with B.1 and B.1.617.2 lineages. Downregulation of the NO pathway could get worse the pro-inflammatory status.

Abstract ID: 2838 / Poster Number: 427.17S

Study of the interaction between microbes and carbonaceous filters to develop sustainable methods to treat contaminated waters and filters

Presenting author: Ms. Eva Donini, University of Bologna, Bologna, Italy

Co-authors: *Stefano Macrelli, Andrea Firrincieli, Andrea Contin, Lucia Poppa, Federico Villani, Martina Cappelletti*

Introduction: Bacteria establish biofilms on filters contributing to the filtering performance in water treatment plants to remove pollutants from contaminated waters. Biochar is an adsorbent carbonaceous material generated through thermal conversion of organic biomasses. Thanks to its peculiar chemical and physical properties, biochar can be used as filter in water treatment plants, providing a sustainable and low-cost alternative of activated carbons (ACs). The mechanisms of the interaction between microbes and filters and their possible use to recover the adsorption capacity of saturated carbonaceous filters are still mostly unknown.

Objectives: Our work aims at i) unravelling the diversity and role of microorganisms adhering on AC and biochar used as filters in two real-scale groundwater treatment plants and ii) at designing and applying synthetic microbial communities to remove contaminants from end-life filters in the framework of circular economy.

Methods: Microbial communities adhering on AC and biochar filters in two groundwater treatment plants (Pump&Treat and Groundwater Treatment Plant (GTP)) were thoroughly analysed over time by applying metabarcoding and metagenomic shotgun sequencing. Synthetic microbial consortia were generated by selecting bacterial strains based on metabolic capacity and conducting compatibility assays and by evolving natural communities thriving in the treatment plants. GC-FID analyses were conducted to evaluate the capacity of the synthetic communities to remove the contaminants from the filters.

Findings: Microbial communities stably colonize filters in the two groundwater treatment plants reaching biomass concentration that remains unchanged over time. The microbial community composition and functionality differ depending on the treatment plant under analysis and the contamination present. Although microbial communities colonizing AC and biochar applied in GTP showed similarity, we observed the enrichment of specific

microbial taxa and functions associated with biofilm growth, stress resistance and organic contaminant biodegradation on each type of filters and water treatment plants.

Biological treatment experiments showed the applicability of microbial synthetic communities for the biological treatment and recovery of end-life filters. In particular, we observed a better performance of contaminant removal on biochar filters as compared to AC, together with a higher removal of aromatic hydrocarbons as compared to chlorinated compounds from the filters.

Conclusion: In conclusion, our results indicate that i) biochar is a valid alternative to ACs in groundwater treatment plants as it can function as efficient support for the adhesion and development of microbial communities that can contribute to contaminants biodegradation, ii) synthetic microbial communities can be applied to at least partially remove adsorbed contaminants from end-life filters, enabling their potential re-use in other bioremediation applications.

Abstract ID: 905 / Poster Number: 190.15P

Study of the microbial biodiversity of the Iberian Pyrite Belt (Spain) deep subsurface by flourescence in situ hybridization

Presenting author: Ignacio Carrasco Ropero, PhD, Universidad Autónoma de Madrid, Madrid, Spain; Centro de Biología Molecular Severo Ochoa, Madrid, Spain

Co-authors: *Cristina Escudero, Nuria Rodríguez, Ricardo Amils*

The deep subsurface is an extreme environment defined by continuous darkness, oligotrophy and anaerobiosis with limited potential to sustain life. Its inaccessibility and dependence on drilling limit our understanding of such important ecosystems.

The Iberian Pyrite Belt (IPB), one of the largest massive metal sulfide deposits on earth, is located 250 km southwest of the Iberian Peninsula (Spain). Here is where Río Tinto raises up, an unusual extreme habitat due to its acidity and high concentration of heavy metals. Despite its extreme conditions, it exhibits a significant microbial diversity. Recent results suggest that the IPB subsurface acts as a huge underground bioreactor, where sulfide minerals are one of the main sources of energy, and the metabolic end-products of the microorganisms present in the solid matrix drain into the river.

Our research group has carried out a 340 meters depth drilling project to study the geomicrobiology of the IPB subsurface. The borehole exhibits a unique geochemical profile where mineralogical, ionic, and occluded gas compositions were measured, showing an acidic surface pH transitioning to neutral values with depth.

This work aims to analyze the microbiological and geochemical processes operating in the IPB deep subsurface by using fluorescence in situ hybridization (FISH) techniques. Through Catalyzed Reporter Deposition (CARD)-FISH we have found the coexistence of Bacteria and Archaea domains, suggesting that their metabolic collaboration is essential for the survival of microorganisms in the IPB subsurface.

Abstract ID: 829 / Poster Number: 310.16F

Study on the potential of Actinobacteria and their co-culture for Polyurethane Biodegradation

Presenting author: Dr. Moyinoluwa Akintunde, University of Applied Sciences, Aalen, Germany; Koladaisi University, Ibadan, Nigeria

Co-authors: *Surong Guo, Iman Taha*

Plastic waste is a major cause of global environmental pollution. Polyurethane (PU) a polymer extensively used in various industries, has significant environmental challenges due to low recycling rates and landfill accumulation. Use of PU as binders or in composites, poses difficulty for mechanical recycling, while chemical recycling presents environmental and health hazards. Biodegradation promises a potential solution to plastic pollution. Microorganisms such as Actinobacteria can utilize polymers as carbon sources, thus, contributing to their degradation. These bacteria produce metabolites capable of biodegrading polymers, providing green alternative for plastic waste management. This study assessed the biodegradation of commercial PU films by two groups of Actinobacteria and their co-cultures. Two Actinobacteria; Streptomyces violaceruber and Rhodococcus ruber were used for the biodegradation of PU films in minimal medium for 14 days. The biodegraded PU film was analysed by investigating changes in film colour, surface morphology, thermal properties, surface wettability, and surface energy. After 14 days of biodegradation, good microbial interaction between the two species was observed; visible colour changes were observed on the PU films, with a difference in colour (ΔE) and lightness (ΔL) values of up to -15.58 and 17.22. Pits and cracks were observed on the surface of the PU film by means of scanning electron microscopy. Further changes were observed in the glass transition and melting temperature. A 30% decrease in hydrophobicity and a 60% increase in surface energy were observed. These changes in the PU film prove the first stage of biodegradation and show the potential for further optimization.

Abstract ID: 1933 / Poster Number: 102.17P

Studying the regulation of spirochetal transcription with fluorescent light-up RNA aptamers

Presenting author: Ms. Vilma Trapp, University of Turku, Turku, Finland

Co-authors: *Janne Mäkinen, Sari Paavilainen, Verneri Nissilä, Juho Kotikoski, Ville Levola, Tingting Wang, Markus Wahl, Georgi Belogurov*

Spirochaetota are long, thin helical-shaped bacteria that are poorly characterized at the molecular level. Pathogenic spirochetes cause serious diseases such as syphilis, borreliosis, and leptospirosis, of which syphilis is showing early signs of antimicrobial resistance. Bacterial RNA polymerase is a validated target for antibacterial drugs and all Spirochaetota are naturally resistant to rifampicin, an inhibitor of transcription initiation. A non-pathogenic and alkalophilic *Spirochaeta africana* is a good model system to study spirochetal transcription and potentially identify new drug targets. To investigate the functionalities of spirochetal transcription factors CarD and DksA, we reconstituted *S. africana* transcription apparatus *in vitro* and monitored the transcription output of several spirochetal promoters using fluorescent light-up RNA aptamers (FLAPs). In our assays, RNA polymerase synthesizes a Broccoli FLAP according to the DNA template, the FLAP adopts a defined three-dimensional fold that binds a fluorogen, increasing its fluorescence. The results of the FLAP assays were further validated by quantifying RNA using Qubit and analyzing the RNA products on denaturing polyacrylamide gel. We show that CarD is an important activator of the ribosomal RNA promoters in *S. africana,* whereas DksA strongly downregulates ribosomal RNA transcription at neutral pH. We also demonstrate that the RNA polymerase β subunit Asn490 is the primary determinant of rifampicin resistance in *S. africana*. This information could be used in drug development against pathogenic spirochetes.

Abstract ID: 226 / Poster Number: 059.17P

Subinhibitory Meropenem Concentrations Alter Membrane Vesicle Production and Immune Response in Bacteroides fragilis

Presenting author: Assoc. Prof. Saniya Kozhakhmetova, National Center for Biotechnology, Astana, Kazakhstan

Co-authors: *Ayazhan Bekbayeva, Elena Zholdybayeva*

Introduction: Bacteroides fragilis is a prevalent intestinal bacterium that produces membrane vesicles (MVs), specialized structures transporting various substances, including virulence factors, from the cell. These vesicles are crucial for host-microbe interactions and bacterial stress adaptation, with their production potentially modified under antibiotic exposure.

Purpose: To investigate the effects of subinhibitory meropenem concentrations on B. fragilis membrane vesicle production and immune response modulation in an animal infection model.

Methods: In this study, we meticulously utilized the multidrug-resistant strain BFR KZ01 (BioProject: PRJNA531645). A total of 42 male Wistar rats were carefully divided into three groups (negative control, positive control, and experimental) for an abdominal infection model. Data were collected on days 8 and 16 post-infection, and a comprehensive analysis was conducted, including vesicle production via transmission electron microscopy alongside haematological, immunological, and histological parameters.

Results: The meropenem-treated group exhibited enhanced MV production compared to the positive control. Vesicle size was significantly larger in the experimental group on day 8 (95.00 nm versus 84.25 nm, $p = 0.053$). Both infected groups showed increased monocytes (21.5%). By day 16, the experimental group demonstrated enhanced macrophage activation, neovascularization, and fibrosis. Pro-inflammatory cytokine levels (IL-5, IL-6, IFN-γ, and G-CSF) increased on day 8 but decreased by day 16 in both infected groups compared to negative control.

Conclusion: Subinhibitory meropenem concentrations significantly influence B.fragilis membrane vesicle production, affecting vesicle size and immune responses, and ultimately promoting disease chronification.

Abstract ID: 2559 / Poster Number: 130.17P

Sub-lethal antibiotic concentrations induce Klebsiella pneumoniae biofilm formation through overproduction of matrix polysaccharides

Presenting author: Ms. Patrícia Coelho, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden; Uppsala Antibiotic Center, Uppsala University, Uppsala, Sweden

Co-authors: *Linus Sandegren*

Sub-lethal concentrations of antibiotics have complex, context-dependent effects on bacteria. While it seems logical that sub-inhibitory concentrations could have beneficial effects in preventing or disrupting bacterial infections, they have been shown to promote mutagenesis, DNA transfer, and adaptive bacterial responses. Additionally, 80–90% of bacteria in natural environments exist in biofilms, and exposure to sub-Minimum Inhibitory Concentrations (MIC) of certain antibiotics has been reported to induce biofilm formation in some species, likely as a protective response to stress. However, this phenomenon remains poorly understood.

To investigate this, we studied *Klebsiella pneumoniae* biofilm formation under sub-lethal antibiotic exposure and analysed its impact on the extracellular matrix and its regulatory mechanisms. Biofilm formation in eight *K. pneumoniae* strains was assessed using nine antibiotics at concentrations from 1/8 to 8 times the MIC. Biofilm biomass and cell viability were measured through crystal violet staining and colony-forming unit analysis, respectively, while matrix components, including polysaccharides, protein, and eDNA, were quantified using fluorescent and spectrophotometric methods.

Sub-MIC exposure to certain antibiotics increased biofilm biomass without affecting viability, suggesting extracellular matrix overproduction. Congo red staining revealed increased polysaccharide production, particularly cellulose, in response to cefotaxime and fluoroquinolones, highlighting cellulose as a key biofilm matrix component, while protein and eDNA levels remained unchanged.

These findings suggest that sub-lethal antibiotic exposure may trigger biofilm regulatory pathways or stress responses, enhancing biofilm formation and reducing susceptibility to treatment. Ongoing transcriptomic studies aim to uncover the regulatory mechanisms involved, underscoring the clinical implications of sub-MIC antibiotic exposure in biofilm-associated infections.

Abstract ID: 1290

Sub-lethal antibiotic concentrations promote biofilm streamer formation in Pseudomonas aeruginosa

Presenting author: Mrs. Alessia Di Claudio, Humanitas University, Pieve Emanuele, Italy

Co-authors: *Giovanni Savorana, Eleonora Secchi, Roberto Rusconi*

Introduction: Biofilms, microbial communities embedded in a self-produced extracellular matrix, represent a major challenge in clinical settings due to their role in developing persistent and chronic infections. Bacterial extracellular DNA (eDNA) has emerged as a fundamental component of biofilms, playing a key role in their structural integrity and antibiotic resistance. Our work focuses on streamers – filamentous, suspended biofilms occurring in flowing environments – where eDNA acts as structural backbone, essential for their assembly and stability.

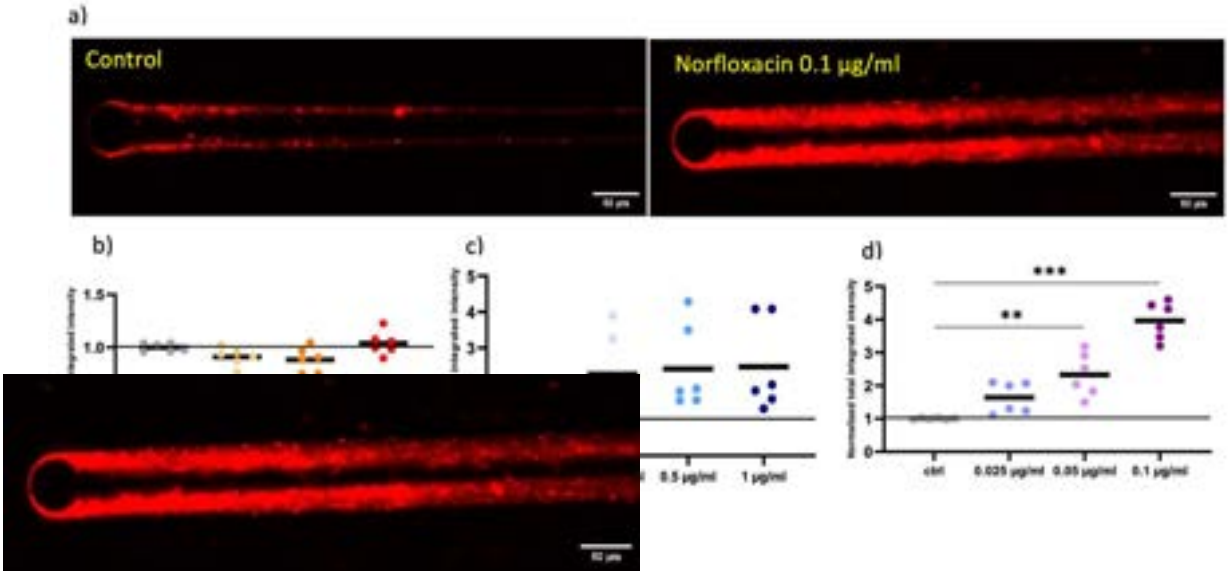
Objective: Despite their ability to lyse bacterial cells, antibiotics can also trigger the release of extracellular DNA (eDNA), which in turn promotes biofilm streamers formation. This study aims to explore the impact of sub-lethal antibiotic concentrations, commonly used for urinary tract infections, on the development of biofilm streamers by *Pseudomonas aeruginosa*.

Method: To investigate *Pseudomonas aeruginosa* biofilm streamers formation, we integrated microfluidics with microscopy and image analysis. Our approach utilizes a cutting-edge microfluidic platform equipped with isolated micropillars designed to induce the growth of streamers. This setup allows for precise control of the microenvironment and real-time observation of biofilm dynamics under sub-MIC antibiotic conditions.

Findings: We demonstrated that, while sub-lethal concentrations of antibiotics such as colistin, tobramycin, and norfloxacin reduce bacterial attachment to surfaces, they simultaneously induce cell lysis, which significantly enhances biofilm streamers formation (Fig.1). This effect is driven by the release of eDNA, which acts as a structural scaffold, facilitating streamer development and promoting biofilm growth in flowing environments.

Conclusion: Our findings highlight the profound impact of bactericidal antibiotics on eDNA-mediated processes and raises crucial questions about bacterial responses to suboptimal antibiotic exposure. Understanding these interactions is vital for refining antibiotic treatments and combating biofilm-related infections.

Figure 1. Sub-lethal antibiotic concentrations increase biofilm streamer production. Rappresentative fluorescence images of the biofilm streamers formed by Pseudomonas aeruginosa (a). Quantification of propidium iodide emission at 10 hours from the starting point of sub-lethal concentration of tobramycin (b), colistin (c) and norfloxacin(d).



Abstract ID: 3496 / Poster Number: 294.15P

Substrate recognition by the type III secretion system of enteropathogenic Escherichia coli

Presenting author: Mrs. Paula Andrea Monsalve-Agudelo, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

Co-authors: *Norma Espinosa, Bertha González-Pedrajo*

Enteropathogenic *Escherichia coli* (EPEC) employs a type III secretion system (T3SS) to translocate type III (T3) effector proteins into enterocyte host cells. During T3SS biogenesis, three types of substrates are secreted in a hierarchical manner: (i) early substrates, which form the inner rod and the needle; (ii) intermediate substrates, which form the filament and the translocation pore; and (iii) late substrates, or virulence effectors, which are translocated into host cells to subvert several signaling pathways and cellular processes.

Type III substrates require an N-terminal signal sequence to be recognized and translocated. However, a consensus sequence has not been identified, and the mechanism of substrate recognition remains poorly understood.

The aim of our study is to investigate the role of the distinct secretion signals present in each class of substrate in their differential recognition by the cytosolic components of the T3SS of EPEC, including the sorting platform, ATPase complex, and export gate components. Using secretion assays in wild-type and mutant strains of EPEC E2348/69, we evaluated various overproduced versions of T3 substrates lacking their recognition sequences, as well as chimeric substrates containing sequences from a distinct substrate category. Our data indicate that substrates may have more than one signal that allows their differential recognition by T3SS components. Overall, our results provide new insights into the complex molecular mechanisms that regulate protein secretion hierarchy during T3SS assembly.

Abstract ID: 427 / Poster Number: 063.17P

Substrate specificity of the Major Facilitator Superfamily efflux pumps of Acinetobacter baumannii in non-antibiotics transport

Presenting author: Dr. Wuen Ee Foong, University of South China, Hengyang, China

Co-authors: *Heng-Keat Tam, Xinxin Xiang*

Introduction: The use of medicine has greatly benefited human health but recent studies discovered that numerous pharmaceutical agents not traditionally prescribed as antimicrobials (hereafter referred to as non-antibiotics) significantly impact the composition of the human gut microbiome and inhibit the growth of primarily Gram-positive bacteria, indicating that non-antibiotics can exhibit antibacterial activity. Interestingly, non-antibiotics show no antimicrobial effects against most Gram-negative bacteria, suggesting an intrinsic tolerance to these compounds. Despite this, the molecular mechanisms, particularly involving single-component efflux pumps like the Major Facilitator Superfamily (MFS) transporters, remain poor.

Objectives: This study aims to elucidate the substrate specificity of non-antibiotics mediated by MFS efflux pumps, including the multidrug efflux pump CraA and the uncharacterized Bcr/CflA family efflux pump (named as DrtA) in *Acinetobacter baumannii*.

Methods: Drug susceptibility assays were conducted using:

2. *baumannii* strains with knockout mutations in MFS efflux pumps.
3. *Escherichia coli* strains overexpressing *A. baumannii* MFS efflux pumps.

Findings: Our results reveal the following:

1. CraA exhibits broad substrate specificity, transporting antibiotics, antiseptics, and antineoplastic agents.
2. DrtA confers resistance to non-antibiotics, including immunosuppressants and antineoplastic drugs.

Conclusion: This study highlights the substrate promiscuity of *A. baumannii* MFS efflux pumps, which mediate the extrusion of antibiotics, antiseptics, and non-antibiotics. Our findings provide valuable insights into the role of MFS transporters in multidrug resistance and suggest potential strategies to combat multidrug resistance by targeting these critical efflux systems.

Abstract ID: 2871 / Poster Number: 014.17S

Sugar Substitute Surprise: Increased Susceptibility to Salmonella Typhimurium Infection

Presenting author: Dr. Bidong Nguyen, ETH Zurich, Institute of Microbiology, Zurich, Switzerland

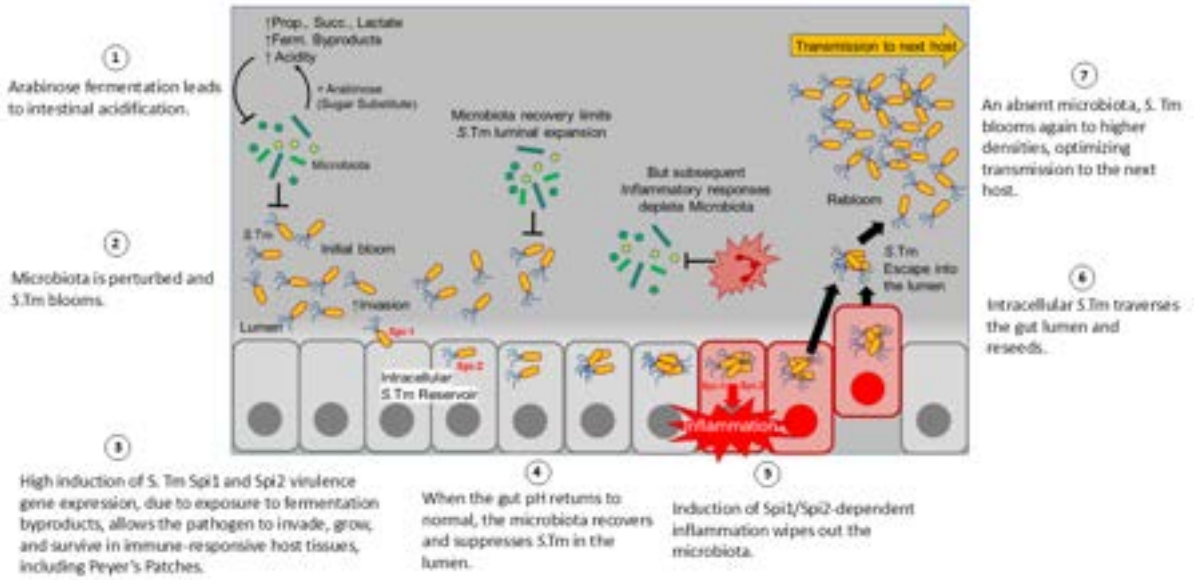
Introduction: Low-digestible carbohydrates (LDCs), which include dietary fibers and sugars that resist absorption, are a major component of our diet and are generally considered beneficial for gut microbiota. However, we found that ingestion of certain LDCs, particularly non-digestible sugars such as arabinose, lactulose, and polyols commonly used as sugar substitutes (e.g., erythritol, maltitol, and sorbitol), reduces colonization resistance against the foodborne pathogen *Salmonella* Typhimurium.

Objectives: To investigate the mechanisms by which LDCs promote *Salmonella* colonization and virulence in the gut.

Methods: Mouse infection models, liquid chromatography-mass spectrometry (LC-MS), 16S rRNA gene sequencing for microbial community profiling, and ELISA for immunological assessments.

Findings: Consumption of these sugars increased fecal shedding of the pathogen, systemic dissemination, and colitis in infected mice. To uncover the mechanisms underlying pathogen colonization, we focused on arabinose. This sugar is rapidly fermented by the microbiota, resulting in intestinal acidification, which leads to dysbiosis and subsequent pathogen blooms. Metabolite analysis revealed shifts in short-chain fatty acid levels that promote virulence gene expression and increased availability of amino acids, serving as carbon and nitrogen sources for pathogen growth. Arabinose consumption further impacts virulence by expanding tissue tropism, enabling efficient invasion of Peyer's patches, and modulating microbiota-pathogen interactions to select for fully virulent, invasive *Salmonella*.

Conclusions: Our findings demonstrate how specific dietary components, such as non-digestible sugars, disrupt the gut microbiota and enhance pathogen virulence. This study underscores the potential risks of certain dietary patterns in exacerbating susceptibility to enteric infections.



Summary Figure. Ingestion of arabinose promotes gut colonization and virulence in *Salmonella* Typhimurium.

Abstract ID: 1878 / Poster Number: 242.15P

Summer drought impacts soil bacterivore dynamics in a rewetted fen peatland

Presenting author: Die Hu, PhD, University of Greifswald, Greifswald, Germany

Co-authors: *Haitao Wang, Tim Urich*

Summer drought greatly affects soil microbiome diversity and functioning, especially in wetland. However, it is poorly characterized how bacterivores, performing crucial roles in the soil food web, respond to drought in rewetted peatlands.

We investigated the long-term dynamics of bacterivores in a temperate rewetted fen using 16s rRNA gene sequencing and quantitative metatranscriptomics. We screened the datasets for SSU rRNA transcripts, respectively of bacterivores such as bacterivorous protists (BPro), nematodes (BNem), bacteria (BBac), as well as Patescibacteria and DPANN archaea (PD) with a symbiotic or parasitic lifestyle. Also, transcripts of bacteriophages in the metatranscriptomes were analysed.

The 2018 summer drought led to a higher diversity of BBac. During the drought period, we found that the SSU rRNA abundances of all bacterivores increased, except for PD, although their peak periods were different. However, despite a small decrease, their abundances did not return to the pre-drought level after the drought ended, suggesting a long-lasting impact of the drought. This was more evident when assessing the relative contributions of bacterivores to prey bacteria dynamics. The contribution of BPro and BBac, the two most abundant bacterivores, increased significantly during the drought. However, all bacterivores had increased abundance in the post-drought period compared to pre-drought.

This study provides a holistic view of diversity and composition of different bacterivores in soil microbiomes, including PD and bacteriophages, and the long-lasting impact of summer drought on their dynamics. The strong responses of these bacterivores may lead to changes in ecosystem functioning through trophic interactions in a changing climate.

Abstract ID: 2866 / Poster Number: 385.17P

Supplementation strategies for the re-use of spent media from a Komagataella phaffii fermentation expressing β-glucosidase

Presenting author: Ms. Laura Murphy, University College Dublin, Conway Institute, Dublin, Ireland; BiOrbic Bioeconomy Research Centre, Dublin, Ireland

This study examined the re-usability of fermentation media from a Komagataella phaffii β-glucosidase expression system. β-glucosidase is a biotechnologically valuable enzyme in processes ranging from producing bioethanol from lignocellulose, to improving wine and fruit juice aromas. Following a 144-hour culture with a β-glucosidase overexpressing clone (IRA1), the secreted enzyme was purified via ion exchange chromatography (IEX). Our previous investigations have determined the consumption of elements and amino acids by K. phaffii during fermentation using ICP-MS and LC-MS/MS methods. These measurements have shown a near total depletion of amino acids with 17 of 20 being significantly reduced.

In this work, the elemental composition of β-glucosidase expression media at 144- hrs was analysed post-IEX purification of the secreted protein. The data showed that concentrations of magnesium, zinc, manganese, and iron were significantly reduced in the spent media to levels below the metabolic requirements of K. phaffii. Based upon this analysis, an optimal supplementation strategy to replace missing elements and amino acids was devised with the aim of recycling this spent media. This resulted in improvements in growth rates following the addition of peptone to cultures, along with increased β-glucosidase expression over unsupplemented spent media.

A potential benefit of this study is the description of a circular bioeconomy approach in an industrially relevant process. In addition, this process was evaluated through analysis of process mass intensity (PMI) and life cycle assessment (LCA) metrics using energy meters and appropriate calculations.

Abstract ID: 3245

Suppression of manure microbiota by a synthetic community mitigates ammonia emissions

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Co-authors: *Xia Li, Mingxiong He, De Ridder Dick, Jing He, Kupczok Anne*

Suppression of manure microbiota by a synthetic community mitigates ammonia emissions

Introduction: Ammonia emissions from livestock manure pose significant environmental and health risks including air pollution and water contamination. Synthetic microbial community (SynCom) has become a promising strategy for reducing these emissions. However, the interactions between members of the SynCom and the native manure microbial community are poorly understood. This hinders the application of SynComs in complex environments.

Objectives: We aimed to build a synthetic community of lactic acid bacteria (LAB SynCom) to mitigate ammonia emissions from manure microbiota. Then, we aimed to reveal the mitigation mechanisms of ammonia emission by the LAB SynCom.

Methods: To reveal the effects of interactions between the LAB SynCom and the manure microbial community on ammonia emissions, metagenomics was used to identify the ammonia-producing bacteria and their encoded biosynthetic pathways. Metatranscriptomics was employed to reveal the influence of the LAB SynCom on the manure microbial community and on the activity of ammonia biosynthetic pathways.

Results: During the treatment, the LAB SynCom rapidly colonized manure within 16 hours and its members became dominant with relative abundance of over 50%. At the early stage of colonization, the LAB SynCom played a leading role in utilizing carbon sources and depleting the availability of carbon sources to native manure microbiota members. The uptake of oligopeptides was significantly decreased for the native manure microbiota when the SynCom was added, compared to the untreated group. In the nitrogen metabolic network reconstructed from metagenomics data, ammonia emissions were generated mainly via the pathways of urea hydrolysis and amino acid deamination from manure microbiota. Transcriptional activities of key genes including ureC, ureB, and ureA encoding ureases in the urea cycle were significantly suppressed by the LAB SynCom in the treated group. In addition, transcript abundances of genes involved in the deamination of 8 amino acids were significantly decreased by the LAB

SynCom in the treated group. By coculturing the LAB SynCom and ammonia-producing bacteria, the LAB SynCom inhibited the growth and ammonia production of ammonia producers. We further revealed that lactate and antimicrobial peptide (PlnA) generated by the LAB SynCom functioned as the inhibitory metabolites in the antagonistic effect.

Conclusion: The LAB SynCom successfully colonized the manure environment due to the fast uptake of carbon sources and oligopeptides. The LAB SynCom mitigated ammonia emissions by decreasing microbial growth and activity of the nitrogen metabolic network of manure microbiota through antagonism.

Abstract ID: 775 / Poster Number: 021.17F

Surfaceome analysis of Bilophila wadsworthia reveals key factors in host-cell interaction

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Co-authors: *Lyzbeth Sayavedra, Arjan Narbad, Inês A.C. Pereira*

The human gut microbiota is crucial for maintaining physiological homeostasis, but its dysregulation (dysbiosis) has been linked to various diseases [1,2]. Sulfidogenic bacteria (SB), including *Bilophila wadsworthia*, produce hydrogen sulfide (H_2S) as a byproduct of their metabolism[3]. Excessive H_2S production by SB is cytotoxic, compromising the colonic epithelial barrier and mucus layer, triggering inflammation and contributing to conditions such as inflammatory bowel disease and colorectal cancer [4–6]. Using *Galleria mellonella* larvae as a model, we previously demonstrated that *B. wadsworthia* induces symptomatic infections, impairs the host immune system, and proliferates within the larvae, ultimately causing death. Despite these findings, the mechanisms by which *B. wadsworthia* interacts with human intestinal cells and promotes disease remain poorly understood.

This study aimed to elucidate the molecular structures involved in *B. wadsworthia's* interaction with host cells during the early stages of infection. A polarized monolayer of differentiated human intestinal epithelial cells was employed as an *in vitro* model. Surface proteins of different *B. wadsworthia* strains were removed using a shaving protocol, and adhesion, invasion, and translocation across the epithelium were assessed before and after proteolytic treatment. The shaved peptides were identified through mass spectrometry.

Our results demonstrate that *B. wadsworthia* adheres to and invades human intestinal cells. Key surface proteins were identified as essential for these bacterial-host interactions during early infection. These findings enhance our understanding of *B. wadsworthia* pathogenesis and highlight potential molecular targets for the development of novel therapeutics aimed at preventing bacterial adhesion and invasion.

Abstract ID: 3023 / Poster Number: 285.15P

Surfactants in glyphosate-based herbicides can reduce glyphosate biodegradation in anoxic soil microcosms

Presenting author: Mr. David Krappmann, ISWA, University of Stuttgart, Stuttgart, Germany

Co-authors: *Olivia F. Sieniawski, Chiara Lesuis, Daniel Straub, Carolin Huhn, Esteban Góngora, Katharine J. Thompson, Sara Kleindienst*

Glyphosate-based herbicides (GBHs) are the most widely used herbicides globally. Other than the active ingredient, formulations contain many other compounds, including surfactants as key co-formulants. Their purpose is to enhance the efficacy of the herbicide by improving spread and facilitating uptake. While surfactants are often referred to as 'inert', prior research on oil-degrading bacteria and wastewater microbial communities has suggested that they can have a direct impact on key microorganisms. Here we show that glyphosate, as well as the surfactant – and organic herbicide – pelargonic acid (PA), are degraded under oxic and anoxic conditions in agriculture soil microcosms. In oxic microcosms, glyphosate was rapidly degraded (in just 3 days) when either pure glyphosate or a GBH was applied. Additionally, the PA decreased over 14 days but remained in concentrations consistent with biotic controls at day 0. This suggests that the formulants have no broad-scale impact on the glyphosate-degrading microorganisms under oxic conditions. Glyphosate was also degraded under anoxic conditions, albeit at a much slower rate (~50 % over 70 days). Unlike under oxic conditions, glyphosate degradation in the anoxic microcosms occurred at differing rates between the pure glyphosate (~50 %) and GBH (~10 %) microcosms. While these results suggest that co-formulants impact glyphosate biodegradation under anoxic conditions, ongoing metagenome and -transcriptome data analyses from both the oxic and anoxic microcosms are needed to further investigate glyphosate-degrading microorganisms and the involved genes. Ultimately, determining the impact of surfactants on glyphosate biodegraders needs to be assessed across more environments and soil types.

Abstract ID: 1762 / Poster Number: 099.17P

Surveillance of Extended-Spectrum Beta-Lactamase-Producing Salmonella spp. Isolated from Human and Animals

Presenting author: Jong-Chan Chae, PhD, Jeonbuk National University, Iksan, South Korea

Co-authors: *Su Min Kwak, Jae Young Oh*

Salmonella enterica is one of the major causes of human foodborne poisoning globally. The present study aimed to evaluate the antimicrobial resistance and genotypes among *Salmonella* serovar isolated from humans and animals. A total of 216 *Salmonella* were isolated from humans. In addition, 73 isolates from pigs, 55 from breeding dogs, 43 from broiler chickens, 42 from layer chickens, and 4 from pets were obtained. Of the total 433 isolates, *S.* Typhimurium (STY) was the most common as 134 (30.0%), followed by 113 (26.1%) of *S.* Infantis (SIN), 49 (11.3%) of *S.* Enteritidis (SEN). STY strains were most prevalently obtained from pigs (n=67, 50.0%), followed by 60 (44.8%) from humans, and 7 (5.2%) from layer chickens, respectively. SIN strains were 43 (38.1%) from broiler chickens, 43 (38.1%) from breeding dogs, 23 (20.3%) from humans, and 4 (3.5%) from pigs. Among 216 human isolates, SENs carrying $bla_{\text{CTX-M-15}}$ were 7 (3.2%), while STYs carrying $bla_{\text{CTX-M-55}}$ were 14 (6.5%). Besides, 8 (3.7%) SINs contained $bla_{\text{CTX-M-65}}$. SINs carrying $bla_{\text{CTX-M-65}}$ account for 81.4% (34/43) from breeding dogs, 50.6% (43/85) from chickens, and 20.5% (15/75) of STYs from pigs. The STY belonging to sequence types (ST) 34 and 19 were predominant in both human and pig origins. The SIN belonging to ST32 were also common in human, breeding dog, and chicken origins.

Abstract ID: 33 / Poster Number: 178.15P

Survival mechanism of pancreatic tumor bacteria and their ability to metabolize chemotherapy drugs

Presenting author: Zara Ahmad Khan, PhD, Karolinska Institutet, Stockholm, Sweden

Co-authors: *Michał J. Sobkowiak, Mahin Ghorbani, Tajpara Poojabahen, Khaled Al-Manei, Asif Halimi, Zeeshan Ateeb, Volkan Özenci, Rainer Heuchel, Matthias Löhr, Margaret Sällberg*

Introduction: Microbes have extraordinary survival skills -their ability to adapt in harsh environment is part of their natural genetic evolution; how they adapt themselves in tumor-microenvironment (TME), metabolize environmental factors, degrade chemotherapeutics drugs and their survival mechanism to cope with harsh environmental conditions of TME is therefore of great interest.

Objective: Here in this project, we tried to explore the phenotypic and genotypic characterization of (Gammaproteobacteria and Bacilli) cultivated from intraductal papillary mucinous neoplasms (IPMNs) from treatment-naïve cases.

Methods: Phenotypic characterization and antimicrobial effect of chemotherapy drugs (5-FU and gemcitabine) was determined through half maximal inhibitory concentration for IPMN strains. We also studied if IPMN derived strain can metabolize them and verify drug's efficacy through different pancreatic cancer cell lines. Furthermore, the mechanism of survival of Gammaproteobacteria and Bacilli in IPMN cysts was studied through Whole Genome Sequencing (WGS).

Finding: Our results indicate that most of IPMN strains were sensitive to gemcitabine and resistant to 5-FU. However, we observe that they were able to degrade both gemcitabine and 5-FU. WGS bacterial profiling reveals that Gammaproteobacteria were two-fold functionally enriched compared to Bacilli. Moreover, specialized genes coding for antibiotic resistance, drug targets, virulence factors, and transporters were enriched in pyrimidine metabolism in Gammaproteobacteria and purine metabolism pathway in Bacilli.

Conclusion: Our discovery of a robust bacterial pyrimidine and purine nucleotide metabolism suggests a mechanism by which IPMN strains can survive within TME, potentially as a counter mechanism against nucleotide biosynthesis in TME.

Abstract ID: 3244 / Poster Number: 228.16P

Surviving unknown waters: The journey of Salmonella`s integral membrane effector proteins SseF and SseG into eukaryotic membranes

Presenting author: Ms. Sophie Schminke, University of Tübingen, Tübingen, Germany

Co-authors: *Sarah Trenz, Samuel Wagner*

Salmonella Typhimurium establishes its intracellular niche by injecting virulent effector proteins through a Type 3 Secretion System (T3SS) into its host cells. Among these T3SS-injected effectors are SseF and SseG. They possess two trans-membrane (TM) domains and are localized in the membrane of the *Salmonella*-containing vacuole by an unknown mechanism.

We hypothesized that SseF and SseG are targeted to the endoplasmic reticulum (ER) and are then inserted by the host cells membrane insertion mechanism. Frist, I used biotin identification (BioID) to analyze the microenvironment of SseF and SseG within a 10 nm radius after T3SS-injection and in-host cell translation of the effectors.

In the microenvironment analysis of T3SS-injected SseF, the signal recognition particle receptor-β (SRPR) and the δ-subunit of the translocon-associated protein complex were found as potential interaction partners. Both proteins play a part in membrane protein insertion and processing at the ER and hint towards a targeting of the effectors to the ER. For in-host cell translated SseF and SseG, both SRPR subunits and the SRP-54 were found. This suggest that they are co-translationally targeted to ER membranes when expressed in the host cell. Additionally, chaperones for protein folding and transport were found after T3SS-injection but not after in-host cell translation of the effectors.

To validate these potential protein-protein interactions, we are currently using fluorescent microscopy and co-immunoprecipitation. Further, we use siRNAs to determine how the knock down of the membrane insertion machinery affects the effector membrane localization.

Abstract ID: 2166

Susceptibility of Bacteriophages Phi 6, MS2, T7, and PhiX174 to a Multilevel Antimicrobial Polymer (MAP-1) Material

Presenting author: Dr. Leslie Anne Lopez Yasis, The Hong Kong University of Science and Technology, Hong Kong, China

Co-authors: *Rans Miguel Nunag Lintag, King Lun Yeung*

Introduction: The demand for antimicrobial materials has significantly increased in light of the recent pandemic and heightened concerns regarding zoonotic spillovers. One promising approach to mitigate these issues is the incorporation of antimicrobial agents onto surfaces, which offers prolonged protection against pathogens. Antimicrobial polymers are gaining traction due to their broad-spectrum efficacy against a variety of microorganisms and their potential to combat pathogens while reducing the risk of antimicrobial resistance.

Objectives: This study aims to assess the effectiveness of a multilevel antimicrobial polymer (MAP-1) assembly, which comprises a polyvinyl alcohol structural/carrier polymer, a polyethyleneimine penetrating polymer, and the antimicrobial agent polyhexamethylene biguanide. The evaluation focuses on four selected bacteriophages—Phi 6, MS2, PhiX174, and T7—representing both enveloped and non-enveloped viruses.

Methods: Virucidal tests were conducted using the MAP-1 system on the four bacteriophages at six contact times (0.5, 1, 5, 10, 30, and 60 minutes). A 100 µl sample of bacteriophage stock was mixed with 100 µl of the MAP-1 solution and incubated at room temperature with standard humidity. After incubation, a neutralizer was added, and the mixture was combined with host bacteria before being overlaid with soft agar on TSA plates. The plates were incubated overnight at 25°C or 37°C until clear plaques formed, which were then counted for analysis. Log reduction values were calculated to create disinfection curves for each bacteriophage, modeled using the Chick-Watson-Hom (CWH) equation. Additionally, high-resolution transmission electron microscopy and advanced spectroscopic techniques were employed to characterize treated and untreated virions and identify the inactivation mechanisms.

Findings: Three of the four bacteriophages demonstrated susceptibility to the MAP-1. Phi 6 achieved complete inactivation (>99.9%) after 10 minutes, T7 reached complete inactivation (>99.9%) after 30 minutes, and MS2 exhibited >99% inactivation after 60

minutes. In contrast, PhiX174 showed no significant reduction in viral load, even after 60 minutes of contact time. All bacteriophages displayed tailing behavior when analyzed with the CWH equation. Damage to the capsid, along with the denaturation of proteins, was observed, suggesting direct reactive interactions between MAP-1 and the viral components, which effectively disrupt the structural integrity of the bacteriophages. The alteration of the capsid structure could compromise the virus's ability to attach to host cells, thereby inhibiting its infectivity. Additionally, the denaturation of proteins is indicative of the breakdown of essential viral functions, which are critical for the virus's replication and survival.

Conclusion: This study underscores the differential susceptibility of the four bacteriophages to the MAP-1 system, with Phi 6 being the most susceptible, followed by T7, MS2, and PhiX174, as confirmed by the mechanistic study. The observed tailing-off behavior in the disinfection curves provides important insights for determining the optimal contact time necessary for effective use of this antimicrobial disinfection system.

Abstract ID: 511 / Poster Number: 172.16P

Susceptibility of Nakaseomyces glabratus to green gold nanoparticles synthetized with gallic acid

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Co-authors: *Maria Fernanda Costa, Anna Carolina Lage, Maíra Terra Garcia, Paulo Henrique Carmo*

Introduction: Nakaseomyces glabratus (formerly known as Candida glabrata) is the second-leading cause of candidiasis with low susceptibility to azole drugs. Gold nanoparticles exhibit antifungal activity, and novel technologies have emerged to produce eco-friendly green nanoparticles through natural compounds.

Objective: To evaluate the susceptibility of N. glabratus to green gold nanoparticles synthetized using gallic acid, a natural polyphenol.

Method: Gold nanorods capped with gallic acid (AuNpGA), previously synthesized and characterized, were tested against N. glabratus in planktonic and biofilm stages. In addition, its mode of action on fungal cells was investigated by the quantification of ergosterol levels and reactive oxygen species (ROS).

Findings: The minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were 1.56 µg/mL, demonstrating fungicidal activity of AuNpGA. Planktonic cultures of N. glabratus were significantly decreased after 2 h of treatment with AuNpGA (1.56 µg/mL) and eradicated after 8 h. Treatment with AuNpGA also reduced N. glabratus biofilms with statistically significant difference in relation to untreated group ($p<0.0001$). These fungal reductions were observed when AuNpGA were employed at 7.80 µg/mL, that corresponded to 5 times of MIC value. However, treatment with AuNpGA did not affect ergosterol levels of N. glabrata cells even in high concentrations. Promisingly, N. glabratus cells exposed to AuNpGA (1.56 µg/mL) had an increased production of ROS, with significant difference compared to untreated cells ($p<0.0001$).

Conclusion: Green gold nanoparticles capped with gallic acid had fungicidal action and anti-biofilm activity on N. glabratus via stimulating the ROS production.

Abstract ID: 1660 / Poster Number: 042.15P

Sustainable Approaches for Mitigating Biodeterioration of Romanian Cultural Heritage Objects and Churches

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Introduction/Objective: This study aims to address the knowledge gap regarding the antimicrobial efficiency of eco-friendly strategies in which plant-derived bioactive compounds and magnesium diboride (MgB_2) (nano)powders are combined. Our strategies are new potential solutions active against a diverse range of biodeteriogenic fungi specific for Romanian geographical/ historical/ architectural context. In this respect we performed different evaluation tests by *in vitro*, *in vivo* and *ex- situ* methods.

Method: Five hydroalcoholic extracts of *Allium sativum, Equisetum arvense, Polygonum hydropiper, Rosmarinus officinalis,* and *Ononis spinosa* characterized by UHPLC–MS/MS alongside four types of MgB_2 (nano)powders were assessed for their antimicrobial and antibiofilm effectiveness by adapted diffusion and minimum inhibitory concentration determination methods on a total number of 100 filamentous fungi isolates from wooden and stone Romanian churches and cultural heritage (CH) artifacts.

Findings: The plant extracts have proven to be rich in phenolic compounds. The quantitative *screening* of hydroalcoholic plant extracts against biodeteriogenic *Penicillium, Aspergillus, Cladosporium, Purpureocillium, Fusarium, Alternaria, Mucor* and *Rhizopus* microfungi demonstrated the efficiency of *E. arvense* > *P. hydropiper* > *R. officinalis* > *A. sativum* > *O. spinosa* (60.50% > 50.10% > 35.95% > 19% > 10.79%). Antifungal screening of (nano)powders identified two formulations with superior activity, particularly against *Cladosporium*, *Aspergillus*, and *Penicillium* spp. Moreover, the plant extracts effectively inhibited the adherence of *Penicillium* and *Aspergillus* strains to inert substratum.

Conclusion: The eco-friendly formulations characterized by a large spectrum of antimicrobial effectiveness could represent a sustainable substitute to conventional practices for CH conservation.

Abstract ID: 2711 / Poster Number: 378.17P

Sustainable On-Farm production of lactic acid bacteria inoculum for pig slurry nitrogen stabilization

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Pig farming generates large quantities of slurry, whose conventional management systems for storage and treatment contribute significantly to environmental pollution through the release of ammonia (NH3), greenhouse gases (GHG) such as N2O, CH4, and CO2, and unpleasant odors. The objective of this study was to develop a user-friendly, on-farm protocol for producing a complex microbial inoculum enriched in lactic acid bacteria (LAB) from pig slurry. This approach promotes acidogenesis in slurry pits and ponds, thereby stabilizing nitrogen and preserving its agronomic value. A three-step sequential protocol was employed in flasks containing 250 mL liquid pig slurry aimed at enhancing LAB growth and increase their concentration. This protocol included initial acidification, intermittent agitation to establish microaerophilic and anaerobic conditions, and the addition of a selective carbon substrate. Flasks were incubated for 24 hours at 30 ºC. Consistent pH decreases and significant LAB population increases measured by qPCR were observed throughout the protocol steps. Metataxonomic analysis confirmed the enrichment of microorganisms involved in organic acid production. This study provides a promising biological alternative for mitigating greenhouse gas emissions from pig slurry management.

Abstract ID: 3079

Sustainable Production of Polyhydroxybutyrate by Burkholderia thailandensis DSM 13276: Exploring Eucalyptus Bark Hydrolysate Valorization and Enzymatic Biopolymer Recovery

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Co-authors: *Thomas Rodrigues, Cristiana Torres, Maria Ascensão Reis, Filomena Freitas, Susana Marques, Francisco Gírio*

The overuse of fossil-based plastics has led to significant environmental challenges, including their persistence in ecosystems and the proliferation of microplastic pollution. Polyhydroxyalkanoates (PHAs) have emerged as a biodegradable alternative; however, their high production costs have delayed large-scale commercialization. Renewable lignocellulosic biomass, such as eucalyptus bark, presents a sustainable solution aligned with circular bioeconomy principles. *Burkholderia thailandensis* DSM 13276 has demonstrated remarkable metabolic versatility in utilizing synthetic C5 and C6 sugars, achieving yields exceeding 60% in PHA accumulation. This makes it a highly promising candidate for sustainable biopolymer synthesis using C5 and C6 sugar-rich eucalyptus bark hydrolysates. This study aimed to evaluate the impact of substrate variability in eucalyptus bark enzymatic hydrolysates on the production of polyhydroxybutyrate (PHB) by *B. thailandensis* DSM 13276. Additionally, an enzymatic extraction method for PHB recovery was implemented as a sustainable alternative to traditional solvent-based methods. Eucalyptus bark was pretreated using steam explosion and enzymatic hydrolysis, generating three hydrolysates characterized for key properties (pH, sugars, ammonium, protein, and inhibitors such as furfural and 5-HMF). *B. thailandensis* was cultured in 2 L batch bioreactors under controlled conditions (30 °C, pH 7.0, 30% dissolved oxygen), with periodic sampling for CDW, PHB content, sugars and ammonium consumption. PHB was extracted using Alcalase enzymatic digestion (pH 8.3, 50-55 °C) and compared to chloroform-based methods. Structural and thermal characterization was performed using FTIR, SEC, DSC, TGA, and XRD. Hydrolysate composition significantly impacted PHB production. High C/N ratios (160.7) and glucose concentrations (>80 g/L) favored cell growth (CDW = 9.48 g/L) but reduced PHB accumulation (19 wt%). Lower C/N ratios (41.5) improved PHB content (60 wt%) and

productivity (1.36 g/L·day). Enzymatic extraction achieved comparable purity (100 ± 3%) and efficiency (96%) to solvent-based methods, demonstrating greater sustainability. PHB properties, including molecular weight (2.77×10^5 to 6.30×10^5 Da), melting temperature (170–175 °C), and thermal stability (T_{deg} = 280–297 °C), were consistent across samples. Eucalyptus bark enzymatic hydrolysates offer a highly promising and sustainable feedstock for efficient PHB production by *B. thailandensis* DSM 13276, with optimal results achieved through C/N ratio adjustments and the elimination of inhibitory compounds. Integrating renewable hydrolysate valorization with eco-friendly PHB recovery methods significantly enhances PHA production efficiency, fostering more sustainable manufacturing and advancing toward a greener, more resilient circular bioeconomy.

Abstract ID: 3110 / Poster Number: 079.16P

Sweet Science: Investigating how carrageenan and a prebiotic fibre alter the gut microbiome

Presenting author: Ms. Hannah Thomas, University of Reading, Reading, United Kingdom

Co-authors: *Anisha Wijeyesekera, Gemma Walton, Jessica Adams*

Carrageenan, a hydrocolloid derived from red seaweeds, is widely used as a functional food ingredient. Despite being deemed “safe for human consumption” by EFSA, its use remains controversial due to potential links with intestinal inflammation. However, as a polysaccharide resistant to enzymatic digestion, carrageenan may also exhibit prebiotic properties. Preliminary in vitro experiments show that at high dosage carrageenan enhances the growth of the genera Bacteroides and Escherichia/Shigella. At dietary relevant low levels, carrageenan has little impact on the microbiome however when the prebiotic fibre inulin is added there is proliferation of beneficial microbial species and an increase in bacterial load.

The objective of this study is to assess the impact of carrageenan on the gut microbiome when integrated into a food product and combined with a prebiotic fibre. This study employs a three-armed, double-blind, parallel intervention design. Participants will receive one of three interventions in the form of gummy sweets: a placebo (using an alternative gelling agent), a carrageenan-containing gummy, or a gummy containing both carrageenan and a prebiotic. Urine and stool samples will be collected at baseline, after a 4-week intervention period, and following a 4-week washout period. Participants will self-report stool frequency, stool consistency, and gastrointestinal symptoms through online surveys.

Microbial composition and abundance will be analysed using 16S rRNA sequencing and FISH-flow cytometry, respectively. Metabolite production in stool and urine samples will be assessed using 1H-NMR spectroscopy.

Abstract ID: 1134 / Poster Number: 084.17P

Symmetrical and Unsymmetrical Bisacridines as Potential Antifungal Agents with Ability to Overcome Fluconazole Resistance

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Co-authors: *Kamila Rząd, Ewa Paluszkiewicz, Mateusz Olszewski, Iwona Gabriel*

Bisacridines, known for their DNA-binding and inhibition of human topoisomerase IIα, have potential as anticancer agents[1], but their antifungal activity is underexplored. Our previous research indicated bisacridines as promising antifungal agents targeting yeast topoisomerase II (yTopoII) [2]. Among the compounds synthesized by us, IE10 exhibited unique properties [2] and thus it was used as a lead compound. Novel synthetized derivatives IKE18, IKE19, and IKE21 exhibited stronger yTopoII inhibitory activity and higher antifungal efficacy against five reference strains compared to IE10. Unlike IE10, these compounds overcame fluconazole resistance. To assess if this phenomenon involves inability of multidrug-resistance(MDR) pumps to efflux these compounds, we used S. cerevisiae AD mutants overexpressing genes encoding CDR1, CDR2, and MDR1 C. albicans drug-efflux pumps and compared them to a reference strain lacking seven MDR transporters [3–5]. The most potent derivatives, IKE18 and IKE21 exhibited weak substrate activity for Cdr1p and Cdr2p efflux pumps while retaining high antifungal activity against strains overexpressing CDR1 and CDR2 genes. None of the bisacridines seemed to be substrates for Mdr1p efflux pump. Cytotoxicity assays identified IKE18 as the most selective antifungal compound. These findings support further development of bisacridines as antifungals by designing and evaluating new yTopoII inhibitors.

Abstract ID: 1803 / Poster Number: 026.16P

Synbiotcis improve gut microbiota composition and SCFA levels in weaned piglets

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Introduction: Weaning stress disrupts gut microbiota in piglets, reducing nutrient digestibility and increasing intestinal inflammation. Synbiotics, combining prebiotics and probiotics, offer a potential strategy to modulate gut microbiota, enhance short-chain fatty acid (SCFA) production, and improve gut health. However, the effects of *Lactobacillus reuteri* and levan-based synbiotics on microbial interactions and SCFA production are not fully understood.

Objective: This study evaluated the effects of a synbiotic treatment combining *L. reuteri* CACC607 and a levan-based prebiotic on gut microbial composition, SCFA production, and gut health in weaned piglets.

Methods: Sixty-eight weaned piglets were randomly assigned to four dietary treatments: (1) a basal diet (Con), (2) a basal diet with 0.5% levan-based prebiotic (Pre), (3) a basal diet with 0.5% *L. reuteri* CACC607 (Pro), and (4) a combination of both (Syn). Microbial diversity, SCFA concentrations, and microbial networks were analyzed using 16S rRNA gene sequencing, ITS sequencing, fecal SCFA profiling, and microbial correlation networks.

Result: The Syn group exhibited the highest total SCFA levels, with significant increases in acetic acid, propionic acid, and butyric acid ($p < 0.0001$). Alpha and beta diversity analyses showed no significant differences between treatments, but the Syn group had significantly enriched genera, including *Anaerostipes*, *Eggerthellaceae_unknown*, and *Tyzzerella 3* ($p < 0.05$), associated with SCFA production and gut health. Microbial networks demonstrated the Syn group fostered the highest positive interactions among beneficial bacteria, with no negative correlations observed.

Conclusion: Synbiotics combining *L. reuteri* CACC607 and a levan-based prebiotic enhance SCFA production, modulate gut microbiota composition, and promote beneficial microbial interactions, improving nutrient utilization and gut health in weaned piglets.

Abstract ID: 2932 / Poster Number: 039.17P

Synergistic antibacterial effect of methyl ester sulphonate detergent and magnetic nanoparticles on antibiotic-resistant bacteria

Presenting author: Dr. Anna Vareschi, Università degli Studi di Verona, Verona, Italy

Co-authors: *Diana Madalina Gaboreanu, Ilda Czobor Barbu, Salvatore Calogero Gaglio, Maria Teresa Valenti, Luca Giuseppe Dalle Carbonare*

Introduction: Hospital infections have a significant economic and clinical impact on the healthcare system.

The high recurrence rates and the rise of antimicrobial resistance demonstrate that the transmission of infectious agents is not effectively counteracted.

Objective: This study aimed at validating the efficacy of a new nanofluidic ecological detergent, formulated starting from waste oil, in combination with magnetic nanoparticles against bacteria commonly found in hospital infections. This is intended then to be used in automated washer-disinfector devices present in hospitals.

Method: The methyl ester sulphonate detergent, as well as the magnetic nanoparticles, were tested either alone and in combination with each other on 30 E. coli strains, which showed several resistance profiles. The effects of the detergent and the nanoparticles were assessed through MIC and MBC tests.

Findings: The detergent was shown to be more effective in combination with the nanoparticles, having either a growth inhibition and a bactericidal effect. In particular, two types of nanoparticles were tested: silver magnetic ones and biomimetic magnetic nanoparticles (BMNPs), mediated by MamC, a *Magnetococcus marinus* MC-1 magnetosome-associated protein. These demonstrated to have similar effects.

Conclusion: The results obtained showed that the detergent and the nanoparticles together not only have an inhibitory effect on bacterial growth, but also a bactericidal one. The sustainability of the process by which the detergent is formulated is another important point of this experimental design. These compounds have to be further tested on hospital washer disinfector, but these results seem promising.

Abstract ID: 3310 / Poster Number: 379.16P

Synergistic effects of Lactobacillus gasseri KML25 and Sasa quelpaertensis leaf extract fermentation on the functional and metabolic activities of milk

Presenting author: Mr. Ji Seung Han, Korea University, Sejong, South Korea

Co-authors: *Nam Su Oh*

We developed synbiotics by combining *Lactobacillus gasseri* KML25 (KML25) and *Sasa quelpaertensis* leaf extract (SQ), both selected for their superior antioxidant activities among six probiotic candidates and four plant extracts, respectively. SQ enhanced KML25 growth in minimal medium, but antioxidant activities (ABTS, DPPH radical scavenging activity, FRAP, TPC, and TFC) did not show a significant synbiotic effect. In milk, KML25 alone exhibited minimal growth, but the addition of SQ significantly promoted its proliferation. Furthermore, fermented milk supplemented with SQ (FMSQ) demonstrated markedly higher antioxidant activities compared to milk (M) and milk supplemented with SQ (MSQ). To further understand the metabolic mechanisms, we analyzed the transformations of phenolic compounds generated during MSQ fermentation. Additionally, peptide sequences generated during fermentation were identified using LC-MS/MS through de novo sequencing. Whole-genome sequencing of KML25 revealed key genes involved in phenolic compound metabolism and bioactive peptide production, highlighting its potential role in the observed functional properties. Anti-inflammatory activity was evaluated using LPS-induced RAW 264.7 macrophages. FMSQ significantly reduced NO production while decreasing the expression of key inflammatory mediators, including TNF-α, COX-2, IL-1β, and IL-6, when compared to MSQ. In a DSS-induced colitis mouse model, FMSQ reduced disease activity, spleen index, and mucosal damage while increasing colon length and tight junction marker expression. These effects were mediated via the TLR4/MyD88/NF-κB pathway. In conclusion, FMSQ demonstrates synergistic antioxidant and anti-inflammatory effects, promoting phenolic compound biotransformation and bioactive peptide generation, highlighting its promise for improving gut health and managing inflammatory diseases.

Abstract ID: 3002 / Poster Number: 204.16P

Synergistic efficacy of phytochemical combinations against Bovine alphaherpesvirus 1 (BoAHV-1)

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Co-authors: *Sonja Gostimirovic, Darija Vukovic, Isidora Nikolic, Verica Aleksic Sabo, Gospava Lazic, Petar Knezevic*

Introduction: Phytochemicals are naturally occurring compounds found in plants that exhibit a wide range of biological activities. The growing interest in using phytochemicals as antiviral agents arises from their diverse mechanisms of action and ability to target various stages of the viral multiplication cycle. Moreover, combining different phytochemicals has emerged as an effective strategy for treating viral infections. *Bovine alphaherpesvirus 1* (BoAHV-1) causes Infectious Bovine Rhinotracheitis in cattle and leads to significant economic losses, while also serving as a model for herpesviruses *in in vitro* studies.

Objective: This study aims to evaluate the potential antiviral activity of phytochemical combinations against the BoAHV-1 and to determine their possible synergistic interactions.

Methods: The phytochemicals used in combinations in this study are quercetin, rutin, baicalein, epicatechin, and ethyl gallate. Synergistic activity was assessed using the Checkerboard method. The synergy was used alongside the calculation of fractional inhibitory concentration indices (FICI) to determine the antiviral activity of the paired phytochemicals.

Findings: Synergistic activity was observed when the FICI was less than 0.5. Significant synergy was demonstrated by quercetin in combination with rutin (FICI=0.28-1.25), baicalein (FICI=0.22-1.13), epicatechin (FICI=0.09-0.1.03), and ethyl gallate (FICI=0.05-1.02). The synergistic effects were also observed with rutin in combination with baicalein (FICI=0.16-1.09), epicatechin (FICI=0.09-1.03), and ethyl gallate (FICI=0.06-1.02), as well as by epicatechin and baicalein (FICI=0.13-1.13) and baicalein and ethyl gallate (FICI=0.09-1.02). At higher concentrations, the synergistic effect was demonstrated between epicatechin and ethyl gallate (FICI=0.19-1.06).

Conclusion: Tested phytochemicals exhibit synergistic effects when combined, suggesting their potential effectiveness in treating viral infections.

Abstract ID: 2602 / Poster Number: 318.17P

Synergistic enhancement of thermal stability and enzymatic activity; Tailored NADES for mutated Trichoderma sp. Cellulase

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Co-authors: *SEUNGJUN KIM, JEONGHEE YUN*

Natural deep eutectic solvents (NADES) are promising biocompatible co-solvents for enzymes, effectively addressing issues of low stability and activity under severe physicochemical conditions. The effect of NADES varies depending on the types of enzyme and environmental factors; therefore, it is crucial to utilize the optimal NADES tailored to the enzyme to achieve superior stability and enhanced activity. This study investigated the impact of NADES on cellulase activity derived from the mutated Trichoderma sp. Mut-4 to determine the optimal NADES. Four types of NADES, formulated by combining a Hydrogen Bond Acceptor (HBA) and a Hydrogen Bond Donor (HBD) were incorporated into the enzyme. The effects on thermal stability and activity were evaluated by varying the molar ratio of HBA to HBD, and the concentration of NADES. The addition of betaine/glycerol NADES resulted in a 1.05-fold higher initial activity and 1.29-fold higher residual activity following 48 hours of incubation at 50°C compared to the control group. While a significant trend in activity enhancement was not observed concerning the molar ratio, an increase in the HBD ratio improved thermal stability. As the concentration of NADES increased, the enhancement of activity decreased, whereas thermal stability improved. This study demonstrated that betaine/glycerol is the optimal NADES for enhancing both the initial and residual activity of Mut-4. It also revealed trends in activity and stability depending on the ratio of HBD and the concentration of NADES. These findings emphasize the importance of optimizing manufacturing and additive conditions to improve enzymatic hydrolysis efficiency.

Abstract ID: 3133 / Poster Number: 168.17P

Synergistic potential of bacteriocins and bacteriophages of Klebsiella pneumoniae MV91-1 for the control of antibiotic resistant-strains

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Co-authors: *Alberto Aragón Ramírez, Rubén Jurado Escobar, Josué Jara Pérez, Belén Órgaz Martín, Leonides Férnandez Álvarez*

Introduction: Antimicrobial resistance (AMR) represents an important health challenge and a serious

global threat. Recent studies suggest that by 2050, AMR could be associated with over

8 million deaths worldwide. Therefore, new strategies targeting pathogens are required

to reduce the dissemination of resistant strains.

Objective: This study aims to combine bacteriocins and bacteriophages from *Klebsiella pneumoniae* MV91-1, a strain isolated from human milk, to inhibit Gram-negative resistant strains.

Method: Genomes of forty Gram-negative strains isolated from food and food contact surfaces were firstly analysed to look for metabolites of interest, antibiotic resistance, virulence genes, and bacteriophage sequences. The production of bacteriocins was confirmed by phenotypic analysis. Isolated and purified bacteriophages were morphologically characterised by transmission electron microscopy. Antimicrobial activity of bacteriocin- and bacteriophage-containing cell free supernatants (CFS) and that of purified bacteriophages was tested towards target strains, monitoring growth curves via microplate reader. Biofilms of sensitive species received the same treatment and dispersion effects were visualised with confocal microscopy.

Findings: Genomic analysis of *K. pneumoniae* MV91-1 revealed two microcin-type bacteriocins and two complete *Caudoviricetes* bacteriophages. After treatment with CFS and purified bacteriophages, growth of target strains was reduced by up to 50% and 40%, respectively. Treatment of *Escherichia coli* biofilms with bacteriocin- and bacteriophage-containing CFS achieved a reduction of up to 82% in biovolume.

Conclusion: Bacteriocins and bacteriophages produced by *K. pneumoniae* MV91-1 act synergistically against genetically related antibiotic-resistant bacteria. This combination therapy could offer new possibilities to overcome AMR.

Abstract ID: 3549 / Poster Number: 397.15P

Synergy of Theory and Experiments in Understanding Viral Population Dynamics and Evolution

Presenting author: Mara Laguna-Castro, PhD, Centro de Astrobiología (CAB), Madrid, Spain

Co-authors: *Ester Lazaro, Jaime Iranzo*

Explanatory models are widely used to describe the dynamics of viral populations, providing insights into viral evolution, host interactions, and the effects of mutations. These models rely on simplifying complex biological systems to isolate key mechanisms driving the process under study. By stripping away variables in a specific hypothetical scenario, it becomes possible to identify which factors are relevant for that process and to stablish causal relationships among them. Determining the simplest form of a model that retains its explanatory power is critical for assessing its value (1). The parallel development of explanatory models and experimental validation of their predictions is a proven methodology for enhancing model quality.

The aim of this study was to experimentally validate a toy model that predicted the dynamics of a virus population propagating in two different scenarios: high host availability and host scarcity. The model predicted that, when hosts are scarce, the fitness of the virus strongly depends on its adsorption-entry capacity. In contrast, when hosts are abundant, such dependency disappears and the viral fitness becomes primarily determined by the virus productivity.

Using experimental evolution techniques, several duplicate virus lineages derived from a common ancestor of the bacteriophage Qβ were propagated through sixteen serial transfers at decreasing concentrations of *E. coli* (Fig. 1). A concentration of 10^8 cfu/mL was used as the control condition (host abundance), while concentrations of 10^7 cfu/mL and below represented increasingly restrictive host scarcity conditions. At the end of the evolution process, lineages were characterized to determine if they had adapted to host scarcity and, if so, which mutations were involved.

Results showed that all virus lineages evolved under host scarcity conditions presented a common mutation at position 2011 of the viral genome, absent in the control lineages. The effects of this mutation were determined in isolation, with no other mutations present in the viral genome. Mutation C2011A was found to significantly enhance the virus's adsorption-entry capacity, though it reduced the burst size (virus productivity) (2).

In light of these observations, it can be concluded that the experimental results confirm the model's prediction. Under host scarcity conditions, where finding hosts to infect is challenging for the virus, a mutation increasing adsorption-entry capacity is essential for virus survival, even at the cost of reduced productivity. Conversely, when hosts are abundant and enhancing the adsorption-entry capacity is unnecessary, the associated decrease in productivity makes the selection of this mutation disadvantageous.

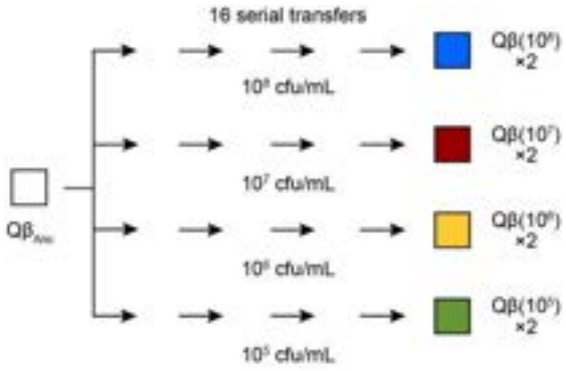


Figure 1: Scheme of the experimental procedure. Starting from the same ancestral virus, several evolved populations were obtained by propagating the viral population at different host concentrations.

Abstract ID: 1251 / Poster Number: 363.17P

Syngas valorization into the fine chemical hydroxyectoine by Hydrogenibacillus schlegelii

Presenting author: Dr. Sara Cantera, University of Valladolid, Valladolid, Spain

Co-authors: *Eva Marcos Rodrigo, Raúl Muñoz, Raquel Lebrero*

The development of innovative green chemical production processes utilizing syngas from waste gasification represents a significant advancement toward establishing sustainable industries based on circular economy. Nevertheless, the limited number of studied microorganisms and products constrains the economic viability of these processes. Hydroxyectoine, a high-value osmolyte for the pharmaceutical industry, has not yet been biologically synthesized as an isolated osmolyte. Our research aimed to establish an innovative process for the valorization of syngas into high-value compounds. First, we identified potential microorganisms using genome mining and corroborate the results using MAGs, selecting the best strain for this process: Hydrogenibacillus schlegelii. Physiological cultivations with syngas as the sole carbon and energy source were established to validate the concept, further optimization in batch bioreactors showed the optimal salinity, temperature and CO:H_2 ratio. Finally, a continuous bioreactor was operated with the optimal conditions to asses the best operational strategies. Laboratory validation confirmed H. schlegelii's ability to convert syngas into hydroxyectoine. Optimal syngas-to-hydroxyectoine conversion was achieved under conditions of 4% NaCl, 65ºC, and a CO:H_2 ratio of 70:10 (% v/v), yielding a maximum hydroxyectoine content of 44.8 ± 10.1 mg·g biomass^{-1}. The continuous bioreactor, operated at 65 °C with 4% NaCl, a gas residence time of 2 hours and a dilution rate of 0.15 d^{-1}, achieved intracellular and extracellular hydroxyectoine concentrations of 22.6 ± 0.5 mg·L^{-1} and 28.4 ± 0.9 mg·L^{-1}, respectively. This research demonstrates the feasibility of the transformation of syngas into high valuable pharmaceuticals.

Abstract ID: 2571 / Poster Number: 468.15S

Synthetic upgrades for modern expression systems

Presenting author: Dr. Andres Felipe Carrillo Rincon, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates

Co-authors: *Andras Gyorgy, Natalie Farny*

The capability to predict protein structures and functions is rapidly gaining attention in the research community. However, the only "chemists" capable of turning these predictions into reality are microbial cells. Current gene expression systems for producing recombinant proteins, such as the pET system, have been around for nearly 40 years. While these systems have been invaluable for advancing biotechnology, they are largely restricted to specific bacterial hosts—most commonly *E. coli*. Moreover, their high expression levels often come at the expense of tight regulation, making it challenging to maintain an effective "OFF" state. These expression systems rely on the efficiency of a promoter, typically composed of two key elements: the operator region, which is recognized by transcriptional regulatory proteins (e.g., LacI, TetR, CymR), and the −10 and −35 consensus sequences that are essential for recruiting the sigma (σ) 70 subunits of RNA polymerase to initiate transcription. Advances in synthetic biology tools, combined with a deeper understanding of transcriptional and translational regulation, have enabled us to improve these expression systems, significantly enhancing both their productivity (yielding higher recombinant protein production) and portability (making them functional across different bacterial chassis). First, transcriptional performance was improved by optimizing the consensus sequences recognized by the σ^{70} factor. These modifications not only boosted the production of target recombinant proteins but also expanded the versatility of the expression systems, allowing them to function across a broader range of bacterial hosts. Second, translational "padlocks" (riboswitches) were incorporated to better control the uninduced state, effectively minimizing basal expression and enhancing regulation. Third, precise regulation of the transcriptional regulators to achieve varying degrees of target gene expression. These modifications significantly enhanced the efficiency of widely used expression systems, as evidenced by several outcomes: increased plasmid copy numbers, higher recombinant protein yields across various bacterial hosts, and successful production of a range of proteins and compounds. We anticipate that this "next generation" of genetic engineering tools will significantly streamline the production of recombinant proteins and bio-compounds.

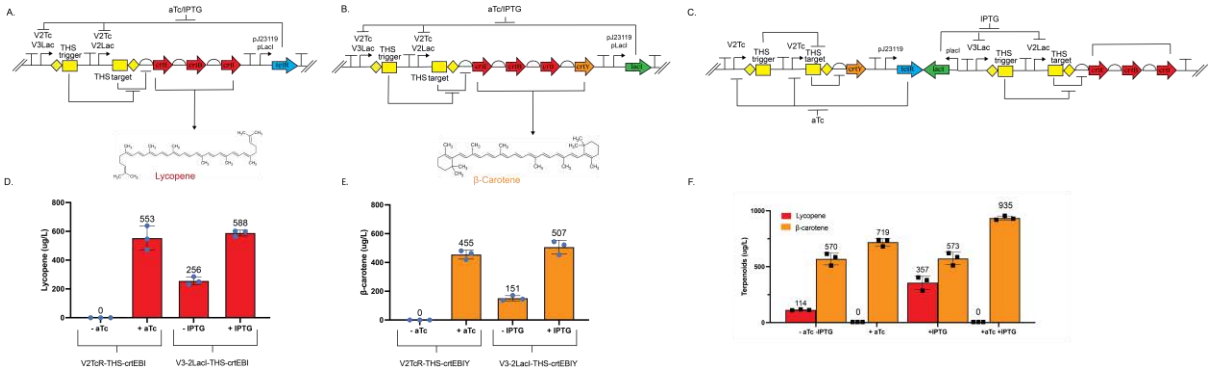


Figure 1: General design of the σ70 expression systems controlling the expression of A. Lycopene B. β-carotene and C. Lycopene & β-carotene. D.E & F. Titters of the terpenoids produced by A. B. & C. respectively.

Abstract ID: 1594 / Poster Number: 352.15F

Systematic exploration of plant-microbe antagonism using the unicellular microalgae Chlamydomonas reinhardtii

Presenting author: Ms. Tzila Vikniansky, The Hebrew University of Jerusalem, Jerusalem, Israel

Co-authors: *Elad Meilin, Noemie Alon, Michal Breker Dekel, Omri Finkel*

Research on negative microbial effects on plants is largely focused on acute plant diseases, but many microbes in the soil can cause more subtle, chronic effects on plants. In order to systematically delve into the complexity of plant-microbiota interactions, we established a unique genome-wide approach utilizing the unicellular green algae *Chlamydomonas reinhardtii*. We tested for the effect of ~150 bacterial strains isolated from *Arabidopsis thaliana* roots on *Chlamydomonas* growth, and found a strong correlation of antagonistic effect towards both *Chlamydomonas* and *Arabidopsis*. To test whether the antagonism was specific to Archaeplastida, we tested their effects on *E.coli*, *B. subtilis* and *S. cereviciae*. We found that *Burkholderia* strains (MF6 and MF7) uniquely inhibited *Arabidopsis* and *Chlamydomonas*. In order to explore which genes within MF6 are involved in the inhibition, we constructed a transposon library of 5000 mutants. We found that 50 mutants lost their inhibition capacity and are now being genetically characterized. Reciprocally, we sought to understand which *Chlamydomonas* functions are targeted by the bacteria. To this end, we isolated a UV-mutagenized *Chlamydomonas* strain that is resistant to MF6 inhibitory effect. Further genetic analysis discovered an early stop codon mutation within Zinc Iron Protein 3 (ZIP3), and its complementation by WT allele recapitulated inhibition. We demonstrate how unicellular model systems can rapidly identify genes in both the host and the bacteria, with a potential for optimizing plant microbiota towards a more cooperative community.

Abstract ID: 1864

Tailor made fermentation of sprouted wheat and barley flours and their application in breadmaking: a comprehensive comparison with conventional approaches in the baking industry

Presenting author: Assoc. Prof. Erica Pontonio, University of Bari-Department of Soil, Plant and Food Sciences, Bari, Italy

Co-authors: *Giuseppe Perri, Lorenzo Ciraldo, Federico Rametta, Gaia Gadaleta-Caldarola, Graziana Difonzo, Marco Gobbetti, Hana Ameur, Maria De Angelis, Francesco Caponio,*

In recent years, the food industry has seen significant changes as consumers now expect more from their food than just basic nutrition. Wheat flour, a key ingredient in most baked goods, is an important source of carbohydrates and contributes to around 20% of the world's daily energy intake. However, refined wheat flour lacks essential amino acids like lysine, methionine, and threonine, which lowers its protein quality compared to other grains. Barley (*Hordeum vulgare* L.) is an excellent alternative due to its higher soluble dietary fiber, particularly β-glucans, and better levels of lysine, an essential amino acid important for human health. Despite this, barley's bread-making potential has been limited by technological challenges. Both sprouting and fermentation have shown promise in improving the performance and sensory qualities of barley-based baked goods, but no research has yet focused on selecting specific lactic acid bacteria (LAB) strains tailored to these flours.

This study aimed to address this gap by selecting and testing twelve strains of LAB for their ability to ferment sprouted wheat and barley flour. The most effective strains were then combined into optimized pools to enhance fermentation. The goal was to develop new bio-ingredients (type III sourdough) from these sprouted flours, which were then used in bread-making trials. The resulting breads were evaluated for their nutritional, technological, and sensory properties, with the novel bio-ingredients compared to conventional solutions used in the baking industry.

Two optimized combinations of bacterial strains were used*: Furfurilactobacillus rossiae* (CR5), *Weissella confusa* (T6B10), and *Lactiplantibacillus plantarum* (SB88.B4) for sprouted wheat flour; and *Leuconostoc pseudomesenteroides* (DSM 20193), L. plantarum (7A), and *F. rossiae* (CR5) for sprouted barley flour. The fermentation process led to significant increases in peptide content (450% in sprouted wheat sourdough and

520% in sprouted barley sourdough) and phenolic compounds (344% and 261%, respectively), along with enhanced antioxidant activity (100% in wheat and 40% in barley). Among the experimental breads, those made with sprouted barley sourdough provided the highest nutritional and functional benefits, including a dietary fiber content of 6.53 g/100 g, superior in vitro protein digestibility (IVPD, 81.14%), a reduced predicted glycemic index (pGI, 84.78%), and strong angiotensin-converting enzyme (ACE) inhibitory activity (73%).

The rheological properties of doughs incorporating novel type III sourdoughs were comparable to those with type II wheat sourdoughs combined with enzymatic enhancers, suggesting their suitability for baking applications. Sensory evaluations revealed that bread made with type III sourdough from sprouted wheat flour was favored for its enhanced crust and crumb color, while bread made with sprouted barley sourdough stood out for its rich bran aroma, toasted notes, and balanced acidity. This study underscores the potential of targeted fermentation of sprouted flours as a key solution to meet the growing demand for health-focused and eco-friendly innovations from both consumers and producers.

Abstract ID: 1250 / Poster Number: 294.16S

Tailored enzymatic-based treatment of wastewater to detoxify heavy metals and degrade antibiotics, addressing water scarcity's issue "A Functional Metagenomic Approach"

Presenting author: Shimaa Farag Ahmed, PhD, The American University in Cairo, New Cairo, Egypt

Co-authors: *Rania Siam, Rehab Abdallah, Amged Ouf*

Background: Biological wastewater treatment plants (WWTPs) are essential for removing organic pollutants. However, detoxifying heavy metals and antibiotics in WWTP effluents remains a significant challenge, posing health and ecological threats. This necessitates innovative biological approaches.

Objective: This study employed a functional metagenomic approach to identify novel enzymes from WWTP sludge microbiomes, focusing on antibiotics-degrading enzymes and mercury-reducing enzymes that can be used in wastewater treatment.

Methods: A Fosmid library of 20,000 clones was constructed from sludge samples from Benisuef's WWTP, in Egypt. Functional screening for laccase activity employed two agar-based methods supplemented with guaiacol or methylene blue. MerA activity was assessed by growing clones on LB media containing mercury at the MIC (0.016 mM). Clones were similarly tested for Ni^{2+} and Cd^{2+} detoxification. PCR screening with degenerate primers for laccases and MerA refined the pool of positive clones.

Findings: Screening ~5000 clones revealed 74 with laccase activity. For MerA, 147 clones displayed 300% mercury resistance, with 18 also resistant to Ni^{2+} and 16 to Cd^{2+} (200% each). PCR identified 17 clones potentially carrying laccase domains and 14 carrying MerA. Clones showing both genes were sent for shotgun sequencing for functional annotation.

Conclusion: We will present the biochemical characterization of these novel genes, highlighting their exceptional capacity for detoxifying antibiotics and specific heavy metals, offering new insights into their potential for wastewater treatment.

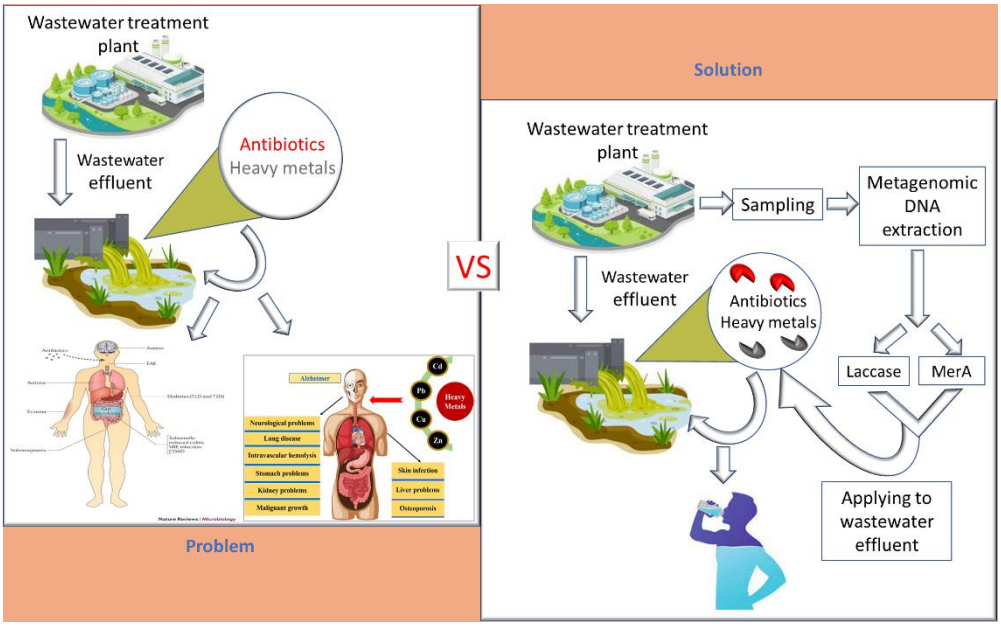


Figure 1: From Problem to Solution: Addressing Deficient Wastewater Effluent with Advanced Enzymatic Treatment for Sustainable Clean Water.

Abstract ID: 1272 / Poster Number: 132.16S

Tailored impact of dietary fibers on gut microbiota: a multi-omics comparison on the lean and obese microbial communities

Presenting author: Dr. Andrea Dell'Olio, Wageningen University and Research, Wageningen, Netherlands

Co-authors: *Josep Rubert*

The gut microbiota plays a crucial role in human health. Obesity is associated with microbial imbalances, such as reduced abundance of key beneficial species and altered microbial metabolic pathways [1]. Dietary fibers can modulate the gut microbiota, improving species diversity and supporting the synthesis of beneficial metabolites, yet the knowledge on how the modulation differ between the lean and obese gut microbiota is still scarce [2]. This study investigates how the whole apple matrix and the pure fibers, pectin and cellulose, differentially affect lean and obese gut microbial communities *in-vitro*. The substrates were subjected to INFOGEST *in-vitro* digestion followed by batch fermentation protocol. Two microbial inocula were obtained by pooling faces form clinically profiled lean and obese individuals after 24 hours dietary recall. Shallow shotgun metagenomics and targeted metabolomics were used to assess microbial composition and functionality changes, while genome-scale metabolic modeling (GSMM) was used to elucidate cross-feeding mechanisms. Apple and Apple Pectin supplementation were characterized by an enrichment of species like *Megasphaera MJR8396C and Akkermansia muciniphila* in the obese gut microbiota. At the same time, we revealed a simultaneous release of indole derivatives such as Indole-3-propionic acid (IPA) and the consumption of several amino-acids as illustrated in Figure 1 for Apple Pectin. Furthermore, GSMM demonstrated how *Bifidobacterium adolescentis* supported the co-enrichment of *Megasphaera sp*. through acetate and lactate cross-feeding mechanisms. In conclusion, Apple and Apple Pectin, modulated the obese gut microbiota by supporting changes in microbial community and promoting health-associated metabolites such as IPA. The presented multi-omics workflow represents a powerful approach for gaining mechanistic insights into the complex interactions between dietary fibers and the gut microbiota *in-vitro*. By integrating metagenomic and metabolomic data, this research highlights the potential of dietary fiber interventions in metabolic health management in obesity.



Figure 1: Volcano plot highlighting increased consumption of aromatic amino acids and synthesis of 3-indolepropionic acid (IPA) compared to the control fermentation, when pectin is supplemented to pooled obese fecal samples. Data are representative of three independent fermentation experiments (n = 3).

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Abstract ID: 3866

Taming Cupriavidus necator as cell factory for one-carbon biomanufacturing

Presenting author: Enrico Orsi, PhD, Danish Technical University, Lyngby, Denmark

Co-authors: *Pablo I. Nikel*

Introduction: A transition to a green economy requires the development of bioprocesses that can mitigate CO_2 emissions. One promising approach is engineering microorganisms capable of growing on CO_2 or its derivatives as cell factories for bioconversions. The β-proteobacterium *Cupriavidus necator* represents an ideal candidate for this purpose, as it can grow via the Calvin-Benson-Bassham (CBB) cycle on two setups obtainable from CO_2 capture and renewable electricity: CO_2 and hydrogen; and formate. Additionally, this microorganism has been demonstrated to support the bioproduction of several classes of compounds. Yet, its full biotechnological potential has been limited by the time-consuming and inefficient genome editing pipeline mediated by suicide vectors. Instead, developing more efficient genome editing tools would streamline this process and enhance the biotechnological exploitation of *C. necator*.

Objective: The objective of this talk is to present our successful efforts in establishing a novel genome engineering pipeline for *C. necator* with improved efficiency and reduced turnover time. Additionally, we showcase its application in the context of biomanufacturing, particularly for the biological conversion of formate into the plastic precursor crotonate (C4 molecule).

Method: To address the limitations of existing genome editing techniques, we developed a Self-splicing Intron-Based Riboswitch-Cas (SIBR-Cas) technology for *C. necator*. This system enables precise control over the translation of the endonuclease Cas, thereby facilitating homologous recombination before counter-selection. Furthermore, we employed the CRISPR-Cas technology to enhance the engineering of this organism for the biological conversion of formate into crotonate. Success of this effort required a combination of strain engineering and process optimization approaches.

Findings: Using the SIBR-Cas system, we achieved genome editing efficiencies of up to 80%, with turnover times reduced by 50% compared to state-of-the-art methods. This streamlined workflow significantly accelerated genome engineering efforts, enhancing the potential of *C. necator* as a platform for the one-carbon biomanufacturing. Then, we

demonstrated valorization of formate into the commodity chemical crotonate. Specifically, *C. necator* was engineered to incorporate a crotonate bioproduction pathway while inactivating competing polymer biosynthesis pathways. Flux through the pathway was enhanced when using a thermodynamically favorable bypass through malonyl-CoA. Additionally, a comprehensive study of formate toxicity and utilization informed the design of a fed-batch process that successfully enabled crotonate synthesis from formate up to 150 mg/L.

Conclusions: These advancements in genetic engineering, metabolic engineering, and bioprocess optimization represent a significant step forward in utilizing *C. necator* for industrial biotechnology, particularly in the field of one-carbon biomanufacturing.

Abstract ID: 3337

Targeted elimination of antibiotic resistance plasmids via phagemid-delivered CRISPR/Cas9 preserving gut microbiota integrity

Presenting author: Ms. Ada Muñoz-Cazalla, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain, Madrid, Spain

Co-authors: *Laura Álvaro Llorente, Ana Elena Pérez Cobas, Laura Jaraba Soto, Cristina Herencias Rodríguez, Jerónimo Rodríguez Beltrán*

Introduction: Antibiotic resistance (AR) genes are tightly linked to plasmids, which serve as a vehicle to transfer horizontally across bacterial populations. Owing to their ability to disseminate, plasmid-mediated AR poses a clinically relevant challenge. In particular, the gut of hospitalized patients is often colonized by resistant bacteria that remain untreated and undetected as they do not cause active infections. These resistant populations spread AR to the rest of the microbiota or to other patients, thereby behaving as reservoirs of resistance.

Objective: To address asymptomatic colonization, we aim to engineer multiplicity-of-infection-dependent phagemids capable of efficiently delivering CRISPR/Cas9 into Escherichia coli (Figure 1A).

Methods: Targeting clinically relevant β-lactamases ($bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, bla_{VIM1}, $bla_{\text{GES-5}}$, $bla_{\text{TEM-1}}$), we demonstrate a dose-dependent relationship between phagemid concentration and the rate of plasmid in vitro elimination, achieving remarkably high specificity.

Findings: Furthermore, we demonstrate that ex vivo phagemid treatment of colonized fecal samples can selectively and highly efficiently (>99.9%) eliminate plasmid populations harboring AR genes (Figure 1C). Importantly, this system targets problematic mobile genetic elements without affecting bacterial cells, thereby preserving the viability, diversity, and richness of the patient's microbiota.

Conclusion: Treated stool samples can then be reintroduced into the colonized patient, displacing AR populations. This novel strategy promises to eradicate the plasmid-mediated AR reservoir while hampering horizontal gene transfer and AR spread.

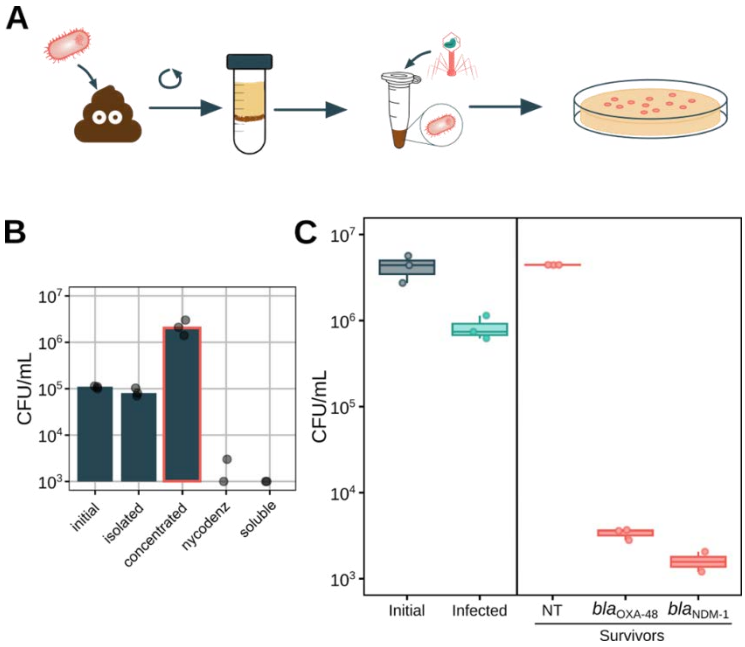


Figure 1: A-B) We can isolate and concentrate microbiota fecal samples to make the cells more accessible for infection. **C)** No killing effect is observed when using a non-targeting phagemid (NT). Treatment of fecal samples colonized by bla_{NDM-1} and bla_{OXA-48} producing Enterobacteriaceae with the phagemid (multiplicity-of-infection = 10^3) resulted in the elimination of more than 99.98% of the target resistance genes.

Abstract ID: 64 / Poster Number: 262.16P

Targeting -1 programmed ribosomal frameshifting (-1PRF) in SARS-CoV-2 via nucleic acid affinity ligands

Presenting author: Ms. Neha Jeena, Central University of Rajasthan, Ajmer, India

Co-authors: *Devesh Sawant, Inshad Ali Khan*

Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is recognized as the causative agent of COVID-19. Targeting the viral genome, rather than proteins, offers a more stable approach due to reduced susceptibility to mutations, thereby disrupting the viral life cycle. The RNA genome of SARS-CoV-2 harbors programmed -1 ribosomal frameshifting (-1 PRF) elements essential for the synthesis of crucial proteins, including RNA-dependent RNA polymerase (RdRp), which plays a pivotal role in viral genome replication and gene transcription.

Objective: This study aims to identify SARS-CoV-2 pseudoknot binding agents that inhibit -1 programmed ribosomal frameshifting.

Method: The RNA pseudoknot structure with PDB ID 7LYJ was downloaded from the RCSB Protein Data Bank (RCSB PDB) and subjected to a 1000 ns molecular dynamics simulation to achieve a stable, energy-minimized structure. The docking process utilized the U21 as active site for grid generation. Subsequently, 58 known compounds with IC50 values were combined with decoys and docked onto the energy-minimized structure obtained post-simulation. After validating the protocol using ROC analysis (0.79), 55,301 compounds from Chemdiv (Coronavirus, RNA binding chemical space, Nucleic acid ligand & Purine based nucleoside mimetics) were screened using the same grid and parameters. The resulting hits from the virtual screening were further analyzed for their ADME properties using the QikProp module of Maestro.

Findings & Conclusion: During the simulation, the RMSD value was 5.7 Å, indicating that the RNA pseudoknot structure remained stable with no significant deviations. The RMSF, Rg, and SASA values were within acceptable ranges, measured between 2.3–3.8nm, 2.3–2.7nm, and 1115–125nm^3, respectively. The FEL plot revealed a free energy range of 0 to 18.70 kJ/mol, from which a stable, energy-minimized structure suitable for virtual screening was obtained.

ChemDiv libraries were screened against the RNA pseudoknot structure's active site (U21), yielding 77 hits with docking scores between -5.21 and -6.51 kcal/mol. Nafamostat, when used as a standard drug, showed a strong binding affinity of -4.221

kcal/mol with critical residues surrounding the U21 site. Detailed interaction analyses of these hits identified 32 novel ligands, among which three (RBC-03, RBC-04 & RBC-17) demonstrated superior absorption properties.

Stable RNA pseudoknot simulations and virtual screening identified 32 potential SARS-CoV-2 inhibitors, with 3 showing superior absorption. These ligands offer promise for antiviral development.

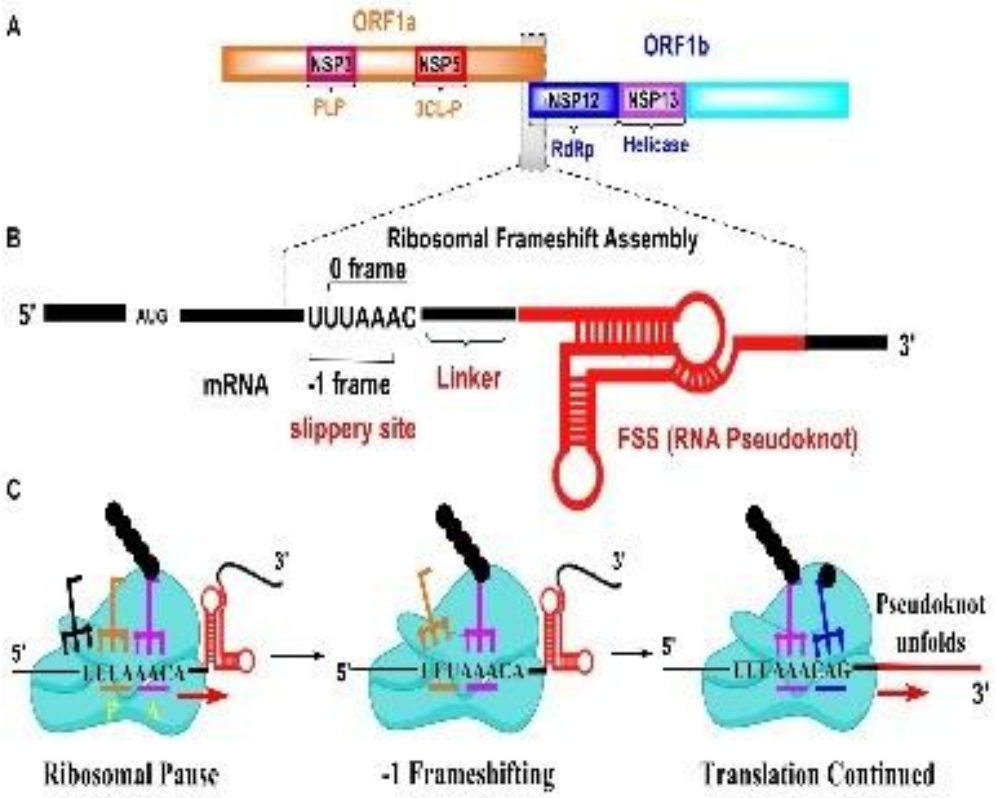


Figure 1: A) Position of frameshifting site in SARS-CoV genome. B) -1 PRF assembly. C) The Programmed -1 ribosomal frameshifting event in SARS-CoV-2.

Abstract ID: 3618 / Poster Number: 240.16P

Targeting ADP-ribosylation to impair bacterial persistence

Presenting author: Dr. Giuliana Catara, Institute of Biochemistry and Cell Biology, National Research Council of Italy, Naples, Italy

Co-authors: *Rocco Caggiano, Luca Palazzo*

Antibiotic-resistant 'superbugs' represent a significant public health concern for the 21st century, with expectations that they will cause tens of millions of deaths per year by 2050. Antimicrobial resistance has been observed in a wide range of infectious diseases from urinary tract infections to tuberculosis and endocarditis, and it is associated with the occurrence of a persistent phenotype formation (1). Persisters pose a substantial challenge in the treatment of chronic and persistent infections, as well as relapsing infections following treatment and the development of biofilm formation, thereby hampering the development of effective treatments. Consequently, combinatorial interventions capable of addressing this challenge are urgently needed (2).

Bacterial toxin-anti-toxin systems are involved in cell regulatory mechanisms in response to stress stimuli by targeting endogenous substrates through post-translational modifications. Among these, ADP-ribosylation is emerging as a new player in the field of TA systems (3). ADP-ribosylation is a highly conserved process throughout evolution, representing an ancient homeostatic and stress-response control system that relies on the transfer of ADP-ribose unit(s) to cellular macromolecules (4). The DarT/DarG toxin/antitoxin pair has recently been described in Thermus aquaticus, Mycobacterium tuberculosis and Escherichia coli. The DarT toxin has been observed to ADP-ribosylate thymidine in single-stranded DNA in a sequence-dependent manner, thereby impairing cell growth, whereas DarG reverses this modification, thereby rescuing cells from toxicity (3). Herein, we discuss the structural and mechanistic aspects of the DarT/DarG toxin-antitoxin-mediated control of DNA ADP-ribosylation, as well as the therapeutic perspectives that chemical inhibition of this specific ADP-ribosylation signalling may have (5).

Abstract ID: 1647 / Poster Number: 189.16P

Targeting Helicobacter pylori RNA Metabolism: Discovering Vulnerabilities for Novel Antimicrobial Therapies Against Resistance

Presenting author: Mr. Jakub Skibiński, University of Lodz, Łódź, Poland

Co-authors: *Yaroslav Lavrynchuk, Przemysław Płocinski, Magdalena Chmiela*

Introduction: The increasing resistance of Helicobacter pylori (Hp) to current antibiotics necessitates the development of novel antimicrobial strategies. RNA metabolism, an underexplored target in bacterial species, holds significant promise. Hp encodes a single RNA helicase, RhpA, from the DEAD-box family, which, unlike in other bacteria, does not interact with the core RNA degradosome protein polynucleotide phosphorylase (PNP). Inhibiting PNP activity has shown potential in altering antibiotic resistance and eradicating pathogens.

Objective: To investigate RNA metabolism pathways in Hp, focusing on PNP and RhpA as potential targets for novel antimicrobials.

Methods: Recombinant RhpA and PNP proteins were expressed in E. coli and purified using chromatography. Bacterial deletion mutants were generated by allelic exchange. Microscale thermophoresis (MST) was used to assess RhpA-PNP interactions. PNP inhibitors were screened in vitro using the MedChemExpress compound library, and active compounds were tested against live Hp.

Findings: Viable PNP and RhpA deletion mutants were generated. MST confirmed the lack of interaction between RhpA and PNP. Transcriptomic analysis revealed extensive transcriptomic alterations in RhpA mutants, with limited changes in PNP mutants. A total of 402 compounds inhibited PNP activity at 100 μM, some of which were active against live Hp.

Conclusion: We identified chemical compounds that inhibit PNP and are active against live Hp, offering a promising foundation for developing new antimicrobial therapies. These findings highlight RNA metabolism as a viable target to combat antibiotic-resistant Hp infections.

Abstract ID: 2817 / Poster Number: 409.15S

Targeting the bacterial SOS response: from structural and mechanistic investigations to inhibitor development

Presenting author: Filippo Vascon, PhD, University of Padua, Padova, Italy

Co-authors: *Sofia De Felice, Monica Chinellato, Younes Bouchiba, Camilla Mazzucco, Riccardo Mezzetti, Lorenzo Maso, Stefan T. Huber, Els Pardon, Gianluca Cioci, Sophie Barbe, Jan Steyaert, Arjen Jakobi, Laura Cendron*

Introduction: The bacterial SOS response is a conserved stress-response pathway elicited by DNA damage and regulated by the DNA-damage sensor RecA and the transcriptional repressor LexA. Under genotoxic stress, RecA oligomerizes on ssDNA and induces LexA autoproteolysis, which derepresses the effector *SOS genes*, including error-prone DNA polymerases, recombinases, virulence factors and antibiotic resistance determinants. Therefore, the SOS response is a potential target for innovative anti-evolutive and anti-virulence approaches. However, development of anti-SOS drugs has been hindered by limited knowledge on the RecA-LexA interaction and LexA's stringent substrate specificity.

Objectives: This work aims to elucidate the molecular events governing the RecA-LexA interplay and to develop potent inhibitors of this pathway (Figure 1), paving the way for novel antimicrobial strategies.

Methods: The atomic structures of *Pseudomonas aeruginosa* LexA autoproteolytic domain, oligomeric RecA and their complex were solved by X-ray crystallography and cryo-electron microscopy, respectively, identifying crucial requisites for LexA proteolysis. Fluorescence polarization assays corroborated this information with the binding affinities of the molecular species involved in the interaction.

Anti-LexA nanobodies were generated via llama immunization and phage display. LexA autoproteolysis assays revealed nanobodies with potent inhibitory activity, while surface plasmon resonance was used to determine their affinity for LexA. The ability of the developed nanobodies to suppress the SOS response was verified by RT-PCR of selected SOS genes in bacterial cultures undergoing antibiotic treatment. Crystallographic structures of LexA-nanobody complexes disclosed the unprecedented mechanism of action of these nanobodies and represented the basis for computer-aided engineering of improved variants.

Findings: The structure of the RecA-LexA complex revealed the determinants required for their interaction, unveiling that a conformational switch of LexA opens a previously inaccessible pocket on its surface. A key phenylalanine residue of RecA inserts in this pocket, stabilizing the conformation of LexA needed for autoproteolysis.

The developed anti-LexA nanobodies proved to be the most potent SOS inhibitors known so far, with IC_{50} values in the low micromolar range and able to abate the expression of SOS genes. Unlike traditional competitive inhibitors that target enzymatic active sites, these nanobodies trap LexA cleavable loop in the inactive conformation, preventing the opening of the RecA hosting cavity. Building on all the structural information gained on this molecular system, nanobodies were rationally engineered, surpassing the affinity and activity of parental ones. These improved variants are being tested in phage-based delivery systems.

Conclusions: Providing fine molecular details on RecA-LexA interaction and discovering potent inhibitors of the SOS system, this work answered longstanding questions in the study of the SOS response and paves the way to groundbreaking antimicrobial strategies.

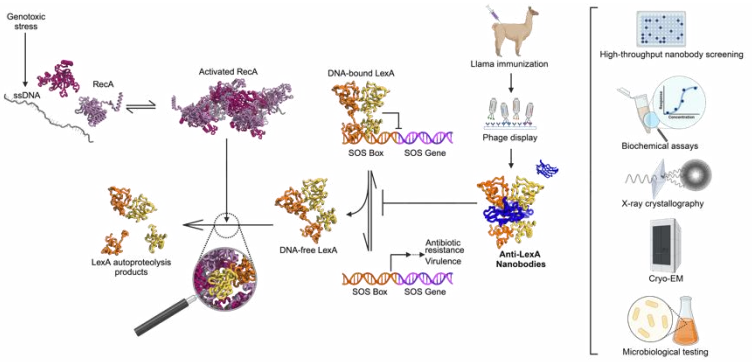


Figure 1: Overview of the work. Mechanistic elucidation and nanobody-based suppression of the SOS response were investigated by structural, biophysical, biochemical and microbiological techniques.

Abstract ID: 3098 / Poster Number: 027.15P

Temperature-dependent biofilm formation in Antarctic plant endophytes

Presenting author: Dr. Olga Iungin, Kyiv National University of Technologies and Desing, Kyiv, UkraineVytautas Magnus University, Kaunas, Lithuania; Institute of molecular Biology and Genetics of The NAS of Ukraine, Kyiv, Ukraine

Co-authors: *Yevheniia Prekrasna-Kviatkovska, Oleksandr Kalinichenko, Olena Moshynets, Marina Sidorenko, Geert Potters, Saulius Mickevičius*

Introduction: Endophytic microbial communities associated with the only two vascular plant species of Antarctic Peninsula exhibit diverse strategies for biofilm formation and maintenance, influenced by environmental conditions and external factors. Biofilms composition within these communities varies with cultivation temperature, impacting cell adhesion, matrix formation, and overall biofilm architecture.

Objective: Biofilms structural components in microbial communities associated with Antarctic vascular plants.

Method: Two types of formulated microbial communities (ALS and LS types) were cultivated in a range 4-42ºC during 3 and 6 days and estimated by CBA. Bacterial architecture (EPS, eDNA, PHA) was analysed with CLSM (Leica TCS SPE Confocal system) and LAS X software. Sequencing of eDNA and bioinformatics analysis was performed using the services of Novogene company. Regression analysis, ANOVA, and response surface methodology were employed to analyze the data using Statistica software.

Findings: The role of eDNA and the polymeric matrix in biofilm formation of endophytes is pivotal. eDNA diversity was highest at 4 and 15°C, with Pseudomonas species dominating at other temperatures. High levels of PHA were observed in ALS biofilms at 4°C and 15°C, while LS biofilms exhibited maximum PHA accumulation at 42°C. These results reveal the complex structure and dynamics of Antarctic plant-associated biofilms, and suggest that temperature and cultivation time are key factors influencing the strength of ALS type, but not LS biofilms.

Conclusion: These findings highlight the complex nature of Antarctic plant-associated microbial communities and their ability to adapt to changing environmental conditions.

Abstract ID: 1229 / Poster Number: 001.17S

Temperature-dependent efficiency of Slow Sand Filters in potable water treatment: biofilm microbial community dynamics and bacterial contaminant reduction

Presenting author: Dr. Juliet Ainomugisha, Universita Cattolica del Sacro Cuore, Piacenza, Italy

Co-authors: *Filippo Vaccari, Giovanni Milani, Claudia Cortimiglia, Andrea Minuti, Giuseppe Bertoni, Pier Sandro Cocconcelli*

Slow sand filters (SSFs) are a widely used technology for the potabilization of water, removing biological and chemical contaminants. This study investigates the effectiveness of SSFs in purifying water, with a focus on the impact of temperature on biofilm development and microbial removal efficiency. Microbiological analysis was used to assess the purification ability of the SSFs, and molecular analysis using 16S rRNA amplicon sequencing was used to characterise the microbial community responsible for the SSFs' potabilization efficiency.

Microbiological analysis of effluent water revealed that SSFs were highly effective in reducing coliforms (4-Log_{10} reduction) compared to enterococci (3-Log_{10}) and aerobic bacteria (2-Log_{10}). The biofilm formation occurred at temperatures below 12°C and took approximately two months. As the temperature rose, a significant increase in biofilm bacterial growth was observed, as well as an improvement in the filtration ability of the SSFs. Moreover, 16S rRNA amplicon sequencing revealed significant shifts in microbial community composition, particularly with temperature fluctuations, influencing the abundance of key taxa like *Nitrospira* and *Pseudomonas*.

To further test the efficiency of the filters, they were inoculated with *Escherichia coli* and MS2 bacteriophage. The results from the challenge tests demonstrated a greater than 6-Log_{10} reduction of *E. coli* but no reduction of MS2 bacteriophage, highlighting temperature and microbial interaction complexities.

These findings support the existence of a relationship between the temperatures, biofilm development and pathogen removal, emphasising the need for further research on the effects of temperature on SSFs in varying environmental conditions.

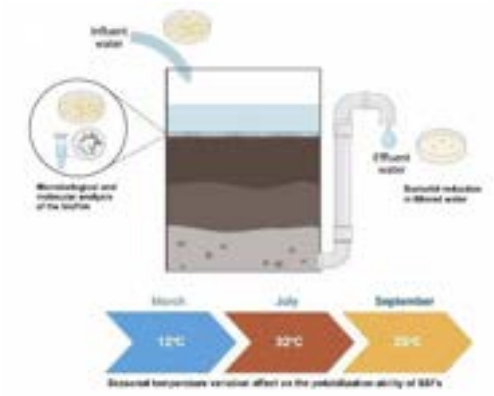


Figure 1: Experimental design of the study highlighting the three seasonal temperatures considered and their effect on both the microbial community in the biofilm and the potabilization efficiency of the slow sand filters.

Abstract ID: 2808 / Poster Number: 037.17F

Temporal and Spatial Resolution of bacterial magnetosome degradation in a 3D lung carcinoma model

Presenting author: Dr. Alicia Gascon Gubieda, Universidad del Pais Vasco (UPV/EHU), Leioa, Spain

Co-authors: *Lucía Gandarias, Mihály Pósfai, Ajith Pattammattel, Malu Fdez-Gubieda, Ana Abad-Díaz-de-Cerio, Ana García Prieto*

Introduction: Magnetotactic bacteria are aquatic organisms capable of synthetising magnetite nanoparticles, called magnetosomes. Their properties make them good candidates for many biomedical applications, such as MRI and magnetic hyperthermia for cancer diagnosis and treatment. Despite their potential, little is known about magnetosome degradation in human cells, and even less so of their degradation within tumours, which are the target of intended treatments.

Objective: To describe how magnetosomes isolated from *Magnetospirillum gryphiswaldense* are transformed within a human lung carcinoma model over a 36 days period.

Method: Magnetosomes were incubated with A549 cells for their internalization. Cells were then used to generate spheroids, and kept for 36 days for analysis. TEM was employed to track the degradation of magnetosomes and the appearance of new nanoparticles within the cells. Hard X-ray probes, nano-XANES and nano-XRF, were employed to determine the transformation process of magnetite within the samples with temporal and spatial resolution.

Findings: Our results reveal two different processes: 1) Degradation: we observed a decrease in magnetosome size and an oxidation of their magnetite to maghemite, which occurred mainly during the first 10 days, and was accompanied by an overexpression of the ferritin H chain. 2) Biosynthesis: we detected the *de novo* mineralization of small nanoparticles (10 nm) of magnetite by ferritin, perhaps as a mechanism of protection against oxidative stress.

Conclusion: The low degradation rate of magnetosomes within tumours and the ability of cancer cells to biosynthesize magnetite suggests that low magnetosome dosages could be used for prolonged periods for cancer treatments.

Abstract ID: 4050 / Poster Number: 099.16P

Temporal Dynamics of Nasopharyngeal and Oral Microbiomes in School-Age Children: Influence of the Built Environment

Presenting author: Dr. Claudia P. Saavedra, Universidad Andres Bello, Santiago, Chile

Co-authors: *Juan Castro-Severyn, Guillermo Valdivia, Gabriel Kruger, Nicolas Pacheco, Francisco Remonsellez, Fernando Valiente, Gloria Arriagada, Aldo Gaggero, Jorge Valdes,*

The "School MicroBE" initiative investigates the influence of the built environment on the microbiomes of school-age children through longitudinal analyses of nasopharyngeal and oral samples collected during three campaigns in 2024 (April, June, and October). The study includes children aged 6 to 12 from a public school in Santiago, Chile.

The samples were analyzed through 16S rRNA gene sequencing to evaluate the diversity and taxonomic composition of the oral and nasopharyngeal microbiota using alpha and beta diversity metrics, hierarchical clustering, and transition networks.

The results indicate significant seasonal variation, with higher alpha diversity observed between April and October. Beta diversity analyses revealed distinct microbial groups associated with seasonal changes and sample origin, with oral samples being more diverse than nasopharyngeal ones. Hierarchical clustering identified dominant taxa such as *Moraxella* in the nasopharynx and *Streptococcus* in the oral cavity. Notable transitions between microbiome groups highlighted dynamic responses to environmental and seasonal changes. Additionally, these results revealed key interactions, suggesting ecological roles for dominant taxa in shaping community structure.

These findings emphasize the importance of temporal and the build environment in microbiome development, with implications for understanding pathogen exposure and microbial health in school settings. Insights from this study may inform public health strategies to understand how respiratory infections alter the microbiomes of healthy individuals.

Abstract ID: 2411 / Poster Number: 018.15P

Temporal microbial community dynamics after rewetting in the peatland Zarnekow, Germany

Presenting author: Dr. Tatjana Živković, GFZ Helmholtz Centre for Geosciences, Potsdam, Germany

Co-authors: *Alexander Bartholomäus, Aram Kalhori, Torsten Sachs, Susanne Liebner*

Peatland rewetting is a widely utilized restoration strategy to mitigate carbon (C) emissions and restore ecosystem functions. Rewetted peatlands generally transition into long term C sinks; however, they may remain net C sources during the initial stages post-rewetting due to increased methane (CH_4) emissions. Research suggests that microbially-driven CH_4 production recovers more rapidly than oxidation, partially driving elevated emissions in the early years after rewetting. Over time, strengthened CH_4 oxidation is expected to mitigate these emissions.

This study examines a rewetted peatland in Zarnekow, Germany. We leverage long-term monitoring data on greenhouse gas fluxes of CH_4 and carbon dioxide (CO_2), alongside yearly 16S rRNA amplicon sequencing to investigate changes in the microbial community composition and their impact on C emission dynamics. Our goal is to elucidate microbial community responses to rewetting and their influence on CH_4 production and oxidation processes within the peat profile. By assessing microbial shifts at different depths—surface layers (>20 cm) with higher oxygen availability and deeper layers (<40 cm) with anaerobic conditions—we aim to characterize the temporal progression of microbial functional recovery. Key microbial taxa involved in methanogenesis, and methane oxidation will be examined to evaluate their abundance, dynamics, and potential lag times.

Understanding these patterns will provide valuable insights into the biogeochemical mechanisms driving C cycling in rewetted peatlands. This study contributes to a broader understanding of temporal and functional trajectories of peatland ecosystems following rewetting, supporting future climate mitigation efforts.

Abstract ID: 1931 / Poster Number: 247.17P

Temporal Shifts in Soil Microbial Communities and Nitrogen Cycling in Minimal Input Oat-Lentil Intercropping

Presenting author: Mr. Eduardo Cerull, Leibniz-Institut für Agrartechnik und Bioökonomie, Potsdam, Germany

Co-authors: *Lena Simon*

Intercropping is known to increase crop yields, control weeds, and retain soil carbon and nitrogen. However, the influence on soil bacterial communities remains less explored. In this study the temporal dynamics of soil bacterial communities and their nitrogen-transformation potential in a minimal input plot experiment with oat, lentil and oat-lentil intercropping over one cropping season was investigated. The soil bacterial community was analysed by quantifying the copy number of genes involved in the nitrogen-cycle, and by high-resolution Nanopore sequencing to capture changes of the bacterial community at species level.

Sampling date, rather than cropping system shaped bacterial community composition and nitrogen-transformation potentials. Early-season samples exhibited a higher potential for dissimilatory nitrate reduction to ammonium (DNRA) – a process associated with nitrogen retention in soils – while late-season samples showed increased nitrification and denitrification potentials. Interestingly, DNRA and nitrification/denitrification potentials displayed contemporaneous seasonal patterns, suggesting tightly coordinated microbial responses to changing nitrogen availability.

Preliminary evaluation of sequencing data are in accordance with qPCR findings, highlighting seasonal shifts in the bacterial community composition between early and late season. Promising candidates potentially involved in nitrogen-transforming processes were observed, waiting for further investigation to evaluate their roles in nitrogen cycling. By revealing potential microbial drivers of nitrogen cycling, this research contributes to eco-innovations in sustainable agriculture and offers a foundation for strategies that leverage microbial communities to improve nitrogen retention as well as soil and crop health.

Abstract ID: 1717 / Poster Number: 352.16P

Terephthalic acid (TPA) as Feedstock for antimicrobial production by plastic-polluted isolated Streptomyces

Presenting author: Ms. Diana Alicia Garza Herrera, Technological University of the Shannon: Midlands Midwest (TUS), Athlone, Ireland

Co-authors: *Marija Mojicevic, Margeret Brennan-Fournet, Jasmina Nikodinovic-Runic*

Introduction: Plastic-contaminated environments as potential harbor places of microbial strains capable of adapting to polymeric substrates[1], with upcycling possibilities, potentially leading to the production of valuable secondary metabolites, such as antimicrobial compounds.

Objective: This study aimed to assess the ability of selected *Streptomyces sp.* isolated from plastic-polluted environments in the presence of polymer-associated substrates, such as TPA to produce bioactive compounds.

Methods: Fermentation experiments were conducted using a soy flour–glucose–starch–mannitol-based medium (JS) with three treatments: unmodified (JS CL), 50% glucose and 50% terephthalic acid (TPA) (JS M), and 100% TPA (JS TPA). Ethyl acetate extraction was employed to maximize metabolite yields from 24 crude extracts. Antimicrobial activity was evaluated against *Escherichia coli* (E. coli), *Staphylococcus aureus* (S. aureus), and *Candida albicans* (C. albicans). Compound identification was conducted using Gas Chromatography-Mass Spectrometry (GC-MS).

Findings: All extracts showed inhibition against *S. aureus*, with 14 extracts active against *E. coli* and six against *C. albicans*. Notably, cultures supplemented with TPA and modified carbon sources exhibited enhanced antimicrobial activity.

Conclusion: Isolated *Streptomyces sp.* demonstrated a remarkable capacity to utilize polymeric substrates to produce bioactive compounds. These findings highlight the potential of strains from plastic-exposed environments in developing novel antimicrobial agent.

Abstract ID: 1308 / Poster Number: 198.16P

Territorial and seasonal variations in the honeybee gut microbiome in Armenia

Presenting author: Mr. Tigran Aleksanyan, Yerevan State University, Yerevan, Armenia

Co-authors: *Ruzanna Harutyunyan, Inesa Semerjyan, Svetoslav Dimov, Inga Bazukyan*

Honeybees are vital pollinators with immense ecological, economic, cultural, and medicinal significance worldwide. In recent years, their health has declined due to various environmental stressors. Understanding the interactions and mechanisms of honeybees' gut microbiome is crucial for enhancing their health. The aim of the study is to observe the distinct patterns in Armenian honeybee gut microbiome influenced by territorial and seasonal factors.

Honeybee samples were collected from six regions of Armenia during spring, summer, and autumn. The gut microbiome was analyzed using V3-V4 16S rRNA amplicon-based metagenomics by "Novogen" CJSC in China.

The results show that the relative abundance of the phyla Firmicutes and Proteobacteria varies across different seasons: Firmicutes are more abundant in spring, whereas Proteobacteria dominate during summer and autumn. In autumn, Alphaproteobacteria dominate Bacilli in two regions, while in the other two regions the abundance of Alphaproteobacteria is similar to that of Bacilli. Alphaproteobacteria supersede Gammaproteobacteria in autumn. Lactobacillaceae is the dominant family in the gut microbiome, however Rhizobiaceae increases in autumn. In two regions an increase in Clostridia is observed in autumn. In contrast, the amount of Bifidobacteria decreases towards autumn, except for one region, where they continue to dominate. Lactobacilli and Bombilactobacilli also decline as autumn approaches, while Commensalibacter and Acetobacteriaceae representatives increase in amount.

The study offers important insights into the composition and spatiotemporal dynamics of the honeybee gut microbiome in Armenia, which are essential for promoting honeybee health and may have practical implementations, such as the creation of probiotics for honeybees.

Abstract ID: 2055 / Poster Number: 440.16P

The 'Microbiome and Stress Resilience Academy' project

Presenting author: Prof. Eleonora Rolli, Università degli Studi di Milano, Milano, Italy

Co-authors: *Francesca Mapelli, Iannis Stringlis, Victor Carrion, Annabel Levert, Laurent Dedieu, Melisa Ozdilek, Christian Espinoza*

Introduction: Society needs to familiarize with microbiome concepts and its benefits for plant fitness to favour adoption of microbiome-based strategies in agriculture. Therefore, it is necessary to train the whole chain of agricultural stakeholders and producers by providing appropriate and reliable pedagogical materials.

Objective: The project will develop high-quality scientific and pedagogical digital training courses intended for secondary schools or university students, training organizations linked with agriculture industries, chambers of agriculture, farming cooperatives, farmers and agricultural stakeholders.

Method: The pedagogical material will be prepared by partners involved in the research, science and digital learning. The consortium includes: My Green Training Box, a provider of digital learning solutions (France); the University of Malaga (Spain); the Agricultural University of Athens (Greece); the University of Milan (Italy) and the French SME AkiNaO.

Results: The project will provide training material about plant-microbiome contribution in stress resilience to enhance sustainable solutions in agriculture and preserve ecosystem biodiversity by: 1) generating 4 digital training courses that will be released for free as learning videos, pdf documents, podcasts and will be available in 5 languages in the MGTB platform; 2) by organizing 8 webinars, focused on specific case studies about microbiome benefit for plant fitness, released for free as web-series; 3) by animating 8 pilot training courses to gain practical experience on microbiome applications.

Conclusion: The project will offer to the final users access to high-quality and reliable digital contents promoting knowledge and future exploitation of the plant microbiome.

Abstract ID: 2336 / Poster Number: 066.16P

The acquisition of pAA plasmid variants may enhance the extraintestinal virulence potential of non-pathogenic Escherichia coli

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Introduction: Recent studies have demonstrated that certain *Escherichia coli* strains can cause diarrhea and extraintestinal infections in the same patient. The frequent isolation of *E. coli* strains with diarrheagenic enteroaggregative *E. coli* (EAEC) markers from extraintestinal sites has raised questions about the role of the EAEC virulence plasmid (pAA) in establishing extraintestinal infections.

Objective: To investigate whether the presence of pAA contributes to the virulence of *E. coli* strains during extraintestinal infections.

Methods: pAA was transferred by conjugation from three EAEC strains (HSP60, LSC52, and UPEC100), which were isolated from symptomatic urinary tract infections, to a non-pathogenic *E. coli* strain MG1655. The isogenic strains of MG1655 with and without pAA plasmids were compared regarding their ability to survive in human serum and their capacity to interact with and invade both intestinal (Caco-2) and extraintestinal cell lineages (T24, EA.hy926, and A549).

Findings: The acquisition of pAA from the LSC52 strain enabled MG1655 to survive human serum, while the others did not. The presence of the pAA plasmids enhanced MG1655's capacity to interact with and invade the extraintestinal but not the intestinal cell lineages. The increase in adherence rates varied from 4 to 15 times, while the invasion rates ranged from 2 to 15,000.

Conclusion: Our preliminary data showed that strains carrying different variants of pAA exhibit different behaviours, which significantly affect and distinctly impact their resistance to human serum and their interaction with and invasion of cells from extraintestinal sites. Therefore, the acquisition of pAA may contribute to the occurrence of extraintestinal infections.

Abstract ID: 1665

The AcrAB Efflux Pump: A Key Driver of Shigella flexneri Epithelial Cell Invasion

Presenting author: Martina Pasqua, PhD, Sapienza University of Rome, Roma, Italy

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Introduction: AcrAB-TolC is the primary multidrug resistance efflux pump (EP) of the resistance-nodulation-division (RND) superfamily in Enterobacteriaceae. Beyond conferring resistance to a wide array of antibiotics, AcrAB plays a pivotal role in human pathogenicity[1]. In *Shigella flexneri*, the causative agent of bacillary dysentery, AcrAB is conserved and actively expressed during the bacterium's intracellular life cycle[2].

Objective: To investigate the role of the AcrAB efflux pump in the infectious process of *S. flexneri* by evaluating bacterial survival within the intracellular environment and its ability to spread between epithelial cells.

Method: Use of CaCo-2 epithelial cells to evaluate intracellular survival and cell-to-cell spreading of *S. flexneri* derivative strains lacking AcrAB encoding genes.

Findings: The data we report reveal that AcrAB plays a critical role in intracellular survival within CaCo-2 epithelial cells. Deletion of both *acrA* and *acrB* genes significantly reduces bacterial viability and impairs cell-to-cell spreading. The importance of the AcrB transporter was further confirmed through an efflux pump inhibitor and a transport-deficient AcrB mutant.

Conclusion: Our findings demonstrate that the AcrAB efflux pump in *S. flexneri* is critical not only for antibiotic resistance but also for virulence[3]. AcrB function is essential for survival within epithelial cells and cell-to-cell spreading. Moreover, the ability to inhibit AcrB with specific inhibitors highlights its potential as a therapeutic target for combating bacterial virulence.

Abstract ID: 2154 / Poster Number: 109.15F

The active microbial communities and functionality on the surface of the Greenland ice sheet

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Co-authors: *Laura Perini, Ate H. Jaarma, Katie Sipes, Helen Feord, Martyn Tranter, Liane G. Benning, Alexandre M. Anesio*

The ablation area of the Greenland Ice Sheet (GrIS) is a biome driven by microbial activity. During the summer melt season, the weathering crust of the ice becomes a wet living skin dominated by eukaryotic glacier ice algae, particularly *Ancylonema* spp., which accelerate ice melt through their dark pigmentation. Cryoconite holes, formed by sediment melting into the weathering crust, also dominate the landscape of the ice surface. They are primarily inhabited by cyanobacteria as the main primary producers and host a diverse community of bacterial, fungal and other microeukaryotic heterotrophs. This study investigates the active microbial communities and functionality of the weathering crust and cryoconites using Total RNA metatranscriptomics. With this approach, we describe the full diversity of ice surface microbial communities, assembling, annotating and analysing jointly full-length 16S rRNA and 18S rRNA genes in addition to transcriptomes. We conducted a seasonal study over a 21-day period during the ablation season, sampling ice and cryoconite habitats. Samples were collected from five cryoconite holes and five 2-meter patches of the weathering crust ca 25km inland on the GrIS, near Ilulissat. Biomass from cryoconite holes and ice surfaces was collected at solar noon on seven sampling days during the summer. The findings highlight the dynamics and spatial variability of very different microbial communities between the weathering crust and cryoconite holes. Notably, the weathering crust is dominated by eukaryotic biomass, and spatial variability is significant; cryoconites are far more diverse, dominated by prokaryotic interactions and relatively stable temporally. A snowfall in late summer provided a window of opportunity to show that cryoconites communities are robust, while the functionality of the weathering crust, including genes associated to carbon, nitrogen and phosphorus cycling all responded to snowfall. The Total RNA approach in this study provides a powerful insight into the entire active microbial community and their functionality on glacial surfaces.

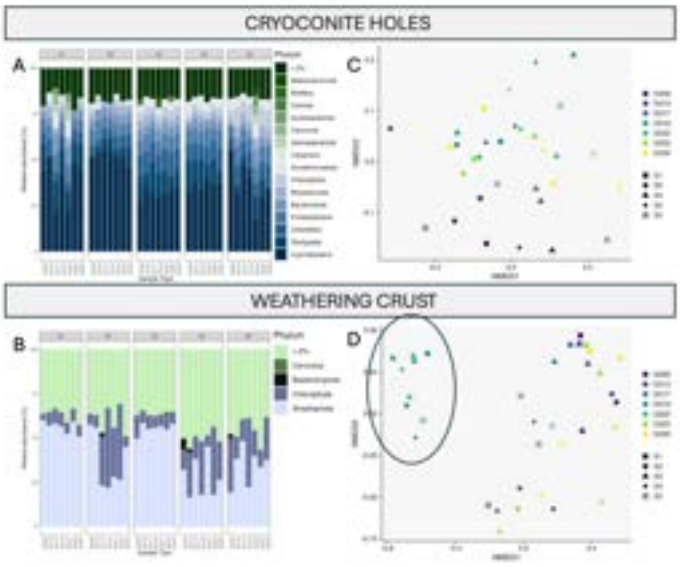


Figure 1: The active community (rRNA) in cryoconite holes (A) is considerably more compared to that of the weathering crust (B). General functions (mRNA) appear very stable in cryoconite holes (C) both over time and irrespective of the snow event, whereas the weathering crust community showed a clear, rapid response to environmental changes and reverted equally rapidly back to its original state (D).

Abstract ID: 3827 / Poster Number: 016.17S

The activity of the quorum sensing regulator HapR is modulated by the bacterial extracellular vesicle (BEV)-associated protein ObfA of Vibrio cholerae

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Co-authors: *Fatih Cakar, Yi-Chi Chen, Katharina Pressler, Leo Eberl, Stefan Schild*

Vibrio cholerae, the causative agent of cholera, exists in diverse environments, transitioning between the human intestine and aquatic ecosystems. In these habitats, it often forms biofilms, which enhance its environmental persistence and facilitate host transmission. While quorum sensing (QS) plays a central role in biofilm regulation, the contribution of bacterial extracellular vesicles (BEVs) to this process remains poorly understood. BEVs are continuously secreted and known to participate in intercellular communication, yet their regulatory mechanisms in biofilm formation are not fully elucidated. This study investigates the role of BEVs in *V. cholerae* biofilm dynamics and identifies ObfA, a BEV-associated protein, as a key modulator of HapR activity, a crucial QS regulator.

This study aims to elucidate the role of BEVs in *V. cholerae* biofilm formation, identify key BEV-associated factors influencing this process, and determine the molecular mechanism by which these factors regulate QS and biofilm dynamics.

BEVs were isolated from both planktonic and biofilm cultures of *V. cholerae* and subjected to comparative proteomic analysis to identify proteins differentially enriched in biofilm-associated BEVs. Gene deletion mutants were constructed to assess the functional role of identified candidates, particularly ObfA, in biofilm formation and QS regulation. Transcriptomic analyses and phenotypic assays were employed to evaluate the influence of ObfA on the HapR-dependent QS regulatory network, including its impact on small regulatory RNAs (CsrC and CsrD) involved in the Csr cascade.

Comparative proteomics revealed that BEVs from biofilm cultures significantly enhance biofilm formation compared to those from planktonic cultures, with ObfA being highly abundant in biofilm-derived BEVs. Deletion of *obfA* led to reduced biofilm formation and impaired colonization efficiency. Further analyses demonstrated that ObfA modulates HapR activity by affecting the Csr-cascade, leading to altered expression of the small regulatory RNAs CsrC and CsrD, which are known to influence biofilm formation and QS-mediated behaviors.

This study uncovers a novel intra-species communication mechanism in *V. cholerae* mediated by BEV-associated ObfA. By modulating HapR activity via the Csr cascade, ObfA plays a critical role in biofilm formation and colonization, emphasizing its importance in the bacterium's life cycle and pathogenicity. These findings provide new insights into BEV-mediated regulation in bacterial communities and highlight potential targets for antimicrobial strategies aimed at disrupting biofilm formation in pathogenic bacteria.

Abstract ID: 3350 / Poster Number: 217.17P

The antidepressant vortioxetine enhances resistance toward multiple azoles

Presenting author: Margherita Cacaci, PhD, Università Cattolica del Sacro Cuore, Rome, Italy

Co-authors: *Silvia Rizzo, Vincenzo Di Pilato, Stefano Di Bella, Umberto Albert, Brunella Posteraro, Maurizio Sanguinetti, Francesca Bugli, Riccardo Torelli*

Introduction: Fungal diseases affect over 6.55 million people annually, with 3.75 million deaths, driven by factors such as antifungal resistance. Recent studies indicate that the class of selective serotonine reuptake inhibitors (SSRIs) can induce bacterial antibiotic resistance. Vortioxetine, approved in 2013 by the FDA and EMA, is an antidepressant in the SSRIs class, with additional action on serotonin receptors transporter inhibition, better tolerate compared to other antidepressants.

Objectives: This study explores whether vortioxetine could impact on the emergence of antifungal resistance. Vortioxetine shows antifungal action against *Candida* species. We investigated whether it induces multiple antifungal resistances in *C. glabrata* underlying the mechanisms.

Method: To define the antifungal efficacy of vortioxetine, minimum inhibitory concentration (MIC) and minimum fungicide concentration (MFC) were determined by CLSI broth microdilution method. The 24h growth and time-kill curves of isolates untreated or treated at 2xMIC, MIC and MIC/2 were obtained by CFU/mL evaluation at defined time-points. Intracellular reactive oxygen species (ROS) generation under vortioxetine exposure was measured using DCFDA-assay. To analyse vortioxetine's effects on an azole-susceptible *C. glabrata* strain, yeast cultures were exposed to sub-MIC concentration (5 µg/mL) over an 80-days evolution. Every 24h for 80 days, a sub-inoculum was performed in fresh liquid medium containing the same antidepressant concentration. Untreated control was included. During this period, the azole-susceptibility profile (fluconazole, itraconazole, voriconazole and posaconazole) of 100 clones from treated and untreated strains was determined by CLSI broth microdilution assay. Probes based qPCR (treated vs untreated samples normalized to beta-actin expression) was performed to study the expression of efflux pumps genes (CDR1, CDR2, SNQ2 and PUP1). Whole-genome sequencing (WGS) was performed using an Illumina NovaSeq X Plus platform on treated and untreated strains.

Findings: Vortioxetine MIC90 and MFC90 values ranged between 4-16 µg/mL and 8-32 µg/mL, respectively, for all selected *Candida* species. At 2xMIC for all species and at MIC for *C. auris* and *C. parapsilosis*, CFU/mL decreased >4 log-units (99.9% killing) by incubation for 24h. Moreover, a vortioxetine dose-dependent increase in the levels of intracellular ROS was observed in treated strains compared to the untreated controls. No ROS production was detected in mammalian intestinal epithelial cells (Caco-2) treated with the same vortioxetine concentrations. After 80 days of exposure to the antidepressant, the 80% of azoles subsceptible *C. glabrata* population became resistant to azoles. The resistance phenotype remained stable over 10 generations without exposure to vortioxetine. An overexpression of efflux pump CDR1 gene was observed in treated strain. WGS results revealed: missense mutations affecting the CDR1 transcriptional factor PDR1 and two uncharacterized genes (one each); a non-sense mutation affecting TUP1. The PDR1 mutation was found in 80% of induced resistant *C. glabrata* population, but not in the remaining 20% of sensitive population and in the untreated sample. CRISPR-Cas9 base editors were designed to convert mutations to understand their role in the resistant phenotype.

Conclusions: These findings highlight the antifungal action of vortioxetine and the increased risk of developing antifungal resistance upon prolonged exposure to this antidepressant.

Abstract ID: 2107

The antimicrobial activity of copper sulphate as an intestinal habitat filter affecting the gut microbiota interactions with Salmonella

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Introduction: Salmonellosis is one of the most common foodborne infections worldwide. Colonisation of pigs by *Salmonella* is a major factor for transmission to humans. During colonisation of pigs, *Salmonella* encounters a hostile environment, including the resident microbiota and antimicrobials such as copper. Copper sulphate is used as a feed additive in the pig industry since EU-wide ban on the use of antibiotics as growth promoters. Widespread use of copper led to development of copper-resistant pathogens and environmental pollution. The most recent pandemic *S.* Typhimurium clone acquired genomic island SGI-4 encoding copper resistance genes.

Objectives: Our central hypothesis is that copper is an intestinal habitat filter affecting the microbiota and providing colonisation advantage for copper-resistant *Salmonella*.

Methods: Four-week-old piglets (n=60) were separated into two groups and fed on diet with high (therapeutic) or low (nutritional) levels of copper sulphate. Faecal samples were collected immediately prior to and on day 7 and 14 following placing piglets on low or high copper diet. Metagenome sequence of total faecal DNA was determined using short read shotgun sequencing and metabolite levels were determined by NMR. Metagenomic analyses were performed with MATAFILER pipeline. Microbiota were isolated from piglets on high and low copper diet using high throughput culturomics methods and sequenced. Copper MIC was determined and niche-competition and niche-invasion co-culture assays performed in anaerobic conditions.

Findings: Differences between high and low copper diet were observed for the 14 species including *Bifidobacterium*, *Escherichia*, and *Lactobacillus*, potential *Salmonella* competitors. A high copper diet affected faecal concentrations of eleven metabolites important for intestinal colonisation by *Salmonella*. Altered abundance of genes responsible for metabolism and copper homeostasis were observed in the gut metagenome of pigs fed high copper diet. 130 microbial species (40 previously

uncultured) were isolated from piglets. Copper MIC and resistance gene prevalence in cultured microbiota revealed phyla associated with lower copper MIC and lower numbers of copper resistance genes. As *Enterobacterales* have been previously shown to compete with *Salmonella* for gut colonisation, 180 isolates were cultured comprising over 170 *E. coli*. Long-read sequencing of selected *Escherichia* revealed the presence of copper resistance clusters integrated mainly into one chromosomal locus but on a mobile genetic element distinct from copper-encoding SGI-4 in *S.* Typhimurium. *In vitro* assays revealed that presence of copper resistance is pivotal for competition between *Salmonella* and *Escherichia* to occur in high copper supplementation, but there are contact-dependent factors that provide *Salmonella* significant advantage over *Escherichia* in copper-formed niche. Experiments with *Salmonella* devoid of T6SS revealed its contribution to competition in presence of copper.

Conclusion: Copper supplementation substantially modifies the gut microbial community and gut environment of piglets that have the potential to alter competition between pathogenic and commensal bacteria.

Abstract ID: 3613 / Poster Number: 232.16P

The application of the Fourier-Transform Infrared spectroscopy (FTIR) in the microbiological management of patients with cystic fibrosis

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Introduction: Fourier-Transform Infrared Spectroscopy (FTIR) is emerging as a faster and cheaper alternative to traditional methods for microorganisms typing and outbreaks detection. In cystic fibrosis (CF), the rapid identification of Pseudomonas aeruginosa (PA) allows for aggressive and early antibiotic eradication therapy, which may delay chronic colonisation and lung function decline. Determining whether the PA reappearing in sputum within months resisted therapy or is a new strain is crucial for improving therapeutic strategy. This is also important for CF patients on new drug treatment re-presenting PA after several negative samples.

Objective: This study assessed FTIR's ability to differentiate between new *Pseudomonas aeruginosa* (PA) infections and recurrence in cystic fibrosis (CF) patients, compared to WGS.

Methods: Twenty-eight PA isolates from 14 CF patients were analyzed. For 11 patients, strains were taken from the first infection and subsequent re-infection (3-33 months apart). Two patients on modulator therapy and one with intermittent colonization were also included. WGS confirmed FTIR results.

Findings: In patients with PA after the first infection, FTIR suggested therapy failure in 10 cases, with overlapping spectra of isolates, and new PA acquisitions in 4 cases. Clonality was found in 1 patient with intermittent colonization and 2 on modulator therapy. WGS confirmed FTIR findings.

Conclusions: FTIR is a cost-effective, rapid tool for assessing eradicant therapy success and guiding anti-Pseudomonas therapy in CF patients. While protocols are being developed to address limitations like the mucoid phenotype, FTIR is a promising method for optimizing treatment strategies.

Abstract ID: 3884 / Poster Number: 303.15P

The Artemisia associated microbiome is interconnected by a hub Rhizobium taxa

Presenting author: Rezaul Karim, African Genome Center, Benguerir, Morocco

Co-authors: *Jean Legeay, Hijri Mohamed, Bulbul Ahmed*

Artemisia species – *A. herba-alba, A. mesatlantica* and *A. nigrei* are important because of their biological, chemical and medicinal properties. They are most abundant and threatened in the High Atlas regions of Morocco. However, mechanisms underlying the effects of associated microbiome on their adaptability, and that affect the plant-associated microbiome on the medicinal properties, remain unquantified. We investigated the microbial communities associated with three Artemisia species across biotypes in three different locations. No significant difference in bacteria and fungi were found between species in the rhizosphere, roots and shoots, suggesting a shared microbiome among three species. Although a significant difference ($p = 0.047$) was observed between locations, no taxa were found to be significatively associated to a specific *Artemisia* species. However, we found *Rhizobium galegae* exclusively in the roots of all samples from all three *Artemisia* species. Through co-occurrence network analysis, *Rhizobium galegae* was also detected to as a hub taxon for bacterial communities in both roots and shoots. Interestingly, *Rhizobium galegae* is originally isolated from the legume genus Galega, which has medicinal properties with *Artemisia*. Our results indicate a key role of *Rhizobium* bacteria in the microbiome of *Artemisia* species and suggests a potential association between *R. galegae* and a diversity of medicinal plants. Our study revealed a hub bacteria associated with Artemisia roots, it can be modeled by structural equation methods, using correlations within the microbiome associated with Artemisia and the environmental factors that influence microbiome community structuring.

Abstract ID: 970

The attenuating effect of riboflavin bio-enriched soymilk by novel probiotic Lactobacillus strains (MTCC 25433,32) mitigates symptoms of DSS- Induced Inflammatory Bowel Disease in mice

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Co-authors: *Tejpal Dhewa, Neetu Taneja*

Drinking probiotic-containing beverages and consuming fermented foods has been shown to improve health and is currently a global trend. A novel plant-based milk that has gained popularity due to its nutritional and health advantages is fermented soymilk (FSM). This study aims to explore whether fermentation by novel probiotic *L.plantarum* strains can increase its anti-inflammatory properties, and analyse the impact of consuming FSM on intestinal immune response prior and during the inflammatory bowel disease (IBD). This clarifies their impact on short-chain fatty acid (SCFA) levels and gut microbiota. In mice with DSS-induced colitis, consuming FSM beforehand can successfully reduce weight loss and bloody stools, and it is linked to a 25% colon length repair rate. Additionally, it inhibits oxidative stress and the immunological response, stops the expansion of the liver and spleen. FSM consumption during IBD, on the other hand, healed colon damage, stopped liver damage, and decreased weight loss. According to an analysis of the gut microbiome composition in mice, the relative abundances of the beneficial bacteria Akkermansiaceae, Alloprevotella, Dubosella, and Lachnospiraceae increased by 27%, 78%, 62%, and 48%, respectively, when FSM was consumed, while lowering by 73%, 45%, and 60% the relative abundance of the harmful bacteria Turicibater, Bacteroide, and Parasutterella, respectively. Additionally, it raised the SCFA levels in the mice gut. According to the results, FSM has scientific support as an effective anti-inflammatory beverage that may improve gut health and address an imbalance in the inflammatory intestinal flora.

Keywords: Inflammatory bowel disease, Gut microbiota, Anti-inflammatory effects, Fermented Soymilk, Probiotics**.**

Abstract ID: 276 / Poster Number: 029.16P

The biological effect of Kombucha-based postbiotic on the in vitro simulated microbiome

Presenting author: Prof. Emanuel Vamanu, University of Agronomical Sciences and Veterinary Medicine, Bucharest, Romania

Co-authors: *Florentina Gatea, Laura Dorina Dinu*

Introduction: We need the human microbiome to stay healthy, and it has a direct effect on our immune systems, the availability of nutrients, and the development of diseases. It's essential to have a natural way to change the microbiome for diagnosis, treatment targets, and new ways to fight antibiotic resistance. Kombucha-based postbiotics represent a promising, sustainable route to enhance microbial patterns and control dysbiosis.

Objective: This study aimed to determine the effects of a Kombucha-derived postbiotic administration on the microbial and metabolomic pattern of a simulated human gut microbiome in a GIS1 simulator.

Method: A controlled in vitro fermentation model used a standardized microbial pattern to represent the gut microbial composition. The postbiotic product, derived from fermented Kombucha beverages on black tea, was used as an atomized powder. Over one month (daily administration of 1 capsule), microbial and metabolomic patterns were assessed through qPCR and zonal electrophoretic method with reverse polarity.

Findings: Early findings showed that Lactobacillus levels increased, Bifidobacterium levels only partially, and opportunistic bacteria levels decreased. Metabolomic data showed the relevant quantity of short-chain fatty acids and other essential chemicals linked to better gut health and antibiotic defense. In the first two weeks after treatment, pH levels varied significantly, and it was impossible to go up over 5.

Conclusion: The Kombucha-based postbiotic might modulate the gut microbiome and create new valuable products as a natural alternative to probiotics.

Abstract ID: 2016 / Poster Number: 250.17P

The challenge of microbial degradation and recycling of recalcitrant natural and synthetic polymers

Presenting author: Ms. Qing Zhou, Leiden university, Leiden, Netherlands

Co-authors: *Sven Lakemeier, Han Winde*

Degradation and re-utilization of recalcitrant natural and synthetic polymers, such as lignin and plastics, is a global challenge. It represents a critical bottleneck in achieving sustainable waste recycling and resource recovery. Lignin, a highly complex and abundant biopolymer, remains largely underutilized due to its structural complexity and rigidity and chemical recalcitrance. Similarly, the accumulation of man-made polymers like polystyrene and polyurethanes poses significant environmental concerns.

This project aims to address these challenges by exploring bacteria as microbial hosts for efficient degradation of recalcitrant polymers. Since those natural and man-made polymers share chemical and structural characteristics, a combined approach promises to be successful. Key objectives include screening and identification of suitable microbial strains, discovering novel enzymes for specific or combined degradation, and engineering hosts to enhance polymer breakdown. Microbial degradation of polystyrene, alifatic and aromatic polyurethanes, lignin and lignin derived compounds is used as target in screening approaches for novel enzymes as well as in enzyme and host engineering. This study also employs CRISPR-Cas3 genome editing, transcriptome analysis, and biochemical characterization using SDS-PAGE, and HPLC.

Preliminary results have identified strains of the soil bacteria Pseusomonas putida and Pseudomonas furukawaii as promising hosts for efficient polymer degradation. These findings lay the groundwork for developing sustainable strategies to recycle and valorize recalcitrant polymers.

Abstract ID: 4032

The Data Hunters Workshop: a scalable solution for microbiome data reuse and Open Science

Presenting author: Sara Fumagalli, PhD, University of Milano-Bicocca, Milano, Italy

Co-authors: *Alice Armanni, Maurizio Casiraghi, Luca Corneo, Giulia Ghisleni, Massimo Labra, Antonia Bruno*

Introduction: Microbiome research is evolving rapidly with advances in sequencing technologies, providing access to vast datasets with unprecedented potential. However, fully leveraging these resources requires accurate metadata, which remains a persistent challenge due to a lack of standardization and adequate training. Curated metadata is part of the broader effort to enable the reusability of microbiome data for large-scale analyses and integrative research necessary to develop microbial solutions for humanity's challenges. Overcoming these limitations is essential to maximizing the impact of microbiome research for societal advancements. To meet this need, we designed the Data Hunters Workshop, a scalable educational model, equipping participants with essential skills in metagenomics and bioinformatics.

Objectives: The Data Hunters Workshop was tested on human skin microbiome metadata. Specific aims include a) Train participants in metagenomics, metadata standardization, and bioinformatics, providing them with critical skills; b) Manually curate and standardize metadata from amplicon-based and shotgun sequencing projects, working on the most comprehensive and up-to-date human skin microbiome datasets; c) Ensure a high-quality, standardized, and reusable resource for future microbiome research. Moreover, it seeks to establish a reproducible model for microbiome studies, applying a participatory framework to enhance (meta)data accessibility, interoperability, and reusability in microbiology.

Method: The Data Hunters Workshop followed a four-phase structure: (i) an in-person lecture covering metagenomics, bioinformatics, and metadata curation; (ii) an autonomous learning phase with Python tutorials and a command-line tool with the metadata curation protocol; (iii) an operative session where participants curated metadata from human skin microbiome projects; (iv) an assessment through surveys evaluating perceived skills acquisition, confidence levels, and knowledge and understanding of the scientific process. Continuous tutoring support was provided, and participants who completed 75% of the workshop activities received certification,

academic credit, and the possibility of co-authorship in a peer-reviewed publication derived from this study.

Findings: A total of 29 students participated, curating metadata from 379 sequencing projects, demonstrating the feasibility of large-scale collaborative metadata curation. 17 students successfully completed at least 75% of their assigned projects, resulting in the curation of 245 high-quality, standardized metadata records, enhancing the reusability of microbiome data. Survey responses (n=12) indicated an increase in perceived skills, with median scores rising from 1.88 to 3.81 (out of 5). Confidence in the metadata curation process showed consistent median scores of 3.88 in both learning and application, demonstrating an effective translation of theoretical knowledge into practice. Knowledge assessment revealed that all students correctly answered at least 50% of the questions, with 75% achieving at least two-thirds accuracy, moreover, showing balanced proficiency in both metagenomics and bioinformatics.

Conclusion: The Data Hunters Workshop successfully demonstrated the feasibility of collaborative engagement in addressing a key bottleneck in microbiome research. Participants improved their scientific and technical skills, contributing to the creation of a high-quality, reusable microbiome dataset. This model provides a scalable and adaptable framework for training researchers and optimizing microbiome data reuse, reinforcing its value in advancing microbiology research.

Abstract ID: 3135

The development of activities for scientific literacy in the Deep-Sea environment derived by microorganisms: a picture book read-aloud performance and ROV ecotourism

Presenting author: Dr. Takako Sato, NPO Team Kujira-go, Yokosuka-city, Japan

Co-authors: *Chiaki Kato, Hanemi Kashiwabara, Satomi Sato, Masahiro Ito, Maya Kadota, Takuya Matsuo*

The science education provided to children, a way to prevent stumbling during the learning process, a recently prominent method is STEAM. This reports on the development of science awareness activities implemented as successful examples.

The purpose of activities is to achieve highly effective educational outcomes by providing children with a unique STEAM-based science education. Additionally, this method is thought to encourage the establishment of high scientific literacy in the parents who participate alongside their children.

The extreme deep-sea ecosystems supported by chemoautotrophic bacteria are still largely unknown to the public. To encourage active interest and further spark curiosity about this ecosystem, we created engaging picture books for preschool and elementary school children and developed a performance combining "reading with live guitar music" and a "quiz". After, a group of 10 elementary school children as part of a "Science Club," where they participated in various scientific activities, with their motivation to learn assessed over time.

Furthermore, an experiential learning approach, we developed a science tour for ocean exploration using ROVs (remotely operated vehicles) to observe the deep-sea environment. This tour was evaluated with 15 parent-child pairs. In these activities, it was found that the participants' attitudes toward science were positively influenced, and their behaviour toward proactive engagement shifted before and after the experience.

These activities demonstrate that elements such as art and exploration in science education create extraordinary experiences that inspire and engage participants, leading to positive effects. This can be seen as a new approach in science outreach.

Abstract ID: 3109

The Dutch Ba(chelor)Ma(ster) symposium; helping students to start a career in Microbiology

Presenting author: Assoc. Prof. Girbe Buist, University Medical Center Groningen, Groningen, Netherlands

Since 2011 the education section of the Royal Dutch Society for Microbiology (KNVM) yearly organizes a scientific meeting for Bachelor and Master students, the so-called 'BaMa symposium'. The meeting, which is free of charge for students, brings together students that have a strong interest in or perform(ed) and internship in Microbiology. Since 2013 the symposium is embedded in the yearly 2-day Spring Meeting of the KNVM and the Dutch Society of Medical Microbiology (NVMM). The objectives of the BaMa symposium are:

1. To give students the opportunity to present their research and gain experience on a national platform.
2. Introducing students and lecturers to the scientific practice, culture and atmosphere of a symposium/conference.
3. To give students and lecturers a better idea of the opportunities for internships at various institutes, institutions and companies in the Netherlands.
4. To give educational institutions, institutes and companies the opportunity to present their microbiological (research) programmes and/or products.

Students who are doing an internship in the field of bacteriology, virology, parasitology, mycology or serology are invited to submit an abstract for an oral/poster presentation. After selection around 8 students are invited for an oral presentation. In-between two sessions with oral presentations a poster session is held. Every year members of the education section select the student with the best oral presentation. Using an online platform, the students vote for the best poster. In 2023 the 'Mike Jetten' award was handed out for the first time for the 3 best Dutch master theses in Microbiology. Last year the 'Fanny Hesse' award was awarded for the 3 best Dutch applied sciences bachelor's theses in the field of Microbiology. The selection for both award is done by the board and education section of the KNVM.



The yearly participation to the BaMa symposium is around hundred students. During the poster session around 30 posters are presented. The students always show an active, enthusiastic, interested but also critical participation in the oral and poster presentations. The number of theses send in for the ‘Mike Jetten’ and ‘Fanny Hesse’ awards has increased since their introduction. As the symposium is embedded in the national Microbiology meeting the student make useful network connections and exchange fruitful ideas with other students and professionals. Students who previously attended a BaMa symposium found their way into a career in the field of Microbiology.

Over the years the ‘BaMa symposium’ has become a meeting where students with a passion for Microbiology can meet, interact and obtain ideas and possibilities for their next career steps in the field of Microbiology.

Abstract ID: 504 / Poster Number: 015.16P

The effect of a probiotic yoghurt on gut microbiome, low-grade inflammation, and weight status of obese South African women

Presenting author: Dr. James Elegbeleye, University of Pretoria, Pretoria, South Africa

The prevalence of obesity and its associated comorbidities, such as low-grade chronic inflammation and altered gut microbiome profiles, is a growing public health challenge in South Africa. The study investigates the impact of a probiotic-enriched yogurt on the gut microbiome, systemic inflammation, and the effects of these enriched-gut microbiome on the weight status of obese South African women. Probiotic-enriched yogurt was prepared using LGG (Lactobacillus rhamnosus GG) and BB-12 (Bifidobacterium animalis subsp. lactis). The probiotic yogurt sample was analyzed at day 0 and 7 to ensure the concentrations are beyond the recommended levels. Using a randomized controlled trial design, participants consumed either probiotic yogurt or a placebo (plain yogurt) for 12 weeks, with assessments conducted at baseline and post-intervention. Gut microbiome composition was analyzed using next-generation sequencing, while markers of inflammation (e.g., C-reactive protein, interleukins) and weight-related parameters (BMI, waist circumference) were measured using standard protocols. Preliminary results suggest that probiotic yogurt consumption leads to significant modulation of the gut microbiota, marked by increased abundance of beneficial bacteria, reduced inflammatory markers, and modest weight improvements. This study highlights the potential of functional foods as an adjunctive strategy for managing obesity and its metabolic consequences in vulnerable populations.

Abstract ID: 3344 / Poster Number: 177.17P

The effect of post-translational modifications on bacteriocin stability

Presenting author: Ms. Katie Bourke, University College Cork, Cork, Ireland; APC Microbiome Ireland, Cork, Ireland

Co-authors: *Paula M O'Connor, Colin Hill, R. Paul Ross*

Introduction: Bacteriocins are ribosomallly-synthesised antimicrobial peptides typically classified into two groups, peptides with post-translational modifications (PTMs) and unmodified peptides. If bacteriocins are to be used as potential therapeutics information on their stability in the gastrointestinal environment is essential. This is also important where bacteriocins are to be used as food biopreservatives where their breakdown during digestion might be preferable so to not impact the microbiome.

Objective: To compare the stability of five bacteriocins with diverse levels of PTMs under conditions mimicking the gastrointestinal tract.

Methods: Capidermicin (leaderless), pediocin PA-1 (pediocin-like), leucocyclocin C (circular), thuricin CD (sactipeptide) and nisin A (lanthipeptide), were analysed for the presence of potential enzyme cleavage sites (pepsin, trypsin, chymotrypsin, and proteinase K). The bacteriocins were then synthesized or purified before enzyme treatment. Aliquots were tested against an indicator strain by well diffusion assay to assess activity followed by matrix-assisted laser desorption/ionization time of flight mass spectrometry analysis to identify fragment/peptide masses. Bacteriocins were also exposed to an array of pH and temperature conditions to assess other aspects of stability.

Findings: Preliminary results showed that when exposed individual GI proteases bacteriocins with more extensive PTMs such as nisin could survive exposure to these proteases for far longer than their less modified counterparts. Exposure to an array of pH displayed that the bacteriocins were active at lower pH conditions such as those encountered during GI transit.

Conclusions: These findings confirmed that the stability of the bacteriocins was correlated to their structural complexity in relation to PTMs.

Abstract ID: 1001 / Poster Number: 191.15P

The effect of shear forces and washing on granular growth and species distribution in water kefir

Presenting author: Ms. Sabine Michielsen, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Co-authors: *Herwig Bachmann*

Background: The water kefir community comprises roughly 30 different species of lactic acid bacteria(LAB), acetic acid bacteria(AAB) and yeasts, contained in a self-produced and maintained granule. The granule is described to improve community stability making granular growth an important community function.

Using the conventional backslopping protocol granular growth can be highly variable if measured over subsequent transfers. Variability could be caused by several factors. One potential cause is a washing step of the granule in between transfers, where shear forces between granules occur. This step may damage the granular structure, change the community balance by washing out community members, or improve nutrient diffusion by preventing buildup of biofilms around the granule.

Here, we investigate the effect of shearing intensity during the washing step on granular growth.

Methods: The growth of water kefir granules was monitored when propagating with and without an intermediate washing step at each transfer. Fluorescent staining combined with microscopy was used to compare the spatial distribution of microbes in the granule.

Results and conclusion: The granules consistently grew faster when washed rigorously. This effect could be reversed after switching the treatment. Investigating community composition with fluorescence microscopy showed that rigorous washing led to a reduction of yeast abundance in the outer layer of the granule. As the exopolysaccharide for granule growth is produced by the lactic acid bacteria of the kefir community this could suggest that the washing step improves granular growth by increasing nutrients available to LABs by diluting the yeast population.

Abstract ID: 1351 / Poster Number: 200.15P

The effects of geographical and nutrient variation on bee microbiome composition: a meta-analysis

Presenting author: Nelli Vardazaryan, PhD, Armenian Bioinformatics Institute, Yerevan, Armenia

Co-authors: *Lilit Nersisyan*

Honey bees are vital pollinators, yet their populations face global declines due to stressors like pesticide exposure and environmental changes. This study examined the effects of geographic location and pesticide treatments on the honeybee gut microbiome composition and network interactions through a meta-analysis of 16S rRNA and WGS datasets from six regions, supplemented with new data from Armenia.

We re-analyzed datasets with a consistent preprocessing pipeline, assessing taxonomic composition, diversity metrics, and differential abundance. To evaluate treatment effects on bacterial co-abundance networks, we developed an R package to build a correlation network based on all studied datasets, identify subnetworks significantly affected by treatments, and link them to biological pathways.

Six previously reported core taxa were identified in the 16S datasets, and an additional six core genera were uncovered with WGS datasets. In Armenian samples, we observed seasonal differences, with increased *Commensalibacter* during autumn. Anthropogenic effects also shaped the microbiome, though without associations to specific bacterial taxa. Among pesticide and sugar supplements treatments, oxalic acid, a widely used insecticide, caused the most pronounced shift in gut microbiome composition; however, sugar supplements and herbicides also induced significant changes. Co-abundance network analysis showed that insecticides of different classes had a similar impact. Treatment with a commonly used herbicide glyphosate, on the other hand, affected different subnetworks in various experiments. Interestingly, in one study, we found pathways potentially facilitating glyphosate degradation by *Pseudomonas*, suggesting novel resistance mechanism.

We explored the impacts of geography and pesticides on the honeybee gut microbiome through a comprehensive meta-analysis.

Abstract ID: 722 / Poster Number: 191.16P

The Effects of Saccharomyces cerevisiae-Derived Vacuoles on Gut Microbial Activity and Metabolite Production

Presenting author: Ms. Jiwoo Lim, Jeonbuk National University, Jeonju, South Korea

Co-authors: *Jiho Min, Yang-Hoon Kim*

Introduction: The composition of gut microbiota is closely linked to host health, playing critical roles in inflammation regulation, gut health, and the development of various diseases. Current methods to modulate gut microbiota, such as prebiotics, probiotics, and fecal microbiota transplantation (FMT), often involve complex procedures or yield unpredictable outcomes. Thus, there is a need for simple and precise therapeutic alternatives.

Vacuoles are cellular organelles in yeast that decompose various toxic substances. *S. cerevisiae*-derived vacuoles are known to perform diverse roles, such as drug carriers, immunomodulators, and possessing antibacterial and antiviral properties.

Objective: This study focused on evaluating the effects of *S. cerevisiae*-derived vacuoles on the activity of beneficial gut bacteria.

Method: Three human gut-beneficial bacteria were selected, and their growth and short-chain fatty acid (SCFA) production were assessed after treatment with *S. cerevisiae*-derived vacuoles. Additionally, changes in bacterial adhesion rates were analyzed using a gut model composed of Caco-2 cells.

Findings: *S. cerevisiae*-derived vacuoles did not stimulate the growth of *Lactobacillus reuteri*, *Lactobacillus casei*, or *Streptococcus thermophilus*, but they promoted SCFA production by *S. thermophilus*. In the Caco-2-based gut model, *S. cerevisiae*-derived vacuoles enhanced the adhesion rates of *L. casei* and S. *thermophilus*.

Conclusion: *S. cerevisiae*-derived vacuoles were found to stimulate the adhesion of beneficial gut bacteria and the production of beneficial metabolites, such as SCFAs. These findings highlight the potential of *S. cerevisiae*-derived vacuoles as a promising tool for modulating gut microbiota and aiding in the treatment of various diseases.

Abstract ID: 2752 / Poster Number: 199.16P

The enhanced pneumococcal LAMP assay: a clinical tool for diagnosing meningitis caused by non-vaccine-serotypes of Streptococcus pneumoniae

Presenting author: Dr. Mitsuko Seki, Meikai University, Saitama, Japan; Nihon University, Tokyo, Japan

Co-authors: *Eun Jin Kim, Dai Kanamori, Hiroko Oguchi, Bin Chang, Chika Takano, Paul Kilgore, Tomonori Hoshino, Dong Wook Kim*

Introduction: *Streptococcus pneumoniae* remains a major pathogen responsible for invasive pneumococcal diseases (IPDs) such as meningitis and bacteraemia, with children under five-year-olds and elderly. The introduction of pneumococcal conjugate vaccines (PCVs) has led to significant reductions in IPD incidence, yet the challenge of serotype replacement persists. Current diagnostic methods for pneumococcal serotyping, such as the Quellung reaction, are constrained by the need for technical expertise and resources, particularly in developing countries. To address these challenges, we previously developed loop-mediated isothermal amplification (LAMP)-based serotyping assays for PCV13 and PPSV23 serotype-specific assays.

Objective: To develop improved LAMP assays targeting fifteen non-vaccine serotypes (6C, 7C, 13, 15A, 16F, 21, 23A, 23B, 24F, 31, 34, 35B, 35F, 38, 39), and assess their suitability for detecting these serotypes in cerebrospinal fluid (CSF) and blood.

Method: The LAMP assays targeting the fifteen non-vaccine-serotype-specific genes were developed. Each assay was evaluated to determine test reactivity, specificity, and sensitivity, and its performance was compared to that of conventional PCR. To support its use in patients with suspected meningitis, we evaluated the detection limits of the assays using bacterial genomic DNA-spiked CSF and blood specimens.

Findings: The specificity of the LAMP assays was confirmed using 49 serotypes of pneumococcal strains. The LAMP assays could detect as few as 100 copies of both purified DNA, spiked CSF and blood specimens with greater sensitivity than conventional PCR.

Conclusion: This specific, sensitive, and robust LAMP method offers significant advantages for screening patients on a population basis particularly in resource limited settings.

Abstract ID: 3035 / Poster Number: 286.15P

The environmental plasmidome of Denmark

Presenting author: Mr. Bertil Sørensen, Aalborg University, Aalborg, Denmark

Co-authors: *Mantas Sereika, Mads Albertsen, Francesco Delogu*

Plasmids are ancillary DNA molecules that play an important role in shaping microbial communities by extending the traits of their hosts. However, the collection of known plasmids is largely populated by sequences recovered from culturable microbes, leaving a large gap of knowledge especially regarding the environmental plasmids. In the past decade, advancements in metagenomics allowed the *in situ* recovery of microbial communities and their plasmidomes. Increasing the knowledge base of plasmids will allow us to peer into their overall diversity and specific contributions to the microbial communities beyond model organisms and systems.

Using the Microflora Danica data collection, spanning 10,000+ metagenomes sampled across Denmark, we clustered the plasmidome of Denmark with plasmids from public databases into Plasmid Taxonomic Units (PTUs) and almost doubled the known plasmidial diversity. We find that 99% of the Danish plasmids do not cluster with previously known plasmids (70% Average Nucleotide Identity and 50% Alignment Fraction of the shortest plasmid in a pair). Moreover, the Danish plasmids had a significantly higher proportion of hypothetical protein-coding genes, accounting for more than 50% of their total genes; more than double of those from databases. Our study vastly increases the set of plasmids recovered from environmental metagenomes, bridging the knowledge gap regarding plasmid diversity in natural environments.

Abstract ID: 4119 / Poster Number: 379.17P

The evolving burden of bacterial antimicrobial resistance in the EU and EFTA: a cross-country comparative analysis over three decades (1990–2021)

Presenting author: Dr. Tomislav Mestrovic, University of Washington, Seattle, United States; Institute for Health Metrics and Evaluation, Seattle, United States; University North, Varazdin, Croatia

Co-authors: *Gisela Robles Aguilar, Lucien R Swetschinski, Authia P Gray, Kevin S Ikuta, Rebecca L Hsu, Chieh Han, Eve E Wool, Erin Chung, Nicole Davis Weaver, Daniel T Araki, Anna Gershberg Hayoon, Ben S Cooper, Christiane Dolecek, Christopher J L Murray, Mohsen Naghavi*

Introduction: Antimicrobial resistance (AMR) is a major global health threat. As part of the IHME/Oxford GRAM project, we provide the first comprehensive estimates of AMR-related mortality across EU/EFTA countries from 1990 to 2021.

Objective: To estimate age-specific deaths attributable to and associated with bacterial AMR for 22 pathogens, 84 pathogen–drug combinations and 11 infectious syndromes, evaluating long-term trends and disparities across the EU/EFTA region.

Methods: We analysed 520 million records across 19,513 study-location-years, using statistical modelling to estimate AMR burden – leveraging sepsis deaths, pathogen-attributable infections, resistance prevalence, and excess mortality risk. Two counterfactual scenarios were modelled: one replacing all drug-resistant infections with drug-susceptible infections, and another replacing them with no infection.

Findings: In 2021, AMR was associated with 283,000 deaths (95% UI 247,000–310,000) and directly attributable to 60,000 deaths (52,400–66,500) in EU/EFTA countries. Bloodstream and lower respiratory infections accounted for the highest mortality burden. Between 1990 and 2021, AMR-related mortality declined by 87.1% in children under five but increased by 5.2% in adults aged 70 and older. Two out of every three bacterial infection-related deaths were linked to AMR. Mortality rates negatively correlated with the Socio-Demographic Index (SDI, r = –0.83 [95% UI –0.91 to –0.67]). *Staphylococcus aureus* was the leading AMR-associated pathogen (60,900 deaths, 95% UI 49,300–73,100), with methicillin-resistant *S. aureus* (MRSA) being the deadliest pathogen-drug combination. Carbapenem resistance exhibited a notable increase over the 31-year period.

Conclusion: AMR remains a significant and evolving threat in the EU and EFTA, disproportionately affecting vulnerable populations.

Abstract ID: 2158 / Poster Number: 408.15S

The expression of integron arrays is shaped by the translation rate of cassettes

Presenting author: André Carvalho, PhD, Universidade Complutense de Madrid, Madrid, Spain; VISAVET Health Surveillance Centre, Madrid, Spain

Co-authors: *Alberto Hipólito, Filipa Trigo da Roza, Lucía García-Pastor, Ester Vergara, Aranzazu Buendía, Teresa García-Seco, José Antonio Escudero*

Introduction: Integrons are bacterial genetic platforms, often associated to plasmids and transposons, that capture cassettes containing promoterless adaptative genes and stockpile them in arrays of variable length. They are key elements in the rise and spread of multidrug resistance in Gram-negative bacteria: 89% of all cassettes carried by class 1 integrons encode antimicrobial resistance genes (ARGs), totalling almost 200 different ARGs conferring resistance to 12 different antibiotic families. Structurally, integrons are composed of a stable platform, which comprises an integrase-coding gene (*intI*) and a recombination site (*attI*) for cassette integration. Downstream of the stable platform lies a variable array of cassettes. Integron cassettes are composed of an open reading frame (ORF) and an *attC* recombination site and are generally promoterless. However, upon expression of the integrase, cassettes can be excised, reshuffled and integrated in the first position of the array at the *attI* site, where their expression is driven by a dedicated promoter (Pc) located upstream in the stable platform. This configuration makes of integron arrays operon-like structures, with a single promoter governing the expression of several cassettes. As a result, it has been generally accepted that expression of integron arrays follows a gradient, with expression decreasing as a function of the distance from the promoter.

Objectives: However, how different cassettes may impact the extent of this gradient has been largely overlooked in the field. We sought to explore in depth this expression gradient model, using a large collection of hundreds of antibiotic resistance cassettes (ARCs).

Methodology: For this, we transformed *Escherichia coli* MG1655 with a vector containing a class 1 integron-like structure with a variable cassette array composed of an ARC in first position followed by a *gfp* "cassette". This allowed us to study the impact of 136 different antibiotic resistance cassettes on GFP expression through flow cytometry and RT-qPCR. We then substituted the *gfp* cassette with other ARCs and assessed how antibiotic resistance levels varied.

Results: Our results show that cassettes can exert different levels of polar effects on the expression of the array (up to 200-fold variation), which can be generally observed at the mRNA level. We also demonstrate that these polar effects determine the resistance levels conferred by cassettes downstream and in some cases can lower their resistance below the clinical breakpoint. We went further and revealed the mechanistic basis of these polar effects: we show that the translation rates of cassettes affect the stability of the whole polycistronic mRNA molecule, thus directly impacting array expression.

Conclusion: Our findings change the working model of the integron, to one where the expression gradient of the array becomes dependent on the identity of the first cassette. Additionally, this work establishes the basis for a novel adaptative role of the integron – that of modulating the fitness costs associated to antibiotic resistance genes.

Abstract ID: 1483 / Poster Number: 349.15F

The ferric uptake regulator (Fur) of Helicobacter pylori: controller of apo and holo direct regulons and mediator of distal chromatin interactions

Presenting author: Dr. Andrea Vannini, University of Bologna, Bologna, Italy

Co-authors: *Eva Pinatel, Stefano Maggi, Giulia Vittoria Ruta, Paolo Costantini, Davide Roncarati, Vincenzo Scarlato, Clelia Peano, Claudio Rivetti, Alberto Danielli*

Introduction: Fur protein controls iron homeostasis by switching between its apo- and holo-forms based on intracellular iron levels, thereby regulating gene expression. Apo-Fur is usually inactive, while holo-Fur represses or, rarely, activates the expression of targeted genes. Peculiarly, in *H. pylori*, both apo- and holo-Fur (HpFur) actively regulate gene expression with distinct DNA binding properties and regulons. Four regulons have been proposed for HpFur: apo-HpFur repressed, holo-HpFur repressed, apo-HpFur activated, and holo-HpFur activated regulons.

Objectives and Methods: A multiomic approach using RNA-seq and ChIP-seq under different strains and iron conditions, along with in vitro protein-DNA binding assays, was employed to identify the four HpFur direct regulons. Atomic force microscopy and Chromosome Conformation Capture assays were used to elucidate the role of holo-HpFur beyond transcription regulation.

Findings: The apo- and holo-HpFur directly repressed regulons were (re)defined, enlisting new coding sequences, virulence factors, unannotated transcripts, putative non-coding RNAs, and bacterial toxins. Both apo- and holo-HpFur activated regulons were found to be indirectly regulated. Analysis of the highly prevalent non-promotorial and non-regulatory holo-HpFur binding sites revealed that it induces DNA compaction and looping at multiple genomic loci, without affecting gene expression. This novel behaviour was further confirmed by the identification of HpFur-mediated interactions between distal genomic sites.

Conclusion: The four HpFur regulons were (re)defined, distinguishing between direct and indirect regulation. A new role of holo-HpFur in bacterial nucleoid organization and modelling is proposed.

Abstract ID: 3631 / Poster Number: 396.17P

The first step for probiotic use to increase rainbow trout (Oncorhynchus mykiss) production efficiency: isolation and characterization of probiotic-candidate bacteria

Presenting author: Assoc. Prof. Menekşe Didem Demircan Akyasan, Istanbul University, Faculty of Aquatic Sciences, Istanbul, Turkey

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Background: Rainbow trout is an economical species cultivated for human consumption. Increasing production efficiency can be possible by increasing the gamete quality.

Objective: One of the applications that would increase gamete quality is to feed the fish with probiotics, which may also keep the health level of the fish high and constitute the hypothesis of this study.

Methods: Eighteen rainbow trout broodfish were sampled from three regions of Turkey between December 2023 and February 2024. Intestine, egg and sperm samples of the fish as well as water samples from the culture environment were taken. Bacterial cultures were grown using the spread plate method on De Man Rogosa & Sharpe Agar to selectively cultivate lactic acid bacteria. Species identification of the bacteria was made with MALDI-TOF and 16SrDNA gene sequencing methods.

Results: To use these bacteria as probiotics, antimicrobial susceptibility profiles against various antibiotics were determined and in vitro antagonistic tests were performed against common pathogens of rainbow trout. Carnobacterium maltaromaticum, Lactococcus lactis subsp lactis, Lactococcus lactis subsp. cremoris and Enterococcus casseliflavus, with low antibiotic resistance and high antagonistic effects were selected and tested for in-vivo pathogenicity in healthy rainbow trout over 21 days. The bacteria were found to be non-pathogenic to the fish.

Future studies will finalize probiotic selection, test probiotic-enriched feed on broodfish, and assess its impact on gamete quality.

Abstract ID: 1505 / Poster Number: 106.15F

The first worldwide analysis of the soil microbial diversity patterns across savanna ecosystems

Presenting author: Dr. Lea Nosalova, Sorbonne University, Paris, France

Co-authors: *Audrey Niboyet, Sebastien Barot, Yoan Marcangeli, David Carmignac, Jean-Christophe Lata*

Savannas are a major biome, covering more than one-sixth of terrestrial habitats, encountered in all tropical regions around the globe. These tropical savannas are characterized by rapid human population growth and are influenced by a range of natural and anthropogenic factors. Although soil nutrient limitation should strongly limit plant growth in these ecosystems, the primary productivity of humid savannas can be as high as that of tropical rainforests. While the microbiota of tropical forests has been extensively studied, knowledge about the savanna microbiome is still developing. To further explore the interactions between savanna grasses and microbial communities, as well as to gain initial insights into global soil microbial diversity in savannas, we conducted the first global sampling of savanna soils. In this study, more than 250 soil samples from six savanna sites and under the rhizosphere of 39 grass species vs. bulk soils, with varying geological, climatic, and ecological conditions were collected and analyzed in terms of soil characteristics (including soil C, N and P contents and soil pH). Additionally, we analyzed microbial composition using metabarcoding, targeting the V4-V5 (for bacteria and archaea) and ITS2 (for fungi) regions. The taxonomic and functional diversity of bacteria, archaea, and fungi, as well as the differences in microbial groups involved in N cycle, were assessed and compared across different sites, climates, and ecosystems. The main environmental drivers shaping microbial communities were identified, with the first results indicating similarities among the sites of West Africa compared to other studied regions.

Abstract ID: 733 / Poster Number: 038.16P

The Gut Microbiome and Incident Hypertension: a 14-Year Cohort Study

Presenting author: Li-Fang Yeo, PhD, University of Turku, Turku, Finland; Turku University Hospital, Turku, Finland

Co-authors: *Joonatan Palmu, Aki Havulinna, Pekka Jousilahti, Ville Laitinen, Katariina Pärnänen, Veikko Salomaa, Leo Lahti, Rob Knight, Teemu Niiranen*

Introduction: Hypertension remains the leading modifiable risk factor attributable to 10.8 million premature deaths. Hence the study of hypertension and gut microbiome as a therapeutic target is very important. Yet the links between the gut microbiome and long-term incidence of hypertension are unknown.

Objective: This study assessed the association between the gut microbiome and incident hypertension.

Methods: The study sample consisted of 3049 non-hypertensive individuals (60.7% women) aged 25-74 years who were drawn from the general population in Finland. In the baseline examination performed in the year 2002, the participants underwent a health examination and provided a stool sample. The gut microbiome was assessed using shallow shotgun metagenomic sequencing. The participants were followed up for incident hypertension until December 31, 2024.

Findings: Over a median follow-up of 13.6 years, 587 participants developed hypertension. Gut microbiome alpha (Shannon index; $p = 0.90$) and beta diversity (Bray-Curtis dissimilarity matrix; $p = 0.69$) were not associated with incident hypertension in the multivariable-adjusted models. In total, 12 genera were significantly associated with incident hypertension in the age- and sex-adjusted models (FDR-$P < 0.05$ for all), but none remained significant in the multivariable-adjusted models ($P \geq 0.05$ for all).

Conclusion: We observed no associations in multvariable-adjusted models between the gut microbiome and incident hypertension in this first-ever prospective study with long term follow up. Our study demonstrates that the gut microbiome does not improve risk prediction for hypertension, nor is there support for the gut microbiome being used as a therapeutic target for hypertension.

Abstract ID: 1047 / Poster Number: 045.16P

The hidden impact of radiation: how urinary microbiome shift in prostate cancer patients

Presenting author: Dr. Michał Złoch, Nicolaus Copernicus University in Toruń, Toruń, Poland

Co-authors: *Ewelina Sibińska, Fernanda Monedeiro, Wioletta Miśta, Adrian Arendowski, Piotr Fijałkowski, Monika Pietrowska, Jolanta Mrochem-Kwarciak, Anna Jędrzejewska, Ewa Telka, Kinga Karoń, Małgorzata Rabsztyn, Paweł Pomastowski, Dorota Gabryś*

Introduction: The urinary microbiome may play an important role in the complications development, however, there is no information about their changes under radiotherapy (RT) of prostate cancer.

Objective: The objective of this study was to evaluate the changes caused by irradiation in the urine microbiome of prostate cancer patients with long-term follow-up.

Methods: Matrix-assisted laser desorption/ionization mass spectrometry (MALDI) alongside diverse culture conditions was applied to identify the urinary microbiome. Moreover, blood and urine biochemical assays were performed to correlate microbiological changes with the health status of the patients.

Findings: Study reveals that RT for prostate cancer induces a dynamic response in the urinary microbiome, characterized by an initial reduction in diversity post-RT followed by a subsequent increase. Glucose levels in urine and blood significantly influenced the urinary microbiota This augmentation in microbial diversity may be attributed to the sterilization effect of radiation, rendering the urinary tract more susceptible to colonization by various microorganisms in the RT-following period.

Conclusions: The robustness of our methodology, employing MALDI identification and multiple culture conditions, has proven helpful in precisely tracking intricate changes in the urinary microbiome during RT. Furthermore, our findings highlight the significant influence of glucose levels in both urine and blood on the urinary microbiota. Considering the prolonged vulnerability of the urinary tract, proactive measures such as probiotic supplementation and careful management of glucose levels could be valuable strategies for maintaining a healthier microbial balance.

Abstract ID: 1484 / Poster Number: 096.15S

The impact of environment and the anthropisation on the fungal exposome in the South-West coastal region of France

Presenting author: Mr. Gautier Chauvin, INSERM U1045, Pessac, France; UMR CNRS 7267, Poitiers, France; Université de Poitiers, Poitiers, France

Co-authors: *Baptiste Defaye, Christine Imbert, Laurence Delhaes*

Introduction: The fungal exposome is a key factor in understanding the emergence and spread of micromycetes (Wei et al. 2022, WHO 2022 report). *Nouvelle-Aquitaine*, a known hotspot for avian pathogen emergence, has experienced multiple avian influenza outbreaks in wild and farmed birds (Briand et al. 2022). This research investigates how anthropization, pollution, and climate change influence fungal biodiversity and infectious emergence within a "One Health" framework.

Objective: To identify environmental factors driving fungal infections and explore the link between anthropogenic pressures, fungal biodiversity, and pathogen emergence in a region critical for human, animals, and environment health research.

Method: Based on Mycosand consortium sampling protocol (Gangneux et al. 2024), samples were collected from 30 sites (sediments, soils, water, birds) along avian migratory corridors, representing a gradient of anthropization. Cultural and metagenomic analyses show a large distribution of different mycobiomes, with notably *Aspergillus* and *Candida spp*. pathogens isolates. Pathogenicity factors such as thermotolerance and antifungal resistance were assessed in *Aspergillus* isolates.

Findings: This study is the first to highlight the fungal biodiversity and exposome in *Nouvelle-Aquitaine*. The results will be analyzed through a "One Health" lens to explore potential fungal-environment-health interactions and advance understanding of pathogenicity and transmission. In all, we position this emerging territory as a valuable resource for studying pathogens dynamics.

Conclusion: These findings emphasize the importance of leveraging *Nouvelle-Aquitaine*'s unique status as a hotspot for avian and fungal pathogen emergence to advance research on microbial-environmental interactions and pathogen control.

Abstract ID: 2404 / Poster Number: 222.17P

The impact of Escherichia coli genetic background on its ability to evolve antimicrobial resistance

Presenting author: Prof. Galardini Galardini, Twincore, Hannover, Germany

Co-authors: *Adam Mulkern, Jenny Fiebig, Thu-Hien Vu,*

Introduction: The rise of antimicrobial resistance (AMR) is a serious threat to global health in the 21st century. Life-threatening multi-drug resistant microbes have emerged and thus new approaches are needed to maximize the “shelf-life” of antimicrobials by reducing the risk of the emergence and spread of resistance.

Objectives: Recent studies have shown how different strains belonging to the same bacterial species possess a different propensity to develop AMR (“evolvability”). The genetic determinants of differences in evolvability have however not been determined in E. coli; our aim is to discover them using laboratory evolution. Additionally, whether different strains acquire the same resistance mutations when exposed to the same drug is also currently unknown.

Methods: We have applied a semi-automated laboratory evolution protocol to 308 E. coli isolates to 3 antimicrobials and 6 biological replicates: Amikacin, Ceftriaxone, Rifampicin. We used a serial passaging protocol with daily doublings in concentration, from 1/8th of the Minimum Inhibitory Concentration (MIC) until 64X MIC. We have preserved one evolved population for each biological replicate and subjected it to genome sequencing and further characterization.

Results: Using the passage at which we last observe growth as a measure of evolvability, we observed substantial variability in the ability of each strain to become resistant, which varies by genetic background and drug class, with Ceftriaxone and Rifampicin showing the largest variance. At the genome level, we observed large differences in the mutations acquired during the experiment, further underscoring the influence of E. coli’s genetic background on evolution. We also identified genetic variants associated with differences in evolvability acorss isolates. We also identified patterns of cross-sensitivity and cross-resistance in the evolved isolates, which have important implications in designing combination therapies.

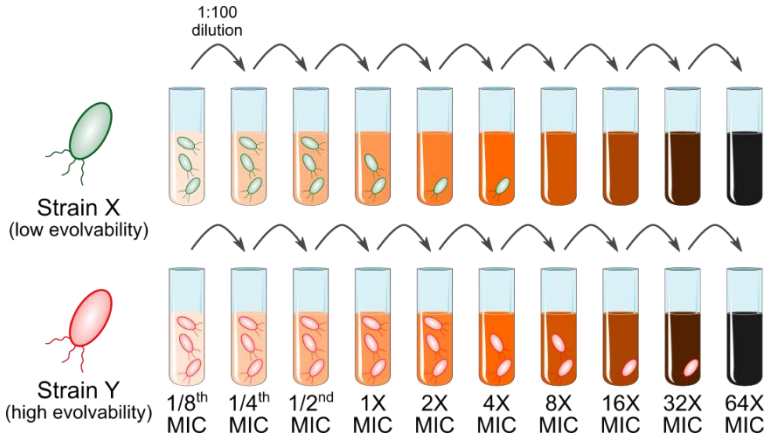


Figure 1: schematics of the laboratory evolution protocol undertaken in this work. Strains that are more “evolvable” survive for a higher number of passages.

Abstract ID: 2210 / Poster Number: 053.15P

The impact of innovative silanized nanoparticle-based materials on the preservation and conservation of Romanian cultural heritage

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Introduction/Objective: Biodeterioration poses a significant hazard to cultural heritage (CH) items, emphasizing the critical need for innovative and sustainable antimicrobial strategies. In light of the extensive damage sustained by artworks and historical monuments as a result of biodeterioration, the study evaluates the antimicrobial and anti-biofilm properties of treated ceramic and concrete materials to combat biodeterioration and preserve Romania's CH.

Method: The antimicrobial and anti-biofilm efficacy, respectively the impact on the capacity to secrete organic acid, of silanized (APTMS) marble fragments functionalized with ZnO, Cu, and TiO_2 nanoparticles (NPs), along with interior plaster surfaces treated with Ag and Cu NPs, was evaluated against strains of *Penicillium chrysogenum*, *Bacillus cereus*, and *B. megaterium*. The *Allium* test was used to determine the materials ecotoxicity. The Griess reaction was used to assess the nitric oxide content in extracellular environment, while calcium ion release was used to indicate microbial degradation.

Findings: Marble and plaster-based materials treated with APTMS functionalized with Cu, ZnO, and TiO_2 nanoparticles (NPs) exhibited stronger antifungal activity rather than antibacterial, particularly by preventing the fungal adherence to inert surfaces, suppressing biofilm formation, and inhibiting NO release, and respectively Ca^{2+} release from concrete-based materials, which was directly correlated with the reduction of an acidic microenvironment. Ecotoxicity assays confirmed that NP-functionalized materials were non-toxic and did not induce significant chromosomal modifications.

Conclusion: The study emphasizes the potential of silanized marble and nanoparticle-coated materials as innovative solutions for conserving CH artifacts against microbial deterioration, providing optimal environmental conditions.

Abstract ID: 2068

The Impact of Land Use on Mycorrhizal Fungi: A 12-Year Time Series Study Across German Ecosystems

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Introduction: Arbuscular mycorrhizal fungi (AMF) are essential for terrestrial ecosystem functioning, playing critical roles in nutrient cycling and plant health (Van Der Heijden et al., 2015). However, land use intensification threatens AMF communities, raising concerns about biodiversity loss and ecosystem stability. While previous studies have highlighted short-term impacts of land use on AMF (Ji et al., 2022; Xu et al., 2017), long-term data remain scarce. This study addresses this gap by evaluating the spatiotemporal variation of AMF communities across forests and grasslands within the large-scale, long-term Biodiversity Exploratories project in Germany (2011–2023) (Fischer et al., 2010).

Objectives: We aimed to:

2. Assess temporal trends in AMF diversity and abundance along a land use gradient;
3. Identify AMF indicator species differentiating ecosystems and land use intensities;
4. Evaluate long-term impacts of land use intensity on AMF community composition.

Methods: A total of 150 grassland and 150 forest plots across three regions (Schwäbische Alb, Hainich-Dün, and Schorfheide-Chorin) were sampled every three years from 2011 to 2023. AMF diversity and abundance were analysed using fungal 18S SSU rDNA amplicon sequencing.

- **Findings:** Grasslands exhibited higher AMF diversity (Shannon and Simpson indices; $p < 0.05$) compared to forests, while Pielou evenness showed no significant differences.
- Indicator species analysis identified unique AMF taxa specific to grasslands and forests, highlighting ecosystem-specific fungal specialization
- PERMANOVA revealed significant effects of ecosystem type ($p < 0.001$), time ($p < 0.01$), and land use intensity ($p < 0.05$) on AMF community composition.
- Temporal trends indicated stable AMF diversity over time but notable compositional shifts under high land use intensity, suggesting resilience in diversity but sensitivity to land use changes. (Figure 1).

Conclusion: This study underscores the critical role of AMF in ecosystem functioning and highlights the long-term impacts of land use on their diversity and composition. Identifying indicator species enhances understanding of AMF specialization and ecosystem resilience. These insights are vital for sustainable biodiversity management and balancing agricultural productivity with conservation efforts under future climate scenarios. By providing unique long-term insights into AMF community responses to land use, this study offers valuable information for ecologists, land managers, and policymakers aiming to promote ecosystem resilience and biodiversity conservation.

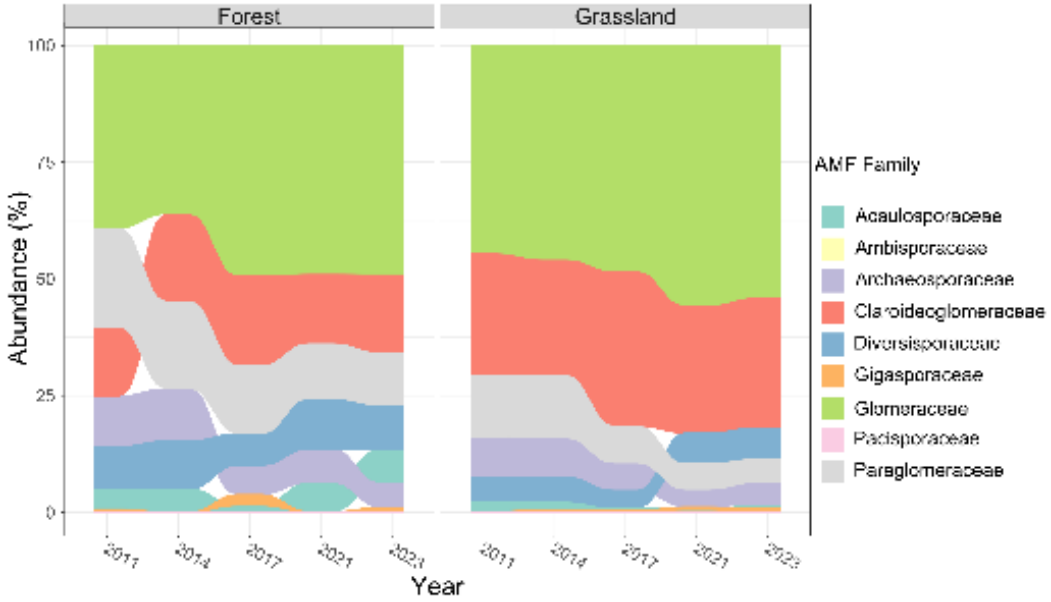


Figure 1: Temporal variation of AMF community composition in the soil of forest and grassland in the three Biodiversity Exploratories in Germany from 2011 to 2023. Species abundance is provided as a percentage upon rarefaction.

Abstract ID: 3361

The impact of low-calorie sweeteners on gut bacteria is modulated by commonly used xenobiotics

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Introduction: The gut microbiota is implicated in the adverse effects of low-calorie sweeteners, but their direct impact on gut bacteria remains underexplored. Additionally, the effects of co-consumed compounds on sweetener-microbe interactions are not well understood.

Objective: This study investigates the interactions between 39 low-calorie sweeteners and 25 gut bacteria, both individually and in combination with four commonly co-consumed compounds: advantame, caffeine, vanillin, and duloxetine.

Methods: We tested the sweeteners against the bacterial strains and examined their effects individually and with the co-consumed compounds. Proteomic, metabolomic, and genetic analyses were conducted, along with tests on a synthetic gut bacterial community and human cell lines to explore underlying mechanisms.

Findings: Three-quarters of the sweeteners impacted the growth of at least one bacterial strain. More than 100 interactions were identified between sweeteners and co-consumed compounds. Notably, isosteviol (a common sweetener) and duloxetine (an antidepressant) synergistically inhibited the growth of *Roseburia intestinalis*, a bacterium linked to glucose homeostasis, and *Parabacteroides merdae*, a commensal bacterium associated with a healthy microbiota. These interactions could be linked to altered small molecule transport, as revealed by proteomic and metabolomic analyses. Furthermore, the isosteviol-duloxetine combination modulated a synthetic gut bacterial community, resulting in increased toxicity to HeLa cells and altered secretion of inflammation-related cytokines IL-6 and IL-8 by Caco-2 cells.

Conclusion: This study provides a mechanistic understanding of sweetener-gut bacteria interactions, emphasizing the importance of considering sweetener-drug interactions in side effect assessments. Further research is needed to explore the combined effects of sweeteners and co-consumed compounds on human health.

Abstract ID: 2996 / Poster Number: 002.15P

The impact of the nitrification inhibitor DMPP on agricultural soil microbial communities

Presenting author: Ms. Anne-Catherine Groven, Thünen Institute for Biodiversity, Braunschweig, Germany

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Agricultural soils contribute as major polluters of nitrous oxide (N_2O) emissions in Europe, which are a result of microbial nitrification and denitrification processes. Nitrification inhibitors (NI) have gained attention by decreasing N_2O emissions and nitrate leaching. However, concerns have been raised about the long-term efficiency of NI at the scale of microbial communities. This work hypothesises that target and non-target microbial taxa develop resistance to NIs over time after showing initial sensitivity to 3,4-Dimethylpyrazole phosphate (DMPP) application. More in depth, specific nitrifying organisms will be less resilient against DMPP in an agricultural field with a high clay/sand ratio. Lastly, different types of fertilizations interact differently with DMPP, creating a change in the response of Ammonia oxidizing bacteria and archaea gene abundance.

The study was conducted on silt loam and silty clay loam agricultural fields over four time point. One of three distinct fertilizers was applied to each field, specifically ammonium sulphate nitrate, slurry or urea ammonium nitrate solution, in combination with or without DMPP. Additional physicochemical parameters including N mineralisation rates, pH, C and N content, and respiration rates of CO_2, N_2O and CH_4 were measured simultaneously. Microbial communities were analysed with qPCR targeting functional genes related to nitrification and denitrification (*amoA* and *nosZ1/2*, respectively). Amplicon sequencing of universal prokaryote taxonomic markers and *amoA* was performed to investigate the response of target and non-target taxa to DMPP addition over time. Furthermore, statistical analyses are being conducted. The results will help address fundamental questions about how NI impact microbial communities.

Abstract ID: 2901 / Poster Number: 453.15P

The infant gut microbiota under the microscope: dissecting interindividual variability across the globe

Presenting author: Chiara Tarracchini, PhD, University of Parma, Parma, Italy

The infant gut microbiome undergoes significant changes during the first year of life, driven by an intricate network of intrinsic and extrinsic factors which converge within a relatively short timeframe, leading to increased taxonomic and functional diversification as well as greater individual divergence.

Here, we investigated the interindividual variability of the infant gut microbiome using a comprehensive database of 5,242 public available fecal metagenomic data from healthy full-term infants across diverse geographic locations. Through our analysis, we identified six Early-Life Community State Types (Eli-CSTs), occurring between a few weeks and one year after birth, which not only capture the heterogeneity of the infant gut microbiome but also reflect the extensive transformation experienced by this developing microbial community over the first year. Indeed, stratifying the data by developmental stages enabled the linkage of specific ELi-CSTs to key developmental moments in the first year of life, contributing to a better understanding of the dynamic nature of the infant gut microbiota. Moreover, Indicative Species analysis and Random Forest modeling assisted the precise identification of unique, key taxonomic signatures that define each ELi-CST, highlighting microbial taxa with pivotal roles in shaping early microbial community structure. Finally, a comprehensive culturing effort allowed us to establish a biobank of 182 infant gut microbiota members, which was used to uncover *in vitro* cross-talk behaviors among recurrently co-occurring early-life gut commensals identified within the aforementioned CSTs, offering novel insights into the ecological interactions behind early microbial community development.

Abstract ID: 159 / Poster Number: 055.17P

The influence of the aminoglycoside acetyltransferase on Leuconostoc citreum resistance to kanamycin

Presenting author: Prof. Do-Won Jeong, Dongduk Women's University, Seoul, South Korea

Background: Resistance to eight antibiotics (ampicillin, chloramphenicol, clindamycin, erythromycin, gentamycin, kanamycin, streptomycin, and tetracycline) was evaluated in Leuconostoc citreum strains isolated from kimchi. The results showed that all strains exhibited resistance to kanamycin above the EFSA-defined cutoff value for Leuconostoc spp.

Objectives: The study aimed to identify resistance genes influencing kanamycin antibiotic resistance in Leuconostoc citreum strains based on comparative genomics. Additionally, it sought to determine whether the gene represents species-level intrinsic resistance or acquired resistance.

Methods: The genome of strains exhibiting resistance to kanamycin was analyzed, and a comparative analysis was conducted to identify genes contributing to kanamycin resistance. Additionally, the identified genes were validated by comparing trends in previously reported genomes, and efforts were made to experimentally verify the findings.

Results: Genomic analysis of Leuconostoc citreum identified the aminoglycoside acetyltransferase (aac6-l) gene, which is presumed to contribute to kanamycin resistance. Additionally, analysis of previously reported genomes revealed that the presence of the aac6-l gene varies among strains. Therefore, the gene was cloned to verify its contribution to kanamycin resistance. In addition, we checked whether the gene was strain-specific or species-specific.

Abstract ID: 2662

The Intake of Sambucus Nigra Extract Differentially Impacts, in Vitro, the Human Gut Ecosystem

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Co-authors: *Andrea Polo, Olga Nikoloudaki, Lena Granehäll, Stephan Plattner, Marco Gobbetti, Raffaella Di Cagno*

Diet plays a key role in shaping the human gut microbiome, influencing its complex structural composition and functionality. In the last decades, some modern diets have been linked to gut dysbiosis resulting in chronic metabolic disorders. In this context, the supplementation with plant-based bioactive compounds like polyphenols show promise as 'duplibiotics': antimicrobial effects on specific pathobionts and prebiotic properties. Despite growing research on polyphenol-gut microbiome interactions, the complexity of polyphenol metabolism and its strict dependence on individual gut microbiota composition challenge generalizations across polyphenols and their sources, particularly in clinical trials or animal studies. Our study examined the impact of a novel polyphenol-rich (22.45%) extract (EBE) from European black elderberry (*Sambucus nigra* L.) on two distinct and representative gut ecosystems (accounting for inter-individual variability), using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). The setup consisted of two identical SHIME® units (S1 and S2) running in parallel, each unit including 3 bioreactors simulating the stomach/small intestine, proximal colon (PC), and distal colon (DC). Each unit was inoculated with faeces from different representative healthy donors selected from a cohort of 40 individuals adhering to the Mediterranean diet. The experiment comprised: (i) colon ecosystems stabilization; (ii) a two-week control period; (iii) a two-week treatment period in which 600 mg/day of elderberry extract was added to the diet; and (iv) a one-week washout period. Lumen samples were collected at the end of control (T0), after one- and two-weeks treatment (T1 and T2), and after washout (T3). Samples were analyzed for short-chain fatty acid profiles via High-Performance Liquid Chromatography with Ultraviolet Detection system, and microbiome changes (both composition and potential functionality) using shotgun metagenomics. EBE intake increased acetic acid synthesis in both gut ecosystems. Propionic acid significantly increased only in S1, while butyric acid remained unchanged. The baseline microbiota composition of each SHIME® unit was key in shaping the temporal effects of EBE. S1 was *Bifidobacterium*-dominated, while S2 was *Prevotella*-dominated. *Enterococcus durans*, *Leifsonia sp.*, and *Phytobacter diazotrophicus* were the core species with consistently significant modulation in both colon tracts of at least one

SHIME® unit, while the Firmicutes-Bacteroidetes ratio was significantly reduced by EBE intake in both units. In at least one SHIME® unit, the relative abundance of species previously associated with beneficial physiological roles like *Akkermansia muciniphila*, *Bifidobacterium longum*, and *Bacteroides dorei* increased, while potentially harmful species like *Acinetobacter communis*, *Enterocloster clostridioformis*, and *Pseudomonas putida* decreased. The ecosystem-specific reshaping of microbiota was reflected in corresponding functional gene changes, with both units showing increased genes for glycine, serine, and threonine metabolism, plant secondary metabolite biosynthesis in the proximal colon, and elevated glutathione metabolism at T2. Among microbiota-dependent effects, S1 had increased gene abundance related to sphingolipid metabolism, neuroactive ligand-receptor interaction, amino acids and energy production pathways. Differentially, S2 had increased genes involved in aminoacyl tRNA biosynthesis, vitamin B6 metabolism, teichoic acid and zeatin biosynthesis, and phenylpropanoid biosynthesis. Both shifts indicate the individualized positive impact of EBE intake, supporting its potential role in personalized nutrition interventions.

Abstract ID: 2410 / Poster Number: 035.17F

The intestinal and urinary pathogenic traits of an enteroaggregative Escherichia coli isolated from bacteremia

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Co-authors: *Claudia A. Freire, Claudia T. P. Moraes, Ana Maria A. Liberatore, Ivan Koh, Jonatas W. Silva, Rosa M. Silva, Cecilia M. Abe, Eneas Carvalho, Waldir P. Elias*

Introduction: Bloodstream infections (BSI) are associated with high mortality worldwide. *Escherichia coli* is among the leading causative bacterial agents of BSI. Enteroaggregative *E. coli* (EAEC) is a pathotype that causes acute/persistent diarrhea. Recent reports also described EAEC strains implicated in BSI and urinary tract infection (UTI); however, the pathogenicity mechanisms involved in these diseases caused by EAEC are still unknown.

Objective: Evaluate the pathogenicity potential of the EAEC strain EC092 isolated from BSI.

Methods: A murine sepsis model was performed to assess EC092 lethality. The bacterial ability to adhere/colonize intestinal cells and fragments (duodenum, jejunum, and ileum) was respectively accessed performing in vitro adherence assays (human/HT-29 cells) and using an ex-vivo rat model. A rat intestinal translocation model was used to evaluate the bacterial capacity to translocate intestinal barriers and disseminate to mesenteric lymph nodes (MLN), liver, spleen, and kidneys. Finally, a murine model was used to access its ability to cause ascending UTI.

Findings: EC092 caused 90% lethality in mice within 24 h. Adherence assays showed a strong aggregative adherence to HT-29 cells. Intestinal colonization was observed with no significant differences in bacterial counts among different fragments. EC092 translocated intestinal barriers, being detected in MLN, liver, and spleen. EC092 was also able to ascend the urinary tract reaching the kidneys.

Conclusion: Although EC092 was isolated from BSI, it was able to adhere/colonize the intestine, cross the intestinal barrier and disseminate systemically, in addition to causing UTI. These findings underscore the clinical relevance and virulence potential of this strain.

Abstract ID: 1786 / Poster Number: 218.15P

The life-history trait trade-offs mediated by reproduction and immunity in the brown planthopper, Nilaparvata lugens Stål

Presenting author: Xiaoping Yu, China Jiliang University, Hangzhou, China

Co-authors: *Kai Sun, Xuping ShenTu,*

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is a global superpest that mostly damages rice crops. Yeast-like symbionts (YLS) exist in the abdominal fat body and are tightly associated with the development, growth, and reproduction of BPH. Our previous research demonstrated that mating behavior promotes the release of YLS from the fat body into the hemolymph, thereby triggering an immune response. Additionally, the fitness costs related to life-history traits of BPH have a strong dependence on the relative abundance of YLS. However, the possible relationship between reproduction and the immune response in BPH has not been identified. In this study, an omics-based approach was used to analyze the transcriptome of fat body in mated and unmated BPH at 72 h post-eclosion, from which two antimicrobial peptide genes, *NlDefensin A* (*NlDfA*) and *NlDefensin B* (*NlDfB*), were selected since they were highly expressed in mated BPH. Spatial-temporal expression analysis indicated the *NlDfA* and *NlDfB* genes were most highly expressed in the fat body at 24 h post-eclosion. Moreover, the symbionts in BPH were significantly inhibited by the in vitro expression of the NlDfA and NlDfB proteins. Furthermore, RNAi-mediated suppression of *NlDfA* and *NlDfB* dramatically increased the relative abundance of YLS in the fat body, while YLS in the hemolymph decreased significantly. Our results indicated that mating could heighten the immunity of BPH by upregulating the expression of the *NlDfA* and *NlDfB* genes. However, the reduced content of YLS may act as a fitness disadvantage in dictating the life-history traits of BPH.

Abstract ID: 1986 / Poster Number: 421.15P

The link of carbon catabolite repression elements, small RNAs CrcY and CrcZ and polyhydroxyalkanoate metabolism in Pseudomonas putida KT2440

Presenting author: Ms. Yixin Che, University College Dublin, Dublin, Ireland

Co-authors: *Tanja Narancic, Kevin O'Connor*

Polyhydroxyalkanoates (PHAs) are biodegradable polymers, which can be synthesized and degraded by a range of microorganisms. As a valuable plastic alternative, the synthesis of PHAs has been extensively studied in *Pseudomonas putida*. However, their industrial application is hindered by their physical properties and production efficiency.

To overcome the limitations, this study investigated the roles of the small RNAs CrcY and CrcZ, key components of the carbon catabolite repression (CCR) system, in PHA metabolism in *P. putida* KT2440. Overexpression and deletion mutants of CrcY and CrcZ were analysed under different carbon and nitrogen conditions. The mutant growth profiles and PHA production were analysed. Proteomic analysis was conducted to identify differentially expressed proteins (DEPs) and uncover regulatory mechanisms involved in PHA metabolism.

The results demonstrated that overexpression of CrcY or CrcZ significantly enhanced PHA accumulation, particularly under nitrogen-limited conditions with octanoate. The CrcY overexpression strain achieved 62.51% PHA content relative to total cell dry weight, a 1.2- and 2-fold increase compared to the CrcZ overexpression and control strains, respectively. Proteomic analysis revealed that CrcY and CrcZ influence similar pathways, with high overlap in DEPs, particularly in ABC transporters and key enzymes involved in fatty acid metabolism and PHA synthesis. These findings highlight the complementary regulatory roles of CrcY and CrcZ in modulating CCR to optimize PHA production. This study provides insights into leveraging CCR elements to enhance the efficiency of PHA biosynthesis, contributing to the development of sustainable bioplastic production.

Abstract ID: 2232

The Lord of The Aromatic Rings: Pseudomonas umsongensis GO16-based catalyst for the biomanufacture of 2,5-furandicarboxylic acid

Presenting author: Tanja Narancic, PhD, University College Dublin, Dublin, Ireland

Co-authors: *Rhys Orimaco, Kevin O'Connor*

While small volume, high value biomanufacturing is truly in force, the production of commodity chemicals, polymers and fuels at high volume is what is needed to tip the scale towards sustainability. In the search for biobased alternatives to traditional fossil plastics, polyethylene 2,5-furanoate (PEF) has emerged. While PEF has similar physical and thermoplastic characteristics to ubiquitous polyethylene terephthalate PET, it is superior in terms of mechanical recycling. PET constituents are terephthalic acid (TPA) and ethylene glycol (EG), while 2,5-furandicarboxylic acid (FDCA) substitutes TPA in PEF.

5-(hydroxymethyl)furfural (HMF), a biomass-derived platform chemical, is toxic to majority microorganisms, hindering biomass valorisation. However, HMF has many applications, including production of FDCA. The conversion of HMF to FDCA requires three oxidation steps, and only a few enzymes that could perform these oxidations are known. To allow for a more competitive production of furan-based plastics, an efficient and cost-effective bioprocess is required.

Discovered from soils surrounding a plastic bottling facility, *Pseudomonas umsongensis* GO16 was shown to convert hydrolysed PET to biodegradable polymers polyhydroxyalkanoates (PHAs), and hydroxyalkanoyloxy-alkanoates (HAAs) used to produce biobased polyurethane. Building on these foundations, we sought to expand GO16's biocatalytic repertoire to produce FDCA using energy and biomass this organism accumulates from PET monomers, TPA and EG.

To achieve the above objective, the HMF oxidation pathway in GO16 was identified and characterised. GO16 grew with HMF, FDCA and 2-furoic acid, suggesting the HMF oxidation is functional and proceeds via these intermediates. PsfA, a putative NAD+ dependent aldehyde dehydrogenase, and PsfG, a putative short chain alcohol dehydrogenase, previously uncharacterised, potentially fulfil the role of HMF oxidation to FDCA. Deletion of *psfA* or *psfG* by CRISPR/Cas9 renders GO16 unable to use HMF as a sole carbon and energy substrate. GO1*ΔpsfA* however grows with FDCA, while GO16*ΔpsfG* grows with formylfurancarboxylic acid (FFCA) and FDCA, suggesting their

roles in HMF oxidation: likely a non-specific periplasmic aldehyde oxidoreductase complex oxidises HMF to 5-hydroxymethyl-2-furancarboxylic acid (HMFCA), subsequently oxidised to FFCA by PsfG, and and FFCA by PsfA. Next, HMF through conversion to α-ketoglutarate was intercepted by deleting HmfF, in this work functionally annotated as FDCA decarboxylase, to facilitate FDCA accumulation.

When GO16*ΔhmfF* cells grown with glycerol were concentrated and used as a biocatalyst in the biotransformation of 50mM HMF, complete depletion of HMF occurred after 6h. This was accompanied by the transient accumulation of HMFCA, followed by its complete conversion into FDCA over the next 18h. When PET mock hydrolysate (TPA+EG mix) was used, only 13mM FDCA was produced after 48h, with HMFCA accumulation noted as a bottleneck. The overexpression of HMFCA transporter HmfT, PsfA and PsfG in GO16*ΔhmfF* resulted in 100% conversion of HMF to FDCA within 24h, a 19-fold improvement in specific productivity.

The engineered, GO16-based microbial platform is the basis of FDCA biomanufacturing using either waste glycerol, or post-consumer PET. It must be recognised that the process relies on water-based environment, and requires energy, so life cycle analysis and costing, as well as techno-economic analysis have to be performed to identify hot-spots and places for improvement.

Abstract ID: 3461 / Poster Number: 292.15P

The marine environment is a rich source of diverse anaerobic organohalide-respiring bacteria

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Organohalogen compounds are widespread in the marine environment as a source of both anthropogenic pollution and natural production: brominated flame retardants are common anthropogenic contaminants of aquatic sediments, while a great variety of natural organobromine compounds are produced by sponges and other organisms. Cleavage of the carbon-halogen bond is a critical step in degradation of organohalides and the natural organohalogen cycle. We have enriched and isolated diverse organohalide-respiring bacteria, from different marine habitats, including organohalide-contaminated sediments, pristine sites, as well as marine sponges. These bacteria utilize organohalides as electron acceptors for energy conservation. Reductive dehalogenase (RDase) genes and the capacity for organohalide respiration were widely distributed in members of marine *Pseudomonadota*. Novel debrominating members of the *Desulfovibrionaceae* and *Desulfuromonadaceae* families were isolated from different estuarine and marine sediments. In contrast, organobromine-rich sponges appear to provide a specialized habitat for organohalide-respiring microbes with different *Desulfoluna* (*Desulfobacteraceae*) species responsible for reductive dehalogenation in geographically widely distributed sponge species (Atlantic, Pacific and Indian Oceans and the Mediterranean) indicating a cosmopolitan distribution. This association between the sponge holobiont and associated microbiota appears to be driven by the organohalogen chemistry of the sponge animal. The identification of diverse bacteria capable of organohalide respiration through functional assays and dehalogenase gene analysis extends our knowledge of their extensive distribution and diversity in the marine environment. Dehalogenating bacteria play an important role in natural organohalide cycling with major impact on the fate of organohalide pollutants in the marine environment.

Abstract ID: 2712

The methane-cycling microbiome in intact and degraded permafrost soils of the pan-Arctic

Presenting author: Dr. Haitao Wang, University of Greifswald, Greifswald, Germany

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The methane-cycling microbiomes in Arctic permafrost-affected soils play crucial roles in the production and consumption of this important greenhouse gas. However, little is known about the distributions of Arctic methanogens and methanotrophs across the regional scale and along the vertical soil profile, as well as their responses to the widespread permafrost thaw.

Using a unique sample set from nine different locations across the pan-Arctic, we identified methanogen and methanotroph phylotypes in 729 datasets of 16S rRNA gene amplicons.

In 621 samples of intact permafrost soils across the pan-Arctic, only 22 methanogen and 26 methanotroph phylotypes were identified. Relative abundances of both functional groups varied significantly between sites and soil horizons. Only four methanogen phylotypes were detected at all locations, with the hydrogenotrophic *Methanobacterium lacus* dominating. Remarkably, the permafrost soil methane filter was almost exclusively comprised of a few phylotypes closely related to the obligate methanotrophic species *Methylobacter tundripaludum*.

In degraded permafrost sites in Alaska, *M. tundripaludum* also dominated the methanotroph microbiome in the wet site. However, in dry, water-drained former permafrost site, *Methylocapsa* phylotypes, closely related with the atmospheric methane oxidizing bacteria, were exclusively found and dominant, indicating a massive restructuring of the methanotroph guild that consequently resulted in functional changes from a soil methane filter to an atmospheric methane sink.

This study provides first insights into the identity and intricate spatial distribution of methanotrophs and methanogens in permafrost soils at a pan-Arctic scale and their responses to different water status after permafrost degradation. These findings point towards a few key microbes particularly relevant for future studies on Arctic CH_4

dynamics in a warming climate and that under future dry conditions more atmospheric CH_4 uptake in Arctic upland soils might happen.

Abstract ID: 2670 / Poster Number: 338.16P

The methylome of WHO Neisseria gonorrhoeae reference strains using Oxford Nanopore sequencing

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The treatment of gonorrhea has become increasingly challenging in recent years due to the increasing rates of antimicrobial resistant *Neisseria gonorrhoeae*. Expanding our understanding of the biology of this pathogen is essential to combat this pathogen. In this context, the study of the gonococcal methylome could provide relevant information that is being overseen by limitations of the current DNA sequencing technologies. Here, we aimed at studying the methylome of the 2024 WHO *N. gonorrhoeae* and superseded reference strains using Oxford Nanopore Technologies (ONT) sequencing.

We cultured *in vitro* three replicates of each of the 29 WHO *N. gonorrhoeae* reference strains (n=15 2024 and n=14 superseded), extracted high molecular weight DNA and sequenced them on a MinION Mk1C instrument. We duplex basecalled the raw data with DNA modification models (6mA, 4mC and 5mC), detected and analysed methylated motifs across the different strains.

Results revealed a complex methylation landscape across all *N. gonorrhoeae* strains but consistent across replicates, with some motifs being constitutively expressed (e.g. 5'-RG5mCGCY-3', NgoAI methylase) and others strain-specific (e.g. 5'-CC6mACC-3', NgoAX methylase). ONT sequencing best identified cytosine methylation compared with previously published PacBio data. For example, we detected 5'-T5mCACC-3' methylation, complementary to the 5'-GGTG6mA-3' motif, produced by two methylases in the NgoAXVI restriction-modification system.

In conclusion, we have obtained the complete methylome of WHO *N. gonorrhoeae* reference strains using ONT sequencing and shown the potential of this technology to analyse different methylation types. This can be key to unveil unexplored mechanisms in the biology of this and other organisms.

Abstract ID: 2183 / Poster Number: 228.15P

The Microbe Atlas database: Global trends and insights into Earth's Microbial Ecosystems

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The quest to better understand the functions and assembly mechanisms of the stunningly diverse microbial communities found around the globe has led to an ongoing explosion of worldwide sampling efforts. Sequenced communities are now spanning all parts of the planet: from the polar regions to the equator, from the deep subsurface to the highest mountain tops, covering a myriad of different hosts, conditions, and biomes. To leverage this data and address fundamental ecological and biogeographic questions, integrative, truly globe-spanning microbiomics is going to be key.

Here, we present a first integrated analysis of the Microbe Atlas database, an extensive resource of more than two million highly diverse microbial community profiles. These profiles are consistently processed to enable full cross-study comparability of ecosystems and taxa. They are enriched with geographic information, such as IUCN biome definitions and extracted keyword information, and freely available through a feature-rich webpage at www.microbeatlas.org.

Our findings indicate that "microbial dark matter" OTUs are mainly environmental, often abundant, and traceable across continents and environments, enabling first ecological characterizations. Taxa frequently exhibit specific geographic patterns, like enrichment in polar or equatorial latitudes, with equatorial regions emerging as hotspots of micro-diversity. Moreover, community clustering by environment reveals under-classified yet highly diverse soil ecosystems and specialist-dominated marine habitats. We furthermore observe a pronounced taxonomic segregation across the generalism spectrum, intriguingly coupled with a prevalence of potential pathogenic groups among generalists. This extensive analysis casts new light on the biogeography of microbial diversity on our planet.

Abstract ID: 381

The microbiome of octocorals in a warming Mediterranean Sea: role in thermotolerance and resilience

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Introduction: Climate change has accelerated the occurrence and severity of marine heatwaves (MHWs) in the Mediterranean Sea which is warming 20% faster than the global average. These recurrent MHWs have devastating effects on coral holobionts, threatening the Mediterranean iconic octocoral species with temperature-dependent diseases and mass mortality.

Objectives: Our goal is to gain a deeper understanding on the role of the associated bacterial and microeukaryotic communities in the resistance and resilience of the key Mediterranean octocoral species *Corallium rubrum* to global warming.

Methods: Using a global approach combining both laboratory experiments and field measurements during the unprecedented 2022 MHWs, we investigated the response of the octocoral holobiont to thermal stress. We assessed both the host physiological status (*i.e.*, (partial) mortality/survival, expression of stress response genes) and the composition of the bacterial microbiome and eukaryome using *16S* and *18S rRNA* gene sequencing and taxon-specific qPCR analyses under high temperature events. For the eukaryome diversity, novel primers were developed to reduce host signal and increase the detection of microeukaryotes.

Findings: MHWs led to a dysbiosis state in the stressed corals, which was observed even before the coral health deteriorates. The dysbiosis started with changes in the eukaryome that responded more rapidly and to lower thermal anomalies compared to the bacterial microbiome. The disruption of the microbial communities grew with stress intensity and duration and favored the proliferation of opportunists and putative pathogens (bacteria and microeukaryotes), while the abundance of the main symbionts (Spirochaetaceae bacteria and Labyrinthulomycetes protists) of *C. rubrum* remained stable. For resilient corals which have not suffered from mortality, microbiome resilience was not complete after two weeks of recovery under the control temperature. Indeed, while corals were no longer stressed based on the expression of stress-response genes, some opportunists (especially *Vibrio* species) persisted within the microbiome.

Conclusions: Our study suggests that mortality in octocorals is not directly caused by the decline of the main symbionts, which may support coral health under stress. Instead,

opportunistic and potentially pathogenic microbes proliferate under hot temperatures, causing a disruption within the microbiome and potentially favoring coral diseases and mortality. The short-term partial resilience of the microbiome after a heatwave might impact the overall coral resistance and resilience to the successive MHWs which are observed every year in the Mediterranean Sea.

Abstract ID: 3912 / Poster Number: 297.15P

The microbiome of the dominant algae in glacier-fed streams

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Hydrurus foetidus is a major primary producer at the base of the food web in glacier-fed streams across the globe. Although it has been reported since 200 years, little is known about this unicellular alga which is able to form long filaments (up to 30 cm) and bloom under oligotrophic conditions. In this study, we hypothesise *H. foetidus* harbours a distinct microbiome with which it may have symbiotic-like interactions, enabling it to thrive under the hard conditions of glacier-fed streams. Various streams of the Swiss Alps were screened and sampled for *H. foetidus* specimens and streamwater samples across three seasons. After DNA extraction, full-length 16S rRNA genes were sequenced using Nanopore technology to evaluate the microbiome composition within *H. foetidus* and streamwater. Preliminary results suggest *H. foetidus* indeed harbours its own microbiome which differs from the streamwater one with an enrichment in *Rhodoferax, Polaromonas, Flavobacterium* genera, as well as a genus from the Oxalobacteraceae family (CM1G08), a family associated with nitrogen-fixation processes. Further analyses will be performed to determine the community assembly processes driving both microbiomes as well as how the distance from the glacier affects *H. foetidus* microbiome composition. Due to climate change and glacier disappearance, glacier-fed streams are now changing towards conditions to which *H. foetidus* may not be adapted. Therefore, it is crucial to intensify research about this alga to better understand its relevance for the ecosystem and the effects climate change will have on its prevalence in glacier-fed streams.

Abstract ID: 1673 / Poster Number: 023.17F

The Misguided Role of PGlyRP-4: From Host Defender to Virulence Enhancer in Shigella

Presenting author: Rita Trirocco, PhD, Sapienza University of Rome, Rome, Italy

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Introduction: Peptidoglycan recognition proteins (PGlyRPs) are innate immunity proteins that detect bacterial peptidoglycan and influence gut microbiota composition through bacteriostatic and bactericidal actions (1). While PGlyRPs typically act as antibacterial agents, pathogens like *Shigella flexneri* have evolved mechanisms to evade these effects. *Shigella*, a highly adapted human enteropathogen, causes shigellosis, a severe diarrheal disease, by invading and replicating within intestinal epithelial cells. This process is regulated by virulence genes, including *virF*, a master regulator essential for pathogenicity (2).

Objectives: This study investigates how PGlyRP-4, unlike other PGlyRPs, enhances *Shigella* virulence by inducing the expression of *virF*. Specifically, we aim to determine whether PGlyRP-4 acts through the CpxA/R two-component system, where the CpxA sensor kinase responds to environmental stimuli by phosphorylating the CpxR response regulator, which then activates *virF* transcription.

Methods: We analyzed *virF* transcriptional and translational levels and infection assays on epithelial cells were performed to evaluate whether PGlyRP-4 increases *Shigella*'s pathogenic potential by enhancing its ability to invade and survive intracellularly.

Finding: Our results reveal that PGlyRP-4 promotes *virF* expression via the CpxA/R pathway. However, CpxA is not the sole regulator, suggesting that additional mechanisms may contribute to *virF* modulation. Additionally, PGRP-4 enhances the infectivity of Shigella by promoting virF expression, which contributes to its ability to invade and survive within host cells

Conclusions: This study identifies a novel role for PGlyRP-4 as a host-derived signal enhancing *Shigella* virulence and provides insights into potential therapeutic strategies for the treatment of shigellosis.

Abstract ID: 3287 / Poster Number: 085.16P

The Mucosodom study: Mucosal and luminal microbiome resolved at species, strain and genome resolution in inflammatory bowel disease

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The gastrointestinal (GI) microbiome plays a critical role in nutrient absorption, immune function, and defence against pathogens[1]. The mucosa represents an important interface between the gastrointestinal lumen and GI lining[2,3]. In inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), inflammation may spread from localized areas to the entire GI tract, and is associated with a decrease in mucosal thickness and change of bacterial composition. Investigation of the mucosa is limited to amplicon sequencing due to nature of samples[4-7]. This restricts a comprehensive characterization of locally enriched microbial species and obscures significant strain-level genetic diversity within a species.

Using spatial mucosal sampling, we aim to identify colonization patterns throughout GI tract, and characterize bacterial species linked to dysbiosis in IBD. We used metagenomic sequencing of paired mucosal and faecal gut microbiomes, to characterize the gut microbiome of 51 IBD patients. Using a newly developed protocol, we could enrich bacteria, enabling mucosal metagenomics; and reconstructed 1090 MAGs that were clustered into 358 metagenomic species. Mucosal biopsies showed greater dysbiosis than faecal samples, especially in CD, with distinct microbial signatures observed between inflammation sites. We found less diverse microbiomes in the mucosa than the lumen, with anaerobes more prevalent in luminal environment.

This study addresses significant gaps in understanding mucosal communities due to restricted sampling, and high ratio of human read contamination. We therefore developed an approach to investigate colonization patterns of bacteria in the human mucosa and host–microbe interactions, while disentangling disease-, location- and inflammation-specific associations.

Abstract ID: 4108 / Poster Number: 102.16P

The neovaginal microbiota and associated inflammation in transfeminine individuals who have undergone penile inversion vaginoplasty

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Transfeminine people (assigned male-at-birth, feminine gender) may use medical care to generate physical characteristics congruent with their gender, including penile inversion vaginoplasty. Transfeminine people with vaginoplasty frequently experience gynecological concerns, but effective care is lacking due to a paucity of knowledge of the neovaginal microenvironment. In this study, we characterized neovaginal bacteria and cytokines from 47 transfeminine people with penile inversion vaginoplasty. Participants provided neovaginal samples and information on behaviours and symptoms; bacteria were characterized by 16S sequencing. Although *Lactobacillus* was often detected, stable *Lactobacillus*-dominant communities were rare. We identified four distinct clusters of co-occurrent bacteria, each associated with unique immune profiles. One cluster, including *Fastidiosiplia*, *Ezakiella*, and *Murdochiella*, was abundant, stable, and linked to lower cytokines, suggesting a potentially beneficial immunomodulatory role. A distinct cluster containing *Howardella*, *Parvimonas*, *Fusobacterium*, and *Lawsonella* was associated with elevated cytokines. These findings highlight the need for evidence-based clinical guidelines tailored to transfeminine gynecology.

Abstract ID: 2310 / Poster Number: 173.15P

The obligate human intracellular pathogen Chlamydia trachomatis modulates metabolic pathways during infection of phagocytes

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Chlamydia trachomatis (*Ctr*) is an obligate intracellular human pathogen and the leading cause of sexually transmitted diseases worldwide. In addition to epithelial cells, the main replicative niche for *Ctr*, *in vivo,* the pathogen also encounters immune cells, such as macrophages. Based on their polarisation status, macrophages are conventionally classified into "M1" *classically activated* or "M2" *alternatively activated*. *Ctr* can survive and replicate in "M2", but not in "M1" macrophages.

Due to its reduced genome and several missing central metabolic pathways, *Ctr* hijacks essential nutrients from the host. We present a systematic investigation of how *Ctr* infection remodels host transcriptional and metabolic landscapes.

Here, we conducted *Ctr* infections of human primary monocyte-derived "M1" and "M2" macrophages and used dual RNAseq coupled with metabolomics and lipidomics to create an unbiased integrated network of regulated host metabolic responses. We additionally investigated *Ctr* gene expression to construct a bacterial metabolic network, during adaptation to a permissive ("M2") or a restrictive ("M1") host niche.

We show by immunofluorescence microscopy and flow cytometry, that although only 8% of "M2" macrophages are infected by *Ctr*, infection results nonetheless in major transcriptome, metabolome and lipidome remodelling. Several metabolic processes (e.g. ceramide *de novo* synthesis, sphingosine accumulation, tryptophane metabolism) are severely impacted upon infection. By contrast, "M1" macrophages remain largely inert to *Ctr*.

Overall, this study gives global insights into the interaction of *Ctr* with primary human phagocytes. The approach we developed here will enable further in depth understanding of metabolic and regulatory networks that govern host-pathogen interactions.

Abstract ID: 1403 / Poster Number: 154.15P

The olive mycorrhizosphere harbor diverse phosphate solubilizing bacteria having multiple growth promoting traits

Presenting author: Dr. Fahad Alotaibi, King Saud University, Riyadh, Saudi Arabia

Introduction/Aim: Phosphate chemical fertilizers are costly and raise concerns about environmental pollution through industrial production. The use of phosphate-solubilizing microorganisms (PSMs) as inoculants can be an option to increase P availability in soils. The aim of this work was to isolate PSMs from olive mycorrhizosphere, having multiple plant growth-promoting (PGP) traits. Materials and **Methods:** Bacteria were isolated from the mycorrhizosphere of olive and tested for P-solubilization (Tricalcium phosphate (TCP), hydroxyapatite, and phosphate rock (PRs)) and PGP traits. Isolates were also identified via the Sanger sequencing of the 16S rRNA gene.

Results: Out of 105 isolates, 12 belonging to *Arthrobacter* sp., *Bacillus* sp., *Pantoea* sp., *Streptomyces* sp., and *Pseudomonas* sp. were found to be the best PSMs. All strains mobilized P from TCP phosphate, hydroxyapatite, and PRs. Additionally, most strains presented various PGP activities like IAA, Nitrogen fixation, Acc deaminase, and siderophore production.

Discussion: Results of this study indicated that the olive mycorrhizosphere contains diverse PSMs with multiple PGP traits. The contribution of PGPR in the P-acquisition strategies of the plant is complex and involves various mechanisms. As observed in results, by selecting the efficient PSMs-solubilizing and PGP abilities of different strains, the potential impact of each component on P nutrition could be increased.

Conclusions: The mycorrhizosphere of olive is a good source for the isolation of efficient PSMs-solubilizing with diverse PGP traits. Bacterial isolates obtained in this study has the potential to be used as inoculants for more sustainable agriculture in low-phosphorus soils such as calcareous soils.

Abstract ID: 1828 / Poster Number: 239.17P

The PHB Equation: Pongamia Oil + Bacteria = Plastic's Sustainable Future

Presenting author: Dr. Long Nguyen, The University of Sydney, Sydney, Australia

Co-authors: *Wilson Ling, Fariba Dehghani*

Introduction: The urgent need to replace petroleum-based plastics has spurred interest in biodegradable alternatives like polyhydroxybutyrate (PHB). PHB, a microbial biopolymer, offers full biodegradability but faces scalability challenges due to reliance on costly substrates. Pongamia oil, grown on marginal lands, presents an eco-friendly alternative that avoids food-fuel conflicts and leverages its carbon sequestration capacity during cultivation.

Objective: This work aims to (1) evaluate Pongamia oil as a substrate for PHB synthesis using bacterial fermentation, (2) characterize the PHB's material properties, and (3) estimate carbon credit gains from avoided fossil plastic emissions and agro-ecological benefits.

Methods: Cupriavidus necator H16 was cultivated in a nitrogen-limited bioreactor using Pongamia oil as the primary carbon source. Thermal properties were analyzed via differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Carbon credit potential was calculated using lifecycle assessment (LCA) comparing PHB production to conventional polyethylene.

Findings: PHB production reached 15.9 g/L (72% of cell dry weight) with 96% purity. The biopolymer exhibited a melting temperature of 172°C, crystallinity of 60%, and thermal stability up to 275°C, suitable for single-use applications. LCA was estimated at an 11.7 tons CO_2-equivalent reduction per ton of PHB, attributed to Pongamia's carbon sequestration (estimating 9 tons CO_2/ha/year) and avoided fossil-derived plastic emissions.

Conclusion: Pongamia oil demonstrates promise as a sustainable PHB feedstock, reducing reliance on fossil fuels and lowering net carbon emissions. The integration of carbon credit frameworks could incentivize adoption by offsetting production costs. This approach aligns with global sustainability goals, offering a dual solution to plastic pollution and climate change.

Abstract ID: 3334 / Poster Number: 333.17P

The potential of Metschnikowia reukaufii in low-alcohol beer production

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Co-authors: *Hye-Jin Hong, Jeong-Ah Yoon, Myoung-Dong Kim*

The increasing demand for low-alcohol beer due to health and wellness trends has driven research into alternative yeast strains for fermentation. This study evaluates the potential of the non-conventional yeast *Metschnikowia reukaufii* for low-alcohol beer production. Thirty-three strains of *M. reukaufii* isolated from natural sources were fermented in wort at 20°C for 5 days, and their ethanol production was analyzed. The *M. reukaufii* KCTC 27374 and *Saccharomycodes ludwigii* NRRL Y-12793 were used as controls. Ethanol analysis results showed that *M. reukaufii* NIY655, NIY670, NIY682, NIY691, NIY697, and NIYL32584 had lower ethanol productivity and higher sugar consumption reduction than the controls, with less than 1%. NIY670 showed the lowest ethanol productivity at 3.42±0.02 g/L. These six strains were identified as promising candidates for low-alcohol beer production, and further evaluation of their flavor compound production potential will be conducted. The results of this study suggest that *M. reukaufii* has the potential to be effectively utilized in low-alcohol beer production.

Abstract ID: 1999 / Poster Number: 249.17P

The potential of the Pseudomonas sp. A3W3 strain for bioremediation supported by its metal accumulation and genomic profile

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Co-authors: *Anna Poladyan, Armine Margaryan*

Introduction: Understanding the mechanisms of heavy metal resistance in bacteria is essential, as it paves the way for innovative applications in the remediation of contaminated environments.

Objective: This study investigates the bioremediation potential of the metal-resistant *Pseudomonas* sp. A3W3 strain, isolated from the Akhtala mine tailing dump in Armenia, through analysing its genomic profile, growth characteristics and metal accumulation.

Methods: The *Pseudomonas* sp. A3W3 strain was cultured in Thiomonas Medium (TM) at a pH of 5.0, under aerobic conditions at 150 rpm and 33°C. The genome of the strain was sequenced using Illumina paired-end technology, and the raw sequence reads were uploaded and analysed on the KBase server. Additionally, the processes of biosorption and bioaccumulation were evaluated using atomic absorption (MP-AES) spectroscopy.

Findings: The *Pseudomonas* sp. A3W3 strain demonstrated high resistance to Cu(II), Cd(III), and Zn(II) ions, with maximum tolerance concentrations (MTCs) of 3 mM, 3 mM, and 2.5 mM, respectively. It was capable to accumulate these ions from the growth media at rates of 70%, 21.3%, and 97.2%, respectively.

The study of the draft genome of A3W3 strain revealed the presence of several genes associated with heavy metal resistance, including those encoding copper sensory histidine kinase, copper-translocating P-type ATPase, cobalt-zinc-cadmium resistance protein, heavy metal resistance transcriptional regulator, and heavy metal sensor histidine kinase, among others. These genes likely contribute to the strain's remarkable resistance to heavy metals.

Conclusion: The results underscore the potential of *Pseudomonas* sp. A3W3 as a promising candidate for bioremediation efforts.

Abstract ID: 2313 / Poster Number: 467.15P

The potential relationship between selected metals and metalloids and obesity in children

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Background: Metals are non-biodegradable natural elements in the environment that can pose significant threats to human health through various exposure routes, including food, drinking water, inhalation, or occupational contact. While some metals, such as copper (Cu), chromium (Cr), zinc (Zn), and iron (Fe), are essential as trace elements and only become harmful when critical doses are exceeded, others, like mercury (Hg) and lead (Pb), are toxic in any amount and have no known physiological function. The impact of metals on human health has traditionally been studied in relation to the circulatory and nervous systems, as well as their effects on the brain, kidneys, and reproductive functions. However, relatively recently, interest has shifted toward understanding their influence on the intestinal microbiota as key mediator in triggering several diseases. This shift coincides with growing evidence that the gut microbiome plays a role far beyond digestion, acting as a cornerstone of both physical and mental health. Dysbiosis, or disturbances in the gut microbiota, has been linked to various metabolic, mental, and autoimmune disorders. Metals can significantly alter the composition of the intestinal microbiota, often reducing its biodiversity, although in some cases, they may also increase it. One increasingly concerning disorder associated with gut composition and structure is obesity, a growing issue in the 21st century, particularly among children. Research exploring the link between metal exposure and obesity in this age group is still limited, and findings are often contradictory or inconclusive. This highlights the urgent need for further investigation to better understand these interactions and their long-term health implications.

Objective: This study aims to validate the methodology to explore potential links between selected metals and metalloids and obesity in children, as well as their overall impact on gut microbiota health.

Methods: Stool samples were immediately frozen after collection and stored until further analysis. Samples designated for metal(loid) concentration analysis were freeze-dried and digested using a mixture of H_2O_2 and HNO_3 in a microwave system, following an improved US EPA procedure. The final concentrations of the selected elements will be

determined using ICP-MS or ICP-OES. The procedure also includes the implementation of blank samples and the use of certified reference materials to ensure methodological precision and data accuracy.

Results: The stool samples are complex matrices to analyse metabolites. Elements successfully analysed for their impact on the intestinal microbiota, including their potential role in obesity, are arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg). Other notable elements include nickel (Ni), cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), zinc (Zn), as well as silver (Ag), barium (Ba), cesium (Cs), iron (Fe), antimony (Sb), thallium (Tl), and tungsten (W). The study cohort consisted of 69 samples from children with normal weight, overweight, and obesity. This cohort is part of a larger project that also includes untargeted organic metabolomics and metagenomics analyses, as well as data collection on the general physical condition of the children, following a multi-omics approach.

Abstract ID: 269

The promiscuity of the Resistance Nodulation cell Division tripartite efflux systems of Acinetobacter baumannii in drug transport

Presenting author: Dr. Heng-Keat Tam, University of South China, Hengyang, China

Co-authors: *Wuen Ee Foong, Xinxin Xiang, Yingqi Jin*

Introduction: Recent research has begun to shed light on the intricate interactions between human-targetted pharmaceuticals (non-antibiotics) and the intrinsic resistance mechanisms in Gram-negative bacteria, particularly through the modulation of multidrug efflux pumps. One such mechanism involves the Resistance Nodulation cell Division (RND)-type tripartite systems. Emerging evidence suggests that non-antibiotics may serve as substrates for these efflux pumps, potentially altering bacterial susceptibility and potentially fostering the development of multidrug resistance.

Objectives: This study aims to: (1) Elucidate the substrate specificity of non-antibiotics mediated by RND efflux pumps in *Acinetobacter baumannii*. (2) Investigate the patterns of cross-resistance between antibiotics and nonantibiotics in *A. baumannii*.

Method: (1) Drug susceptibility assays were performed using *A. baumannii* strains with knockout mutations in RND efflux pumps, as well as strains overexpressing these pumps. (2) Experimental evolution was conducted by treating *A. baumannii* strains with non-antibiotics, followed by drug susceptibility assays to access changes in resistance patterns in the evolved strains.

Findings: Our results demonstrate that two RND efflux pumps, AdeABC and AdeIJK, exhibit distinct substrate specificities toward a diverse array of non-antibiotics. AdeABC specifically recognizes lipophilic drugs with a low topological polar surface area, while AdeIJK preferentially transports drugs with a higher TPSA (Figure 1). Notably, cross-resistance to antibiotics was observed in *A. baumannii* strains exposed to non-antibiotics.

Conclusion: This study provides valuable insights into the role of non-antibiotics in modulating the activity of efflux pumps in *A. baumannii*. Our findings suggest potential strategies for combating multidrug resistance by targeting these critical efflux systems.

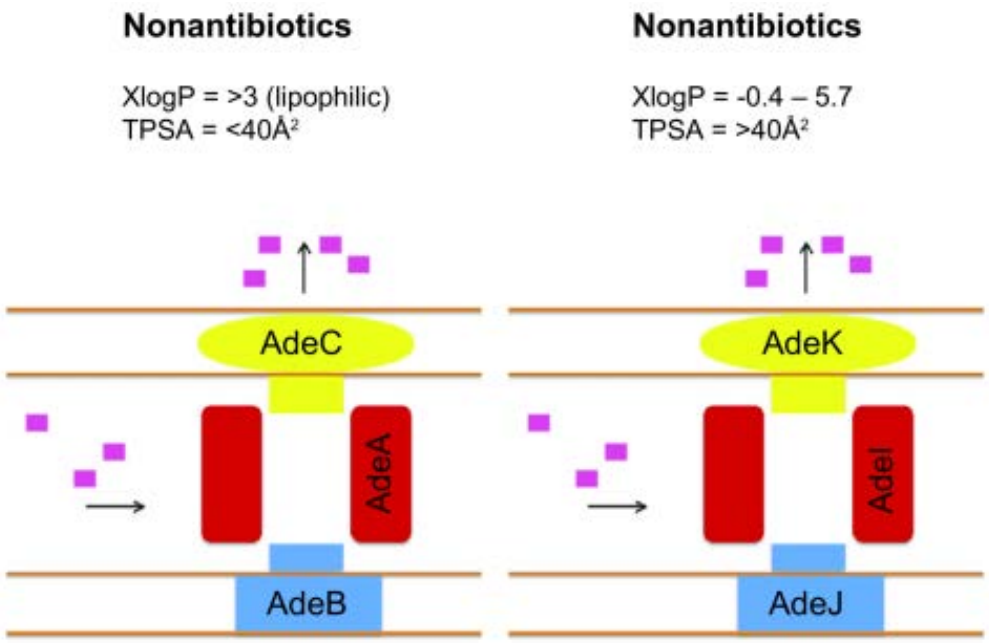


Figure 1: Substrate specificity of *A. baumannii* RND efflux pumps AdeABC and AdeIJK.

Abstract ID: 4121 / Poster Number: 308.15P

The P-type ATPase ZntA and its regulator ZntR defend *Chromobacterium violaceum* from zinc and cadmium toxicity

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The transition metal zinc is an essential trace element for life but can be toxic at high concentrations. Bacteria have many mechanisms to manage zinc toxicity, ensuring their growth and survival under zinc-stressful conditions, such as within the vacuole of host cells. In this work, we aim to characterize the roles of the genes CV_1154 and CV_1153, named here as *zntA* and *zntR*, in protecting *Chromobacterium violaceum* from zinc intoxication. *C. violaceum* is an environmental bacterium that causes rare but deadly infections in humans. Mutant strains with deletions of *zntA* or *zntR* were obtained by allelic exchange and characterized *in vitro* by growth curves, disk diffusion, and CFU assays, as well as *in vivo* in murine and amoeba infection models. Both mutant strains exhibited impaired growth and survival in LB medium supplement with zinc or cadmium, compared to the wild-type and complemented strains. Consistently, a *zntA* mutant showed increased zinc accumulation, as revealed by inductively coupled plasma mass spectrometry. Mice infected with a *zntA* mutant survived similarly to those infected with wild-type *C. violaceum*. Phagocytic plaque assays indicated that a z*ntA* mutant strain was more susceptible to predation by the social amoeba *Dictyostelium discoideum*, particularly when *D. discoideum* knockouts with disrupted zinc transport were used. Expression analysis by RT-qPCR and beta-galactosidase assays showed that *zntA* is activated by ZntR under conditions of zinc or cadmium excess. In conclusion, we demonstrated that the P_{1B-2}-type ATPase ZntA and its transcriptional activator ZntR protect *C. violaceum* against zinc and cadmium toxicity.

Abstract ID: 1772 / Poster Number: 215.15P

The ratio of protein to carbohydrate in the extracellular polymeric substances of marine diatoms

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Marine particles consisting of extracellular polymeric substances (EPS) include transparent exopolymer particles (TEP), made of acidic polysaccharides, and Coomassie Brilliant Blue-stained particles (CSP), containing proteinaceous materials. The ratio of protein to carbohydrate, or CSP/TEP ratio, serves as an indicator of the hydrophobicity, stickiness, aggregation efficiency, and sedimentation of EPS in seawater. However, the relationship between this ratio and the processes of EPS production, degradation, and disappearance in marine environments remains unclear. This study aimed to examine the CSP/TEP ratio of EPS produced by diatoms, which are key primary producers, by incubating two diatom species, *Thalassiosira weissflogii* and *Thalassiosira pseudonana*, under varying nutrient conditions over four weeks. The concentrations of EPS and the CSP/TEP ratio were measured at twice a week. Both TEP and CSP concentrations generally increased over time, ranging from 1.1 to 30.3 GXeq. mg L^{-1} and 0.1 to 21.3 BSAeq. mg L^{-1}, respectively. Although the CSP/TEP ratio roughly increased over time, ranging from 0.1 to 1.0, it peaked at 2 weeks and 3.5 weeks for *T. weissflogii* under different nutrient levels, while for *T. pseudonana*, the peak occurred at 3.5 weeks, regardless of nutrient concentration. Microscopic observations and cell counts revealed that cell abundance peaked during weeks 3–4, with cell lysis becoming significant during weeks 2–3. This suggests that the rise in CSP/TEP ratio is linked to cell lysis. Our results highlight that EPS characteristics change depending on diatom growth stages, potentially influencing marine biogeochemical cycles through particle aggregation and sedimentation processes.

Abstract ID: 2763 / Poster Number: 395.16P

The recombinant production of antiviral peptide Trypstatin with Corynebacterium glutamicum

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Co-authors: *Jan Lawrenz, Benjamin Dehner, Anton Buschmann, Jan Münch, Christian Riedel*

Introduction: In recent years, peptides with antimicrobial, antiviral, or other activities have become interesting alternatives for therapeutic applications. Thus, their biotechnological production is an interesting challenge both from a scientific and an economic point of view.

Objective: Although bioactive peptides can be produced by chemical synthesis, there are considerable limitations regarding production yields, and this may be overcome using recombinant biotechnological workhorse organisms. This study aimed to establish *Corynebacterium glutamicum* to produce Trypstatin, a peptide with potent activity against various respiratory viruses, including SARS-CoV-2.

Method: Different signal peptides were tested for efficient secretion of Trypstatin by *C. glutamicum* into the culture supernatant. The highest levels of secreted Trypstatin were obtained with the secretion signal of Cg1514. Further experiments in shake flasks and bioreactors were performed to optimize upstream process parameters, including concentrations of inducer, carbon source, and pH in the culture media.

Findings: The highest yields of Trypstatin were obtained in fermentations using 1 mM IPTG, 6% glucose at pH 6 in a Biostat bioreactor in a 4 L scale. At the end of fermentation, Trypstatin was successfully purified using cation exchange and reverse-phase chromatography. The purified peptide was successfully tested for activity in an *in vitro* model of SARS-CoV-2 infection using viral pseudoparticles.

Conclusion: Our results show that *C. glutamicum* is a suitable candidate for producing active antiviral peptides with good yields.

Abstract ID: 2086 / Poster Number: 226.15P

The Redox Cofactor Mycofactocin, its Biosynthesis and Regeneration via the Respiratory Chain in Mycobacteria

Presenting author: Mr. Mark Ellerhorst, University of Bayreuth, Kulmbach, Germany

Co-authors: *Ana Patrícia Graça, Vadim Nikitushkin, Gerald Lackner*

Introduction: Mycofactocin is a long-overlooked redox cofactor of actinobacteria, such as *Mycobacterium tuberculosis* (*Mtb*), the bacterial agent behind tuberculosis infections. Its biosynthesis follows the scheme of a ribosomally synthesized and post-translationally modified peptide (RiPP) exhibiting remarkable biochemical modifications, ranging from a carbon-carbon bond formation over glycosylation to site-specific *O*-methylation. Several studies provided evidence for an involvement of mycofactocin in alcohol degradation.

Objective: Intrigued by an ethanol-dependent regulation of mycofactocin biosynthesis and the discovery of a highly modified mycofactocin redox core, we sought to find an elusive enzyme behind the methylation of mycofactocin. Furthermore, we aimed to establish the function of the *mftG* gene in mycobacteria.

Method: A combination of genome manipulation and mass spectrometry guided by bioinformatic correlation studies, biochemical assays and growth monitoring was employed to investigate the biosynthesis and metabolic implications of mycofactocin in actinobacteria.

Findings: We were able to identify MftM, the missing methyltransferase in the biosynthesis of mycofactocin, provide evidence for MFT biosynthesis in, e.g., *Mtb*, *Mycolicibacterium smegmatis* and *Rhodococcus erythropolis*, and could show how a the flavoprotein MftG acts as a link between the redox cofactor mycofactocin and the mycobacterial electron transport chain.

Conclusion: The presented studies shed light on a missing part of the biosynthesis of mycofactocin, demonstrate a functional biosynthetic route of mycofactocin in *Mtb* and related bacteria and provide first insights into the mechanism of respiration-coupled mycofactocin regeneration in mycobacteria.

Abstract ID: 3798 / Poster Number: 455.15P

The relationship between Lon protease, antibiotic production, and growth phase in Streptomyces coelicolor A3(2)

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Co-authors: *Sedef Tunca, Sude Kara*

Streptomyces is a unique microorganism recognized as a prolific source of natural compounds such as antibiotics, immunosuppressants, and herbicides. The biosynthesis of secondary metabolites, which is closely related to the life cycle involving programmed cell death and hyphal differentiation, is regulated by complex molecular networks. Lon protease is essential for several physiological processes, such as pathogenicity, stress response, differentiation, and secondary metabolite synthesis in bacteria. The aim of this study was to elucidate the effect of Lon on the secondary metabolism and growth cycle of *Streptomyces coelicolor* A3(2). For this, cDNA libraries from different developmental stages of the wild type and the recombinant strain overexpressing the *lon* gene were used. The accuracy of the RNA-seq data was validated by qRT-PCR, and gene expression levels were calculated by RSEM. Based on DEG analyses, significant increases in the expression of secondary metabolite biosynthetic gene clusters, stress response, and cellular differentiation-related genes were found in the high antibiotic producer recombinant strain. It was also found that the expression of genes related to primary metabolism tended to decrease. To observe the morphological differentiation of *Streptomyces* strains, CSLM analysis was performed using SYTO 9 and PI stains. It was shown that the recombinant strain exhibited a higher density of pellet and clump formation with viable secondary mycelium, characterized by antibiotic production. The results of this study offer significant insights into strategies for enhancing the production of biotechnologically valuable bioactive metabolites in *Streptomyces* strains and potentially in other microorganisms.

Abstract ID: 1925 / Poster Number: 277.16F

The RNA atlas of the biotechnological chassis Vibrio natriegens

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Co-authors: *Sebastian Krautwurst, Kai Papenfort*

Vibrio natriegens is a rapidly growing, non-pathogenic marine bacterium, renowned for its remarkably short generation time, making it one of the fastest-growing organisms known. This unique trait has garnered significant interest in biotechnology and molecular biology. Although previous whole genome sequencing revealed a wealth of rRNAs and tRNAs in *V. natriegens*, an in-depth analysis of its global transcriptional output, including non-coding RNAs (ncRNAs), was previously missing. In this work, we combined differential RNA-seq (dRNA-seq), RNA immunoprecipitation and sequencing (RIP-seq), and RNA interaction by ligation and sequencing (RIL-seq) analyses to chart an atlas of transcriptional and post-transcriptional regulation in *V. natriegens*. This integrated approach enabled the genome-wide mapping of transcription starts sites (TSS), operons and RNA regulatory elements. In addition, we were able to identify Hfq-dependent small RNAs (sRNAs), discover their base-pairing partners, and to link post-transcriptional regulation to collective behaviours such as biofilm formation. We expect that studying the transcriptional and post-transcriptional landscape of *V. natriegens* will allow us to expand its utility as a biotechnology platform, promote targeted metabolic engineering approaches, and enable the design of novel synthetic biology circuits.

Abstract ID: 508 / Poster Number: 066.17P

The role of *Acinetobacter baumannii* capsular polysaccharides in antimicrobial resistance

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Introduction: The opportunistic pathogen *Acinetobacter baumannii* presents a significant challenge in managing hospital-acquired infections due to multidrug resistance [1]. The capacity to form biofilms on medical devices further enhances its resistance to antibiotics and disinfectants [2]. However, its virulence mechanisms remain poorly understood. Unravelling the role of virulence factors in pathogenesis may unlock new opportunities for developing innovative therapeutic strategies.

Objective: This study aimed to investigate the role of *A. baumannii* capsular polysaccharides in antimicrobial resistance.

Methods: We investigated a clinical *A. baumannii* strain belonging to ST2 and its *galU* gene-deletion mutant, which lacks the ability to produce capsular polysaccharides. The survival of these strains was evaluated under different antimicrobial stress conditions. The expression of capsule locus-related genes was analysed by quantitative PCR.

Findings: Capsule-depletion mutant formed a thicker biofilm compared to its wild-type strain. However, the capsule loss reduced *A. baumannii* resistance to sodium dodecyl sulphate. Interestingly, the biofilm formed by the capsule-depleted mutant exhibited enhanced resistance to colistin, chlorhexidine and hydrogen peroxide. Further analysis revealed the essential role of the capsule in bacterial survival following exposure to photodynamic therapy and antimicrobial components of innate immunity (serum complement and phagocytic immune cells). qPCR analysis showed the distinct regulation of capsule-locus related genes during the antimicrobial stress.

Conclusion: Capsular polysaccharides can either increase or impair the antimicrobial resistance of *A. baumannii,* depending on the mechanism of antimicrobial effect. These findings suggest that targeting capsular polysaccharides could be a promising strategy for managing *A. baumannii* infections.

Abstract ID: 3184 / Poster Number: 361.16P

The role of Antarctic psychro-halotolerant bacteria and their osmoprotectants in the regeneration of saline soils with agricultural potential

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Co-authors: *Aleksandra Goszcz, Marcin Musiałowski, Robert Stasiuk, Silvia Celletti, Michela Schiavon*

Introduction: Soil salinization is an escalating threat to global agriculture, requiring sustainable remediation approaches. Antarctic psychro-halotolerant bacteria (PHTB) stand out for their adaptive capabilities to salinity and low temperatures, offering the potential for soil recovery.

Objective: This study aimed to investigate the efficacy of PHTB-derived osmoprotectants in ameliorating saline soils and mitigating the detrimental effects of salinity on plants.

Method: Adaptive Laboratory Evolution (ALE) and Response Surface Methodology (RSM) were employed to optimize bacterial strains with enhanced osmoprotectant production. Soil microcosms were supplemented with osmoprotectants from optimized strains, and salinity, pH, organic carbon, and soil microbial quality parameters were measured before and after the soil treatment. Plant (lettuce, tomato) growth responses were also evaluated under greenhouse conditions.

Findings: PHTB supplementation improved soil health indicators, including increased organic carbon and dehydrogenase activity. Notably, plants exposed to saline conditions alongside PHTB-derived osmoprotectants exhibited higher growth rates, reduced stress markers, and improved cell division compared to the controls. These results indicate that osmoprotectants of microbial origin can improve soil quality and enhance plant resilience under salinity stress.

Conclusion: By boosting soil fertility and promoting plant growth, PHTB-derived osmoprotectants represent an innovative, eco-friendly strategy for mitigating soil salinization. Their capacity to enhance overall microbial activity and crop performance under extreme conditions underscores their potential for addressing global food security challenges in a changing climate..

Abstract ID: 2822 / Poster Number: 216.16P

The role of microbiota metabolite Urolithin A in fighting Multiple Myeloma

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Introduction: Our group has recently associated short chain fatty acids (SCFAs) and urolithins with Multiple Myeloma (MM) progression and response to treatment. Moreover, urolithin A (UA) presented an antiproliferative effect in MM cells *in vitro* and *in vivo* (Rodríguez-García et al. CCR, 2024 and Rodríguez-García et al. Microbiome, 2025).

Objective: We explored the potential use of UA as serum biomarker and characterize its molecular mechanisms of action. Also, we studied possible synergies with standard treatments.

Method: UA was measured in patients' serum at different stages of MM (n=26) using liquid chromatography and mass spectrometry. *In vitro* toxicity and synergy studies were conducted in cell lines. Molecular pathways were studied by annexin V assay and western blot. Stimulation assays were performed in T-cells from healthy donors.

Findings: UA was associated with asymptomatic stages and complete response. UA was cytotoxic in MM cell lines with IC50 values in the lower micromolar range, producing an increase in apoptotic cells and the expression of BAX. Additionally, UA treatment caused an increase in expression of the antioxidant NRF2, and cell cycle protein p27. Finally, UA was synergic with lenalidomide and venetoclax and activated T-cells after 24h and 12 days of treatment.

Conclusion: Association between UA and a better prognosis of MM implies new diagnostic opportunities. Furthermore, its anti-proliferative effect on MM cell lines, the modulation of several signalling pathways involved in MM pathogenesis and the stimulation of T-cells confirm the therapeutic potential of this metabolite individually and in combination with approved treatments of disease.

Abstract ID: 756 / Poster Number: 039.16P

The Role of NTHi OmpA Orthologue P5 in Complement Inhibitor Binding and Immune Evasion

Presenting author: Ms. Sandra Jonsson, Lunds universitet, Lund, Sweden

Co-authors: *Martina Janoušková, Yu-Ching Su, Oskar Thofte, Kristian Riesbeck*

Recruitment of complement inhibitors to the surface of bacteria is an important mechanism of immune evasion. Non-typeable Haemophilus influenzae (NTHi) has been shown to bind various complement inhibitors, which enhances its survival in serum. Here we report the role of the NTHi OmpA orthologue P5 in binding to complement inhibitors as a strategy to evade immune system clearance. P5 is a transmembrane protein with four surface-exposed hypervariable loops, a transmembrane domain, and a periplasmic C-terminal domain. Isogenic mutants lacking P5 were generated to assess its role in complement binding and its impact on serum survival. Biolayer interferometry and ELISA were used to identify the surface-exposed loops involved in the interaction with complement inhibitors. Our results show that P5-deficient NTHi strains exhibit reduced binding to complement inhibitors involved in the classical and terminal pathways. Further analysis revealed that extracellular loop 2 is the primary binding site for the complement inhibitors tested. P5-deficient mutants with reduced complement binding were more susceptible to serum-mediated killing. Finally, expressing NTHi P5 on E. coli BL21 (DE3) enhanced complement inhibitor binding and serum survival. In summary, our findings suggest that P5 binds complement inhibitors via loop 2, providing protection against serum-mediated killing and contributing to NTHi immune evasion.

Abstract ID: 2652 / Poster Number: 256.17P

The role of plant growth-promoting bacteria in the degradation of biodegradable polymers

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Co-authors: *Maria Swiontek Brzezinska, Joanna Karbowska-Berent*

Polymeric materials are produced in vast quantities, contributing to an escalating global waste management issue. Biodegradable polymers, including poly(lactic acid) (PLA), poly(hydroxybutyric acid) (PHB), and polycaprolactone (PCL), which undergo environmental degradation, are gaining increased attention. However, their production and processing remain costly, particularly when the waste comprises mixtures of different polymers.

Aliphatic polyesters are more susceptible to microbial degradation, with the biodegradation process influenced by numerous chemical and physical factors. Plant growth-promoting rhizobacteria (PGPR) have been observed to thrive in soils containing polymer waste, facilitating polymer degradation through their high metabolic activity and biofilm-forming capabilities.

This study aimed to evaluate the biodegradation potential of the bacterium *Pseudomonas atacamensis* **R38**, isolated from the rhizosphere of rapeseed (*Brassica napus*), toward PHB, PLA, PCL, and their blends. The research focused on the strain's ability to form biofilm, as well as its enzymatic and respiratory activities.

The R38 strain created biofilms of varying densities on all tested polymers, with the most robust formation observed on PCL50/PHB50 and PCL75/PHB25 blends. The highest oxygen consumption was recorded for PLA25/PCL75 and PLA50/PCL50 blends. These findings demonstrate that the strain exhibited a higher degradation efficiency for polymer blends compared to pure polymers.

The results suggest that *Pseudomonas atacamensis* **R38, as a PGPR bacterium, holds promise for enhancing polymer biodegradation in environmental applications.**

Abstract ID: 3020 / Poster Number: 112.15F

The role of prokaryotic mercury methylators and demethylators in Canadian Arctic thermokarst lakes

Presenting author: Mr. Nicola Gambardella, CIIMAR, Porto, Portugal

Co-authors: *Joana Costa, Beatriz Martins, Diogo Folhas, Ana Patrícia Ribeiro, Holger Hintelmann, Joao Canario, Catarina Magalhaes*

Permafrost soils are critical reservoirs for mercury (Hg), with the thawing process leading to the release of this element into the environment, posing significant environmental risks. Of particular concern is the methylated form of mercury, monomethylmercury (MMHg), known for its adverse effects on Human health. Microbial communities play a pivotal role in the formation of MMHg by facilitating Hg methylation and in the demethylation of MMHg, slowing the crossing of toxic threshold concentration in the environment. However, the specific microbes involved still need to be understood. This study aimed to identify the microbial drivers behind changes in Hg speciation (MMHg and Hg) in permafrost thaw lakes and assess the significance of the biotic component in Hg biogeochemistry. Sediment samples from two thermokarst lakes in the Canadian sub-Arctic were collected during the winter and summer of 2022. Gene-centric metagenomics using whole-genome sequencing (WGS) was employed to identify key genes involved in mercury methylation (hgcA and hgcB) and demethylation (merA and merB), supported by qPCR analyses. A seasonal decline in microbial diversity, involved in the Hg methylation, and hgcA gene coverage was observed from winter to summer, mirroring patterns in mercury methylation rates. Notably, hgcA sequences were significantly more abundant than merAB sequences, with contrasting seasonal trends. These results indicate a seasonal shift in the microbial community, transitioning from a dominance of mercury methylation in winter to a predominance of mercury demethylation in summer. Environmental drivers of these dynamics were integrated into a conceptual model. This study provide new insights on the microbial processes influencing the Hg cycle in Arctic permafrost undergoing degradation.

Abstract ID: 2686 / Poster Number: 251.15P

The Role of Spatial Configuration in Modulating Microbial Interactions and Resource Allocation

Presenting author: Ms. Jin Gyeong Jeong, Yonsei University, Wonju, South Korea

Co-authors: *Tae Kwon Lee*

The spatial arrangement of microbial cells can significantly influence their interactions, as proximity and initial positioning can affect resource competition, signaling, and other forms of interspecies communication. This research aims to investigate how the initial spatial arrangement of two microbial species on solid media affects their interactions. We examined the effect of richness on colony size and intercolony distance (or Voronoi area) in mixed cultures of *Staphylococcus aureus* and competing strains with different competitive strengths by modulating the initial culture concentration. We conducted weighted Voronoi analysis to examine spatial patterns of microbial interactions, revealing distinct colony distributions that varied with strain richness and competitive dynamics. At high richness, competitive strains dramatically constrained *S. aureus* colony size, effectively reducing its spatial occupation and demonstrating localized, strong growth inhibition. This spatial compression correlated directly with a significant upregulation of *S. aureus* virulence genes, suggesting an adaptive survival response to intense competitive environments. Conversely, low-richness conditions showed more dispersed colony distributions, with weaker competitive inhibition and consequently larger *S. aureus* colony sizes. The findings underscore how microbial interactions not only modulate spatial organization but also trigger complex virulence responses, with strain richness serving as a critical determinant of bacterial survival strategies.

Abstract ID: 2827 / Poster Number: 073.16P

The role of staphylococcal accessory regulator SarA on phage replication and phage infection

Presenting author: Ms. Ronja Dobritz, University of Tübingen, Tübingen, Germany

Co-authors: *Carina Rohmer, Christiane Wolz*

As a major opportunistic pathogen of human and animals *Staphylococcus aureus* asymptomatically colonizes the nasal cavity but is also a leading cause of life-threatening acute and chronic infections. More than 90% of the human nasal isolates of *S. aureus* were found to carry Sa3-phages, which integrate into the bacterial *hlb* gene disrupting an important virulence factor. On the other hand, Sa3-phages encode highly human specific virulence factors that are probably essential for bacterial survival in the human host. However, how the *S. aureus* host modulates the life cycle of different temperate phages remains largely unknown. Our data suggest that this regulation is strain specific and host dependent. In order to identify host factors that impact the phage life cycle, different global regulator mutants of *S. aureus* were tested for their ability to produce phages. Strains mutated in the staphylococcal accessory regulator SarA showed reduced phage replication of Φ11 and the Sa3-phage Φ13. This effect was also observed when phage free strains were infected with phages and bacterial growth and phage numbers were monitored. Adsorption assays revealed reduced Φ11 binding to the SarA deficient mutant, while Φ13 was able to bind equally to wildtype and SarA mutated *S. aureus*. Analysis of the gene expression of glycosyl transferases showed altered glycosylation patterns in the SarA mutant, could be responsible for impaired Φ11 binding. The life cycle of Φ13 is influenced by SarA at different levels including replication and gene expression regulation.

Abstract ID: 2930 / Poster Number: 156.17P

The role of the fosfomycin resistance fosB gene in Staphylococcus epidermidis

Presenting author: Ms. Giulia Cattabriga, University of Bologna, Bologna, Italy; University of Siena, Siena, Italy

Co-authors: *Francesco Flandi, Alessandro Poletti, Valeria Alonzi, Ijeoma Okoliegbe, Francesca Bonvicini, Vincenzo Di Pilato, Karolin Hijazi, Marco Rinaldo Oggioni*

Introduction and objective: Fosfomycin is a broad spectrum antimicrobial compound that inhibits cell wall biosynthesis through binding of MurA. Several mechanisms are known to confer fosfomycin resistance, including the enzyme FosB which inactivates fosfomycin transferring bacillithiol on the molecule's ring. In *Staphylococcus epidermidis fosB* can be found both in the core genome (*cg*-fosB) and in accessory-elements (*ag*-fosB), with a nucleotide identity of 69%. Our aim is to uncover the role of *S. epidermidis* FosB in fosfomycin resistance.

Methods: We collected, sequenced and analyzed 147 emoculture clinical isolates and 39 carriage isolates. Fosfomycin susceptibility was tested using MIC Test Strips.

Findings: *cg-fosB* was universally present amongst the isolates, albeit often carrying stop-codon mutations or frame-shifts, but only 8 clinical isolates showed the presence of *ag-fosB*. Isolates were tested for fosfomycin susceptibility and *ag-fosB* carrying isolates showed MICs of 128mg/L, while the other isolates showed MICs of 0.25-32 mg/L, without any impact on the susceptibility conferred by *cg-fosB* mutations. Fosfomycin MIC values of carriage isolates from Italy were about four-fold lower than those of blood culture isolates. Absence of resistance and low overall susceptibility is surprising due to the utilization of fosfomycin for urinary tract infections in the community in Italy.

Conclusions: Our data indicate that, in contrast to the *ag-fosB* gene, the *cg-fosB* paralog does not show any fosfomycin related phenotype in *S. epidermidis*. Combined with the data indicating lower overall fosfomycin susceptibility in community isolates indicates that likely other factors influence fosfomycin susceptibility in *S. epidermidis*.

Abstract ID: 4096 / Poster Number: 101.16P

The Role of the Gut Microbiome in Pemphigoid Diseases: Exploring the Gut-Skin Axis and Disease Susceptibility

Presenting author: Ms. Xiaolin Liu, Max Planck Institute for Evolutionary Biology, Ploen, Germany; Kiel University, Kiel, Germany

Background: Pemphigoid diseases (PD), particularly bullous pemphigoid (BP), are autoimmune blistering dermatoses associated with decreased life quality and increased mortality risk. Previous studies implicated the skin microbiome, including increased *Staphylococcus aureus* abundance, as a risk factor modifying disease severity. However, despite a growing appreciation for the gut microbiome and its contribution to the gut-skin axis in health and disease, in potential role in PD remains largely unexplored.

Objectives: We aim to investigate the gut microbiome and its alterations during disease progression in PD, and determine its causal role and potential pathophysiological mechanisms.

Methods: Here, we compared the gut microbiome of 66 BP patients and corresponding controls, and then investigated the gut microbiome changing with longitudinal samples from a BP-like EBA (Epidermolysis bullosa acquisita) mouse model. Moreover, we induced EBA in mice with different initial gut microbiomes, but same genetic background, and compared their responses to EBA immunization.

Results: We observe a reduction in alpha-diversity and an overall altered microbial community in the gut of BP patients. Other key features include decreased *Faecalibacterium prausnitzii* and increased *Flavonifractor plautii*, and elevated pathways related to with GABA metabolism. In EBA mice, gut microbiome changes occur over the course of disease progression, with an increase in *F. plautii* also observed in mice. Further, we find that mice with different initial gut microbiomes developed different EBA disease scores, and the co-housing results both in a homogenized gut microbiome and an intermediate EBA disease level, suggesting a causal role of gut microbiome in BP disease severity.

Abstract ID: 1364 / Poster Number: 287.17P

The role of the HCN-producing Pseudomonas putida A32 in the induction of systemic resistance in pepper plants

Presenting author: Ms. Aleksandra Mesaroš, University of Belgrade, Faculty of Biology, Belgrade, Serbia; Centre for Biological Control and Plant Growth Promotion, University of Belgrade, Belgrade, Serbia

Co-authors: *Marija Nedeljković, Iva Atanasković, Marija Anđelković, Slaviša Stanković, Jelena Lozo*

Introduction and Objective: Plant-beneficial bacteria can enhance plants' resistance to pathogens, reducing reliance on chemical treatments. This study explored the role of the pepper endophyte *Pseudomonas putida* A32, a hydrogen cyanide (HCN)-producing strain, in the induction of systemic resistance (ISR) and modulation of antioxidant enzyme activity.

Methods: To determine HCN's role in plant defence, an HCN mutant strain of *P. putida* A32 was constructed. Pepper seeds were treated with either the wild type (wt) or the mutant strain and systemic resistance was analysed by infecting the plants with *P. syringae* and *Xanthomonas euvesicatoria*. The levels of hydrogen peroxide (H_2O_2), lipid peroxidation and the activity of the main antioxidant enzymes (superoxide dismutase, ascorbate peroxidase and phenylalanine ammonia lyase) were measured in the treated and control plants.

Findings: Plants treated with the wt strain exhibited enhanced systemic resistance, as evidenced by significantly smaller leaf lesions than the control and mutant-treated plants. H_2O_2 content and lipid peroxidation were reduced in the treated groups, with no difference between wt and mutant. In contrast, the activity of antioxidant enzymes was altered in the wt-treated plants, while the mutant-treated plants showed no significant difference from the controls, indicating the role of HCN in ISR.

Conclusion: The results emphasise the role of HCN production by *P. putida* A32 in reducing pathogen-induced damage by modulating the activity of antioxidant enzymes. Further analyses of HCN-dependent mechanisms and gene expression in the context of ISR will deepen our understanding of the interactions between endophyte bacteria and their host plants.

Abstract ID: 1622 / Poster Number: 022.17F

The role of the Rhs insertion in bacterial competition of Listeria monocytogenes

Presenting author: Ms. Yasar Özge Delikkafa, University of Veterinary Medicine Vienna, Vienna, Austria

Co-authors: *Kathrin Kober-Ryhcli, Sarah Thalguter*

Recently, we have identified a 12.5 kbp insertion in *L. monocytogenes*, a human food-borne pathogen, ST121 genomes and named it rearrangement hot spot (Rhs) insertion. The Rhs proteins usually form toxin-antitoxin (TA) modules in which antitoxins/immunity proteins block the toxic activity. In our study, the Rhs insertion harbours a *rhs*/*rhsI* TA system, a potential accessory gene *rhsI2*, an orphan *rhs*/*rhsI* system and a *kptA*. We hypothesise that the Rhs insertion mediates bacterial competition and supports virulence.

First, we analysed the Rhs locus of 35 *L. monocytogenes* ST121 strains. Amino acid sequence comparison revealed that five strains harbour a longer Rhs protein. In addition, we observed that the orphan Rhs proteins and KptA have an extended N terminus in several strains.

Next, we cloned the Rhs C terminus (CT), the toxin domain, and the rest of the Rhs insertion proteins into expression vectors. Expression of Rhs-CT or its extended variant (RhsCT_L) impaired the growth of *E. coli*, whereas expression of RhsI1, RhsI2 or KptA alone did not have any effect. However, co-expression of RhsCT/RhsCT_L and RhsI1/RhsI2 showed a secondary growth inhibition effect. Therefore, we concluded that proteins named RhsI1 and RhsI2 act like accessory factors of any variant of Rhs protein instead of immunity proteins. After testing the effect of the orphan Rhs TA module in *E. coli,* we plan to clone the modules of the Rhs insertion into an *L. monocytogenes* strain that lacks the Rhs insertion and to characterise the biochemical function of the Rhs insertion genes.

Abstract ID: 3670 / Poster Number: 188.17P

The second messenger c-di-GMP regulates ciprofloxacin susceptibility in Pseudomonas aeruginosa

Presenting author: Dr. Tarcisio Brignoli, Università degli Studi di Milano, Milano, Italy

Co-authors: *Silvia Ferrara, Giovanni Bertoni*

The second messenger c-di-GMP influences many bacterial functions. *In P. aeruginosa*, c-di-GMP is a key regulator of biofilm development, with high and low levels being associated with increased biofilm formation and planktonic growth, respectively. We investigated the role of c-di-GMP in antibiotic resistance. We evaluated the impact of c-di-GMP on the susceptibility to the fluoroquinolone antibiotic ciprofloxacin using genetically engineered strains that have low (PA2133Δpp), high (YFINΔpp), or physiological levels (Δpp) of c-di-GMP. These effects were uncoupled from biofilm formation thanks to the deletion of the *pel* and *psl* operons, which prevent biofilm formation. Interestingly, we observed that c-di-GMP levels are inversely correlated with ciprofloxacin tolerance. The PA2133Δpp strain was more resistant to ciprofloxacin than the Δpp strain, while the YFINΔpp was more susceptible. These results correlate well with previous reports showing the efficacy of ciprofloxacin in treating *P. aeruginosa* biofilms. We suggest that the reasons behind these phenotypes are multifactorial. Indeed, we observed that the MexC efflux pump is downregulated at high c-di-GMP levels, while no changes were detected between physiological and high levels of c-di-GMP. This explains the increased sensitivity in the YFINΔpp strain, but not the increased resistance of the PA2133Δpp. Thus, these results suggest that depending on its levels, c-di-GMP can influence more than one factor involved in ciprofloxacin tolerance.

Abstract ID: 3199 / Poster Number: 052.16P

The secreted proteases Aur, ScpA, SspA and SspB suppress the virulence of Staphylococcus aureus USA300 by shaping the extracellular proteome

Presenting author: Assoc. Prof. Girbe Buist, University Medical Center Groningen, Groningen, Netherlands

Surface-located and secreted virulence factors of the Gram-positive bacterial pathogen *Staphylococcus aureus* are key drivers for infection of the human host. Proteolytic enzymes may contribute to virulence by breaking primary barriers and host immune defenses. Therefore, the objective of our present study was to chart the contributions of different proteases to *S. aureus* virulence, and to assess their roles in shaping the staphylococcal surface proteome (the 'surfacome') and extracellular proteome (the 'secretome').

To this end, we applied 12 protease mutants of the *S. aureus* USA300 lineage. Four mutants lacking the metalloprotease aureolysin (Aur), the cysteine proteases staphopain A (ScpA) and SspB, or the serine protease SspA displayed enhanced cytotoxicity towards human lung epithelial cells, showing that they serve to suppress virulence.

Profiling of the surfacomes and secretomes of the four mutants allowed correlation of their increased cytotoxicity to altered virulence factor profiles. Furthermore, enhanced levels of virulence factors were detected in the mutants' surfacomes, which was shown to be relevant as all four mutants displayed enhanced lung epithelial cell invasion. Enhanced levels of cytoplasmic and membrane proteins in the mutant's surfacomes showed that Aur, ScpA, SspA and SspB set limits to autolysis by reducing the levels of peptidoglycan hydrolases.

We conclude that Aur, ScpA, SspA and SspB have key roles in shaping the surfacome and secretome of *S. aureus*, thereby controlling the virulence of this major pathogen. This implies that novel antimicrobial agents or vaccines should not target these proteases.

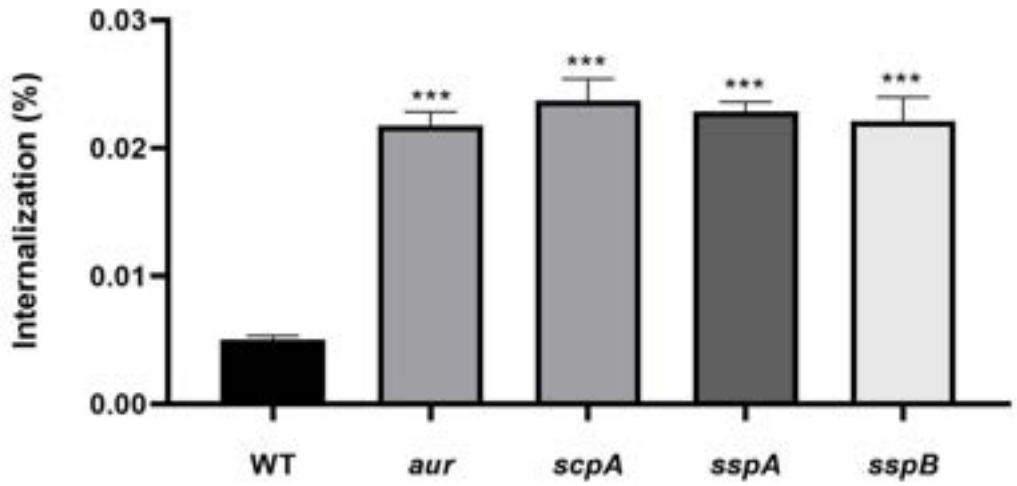


Figure 1: **Invasive behavior of *S. aureus* USA300 WT and the *aur*, *scpA*, *sspA* or *sspB* mutant strains towards human lung epithelial cells.** The numbers of internalized WT or single protease mutant bacteria were determined after 1 h of infection and 30 min incubation with lysostaphin. To this end, the cells were lysed with 0.05% SDS and the released bacteria were collected and counted by plating on TSA plates. The internalization of the WT and mutant bacteria is presented as the proportion of the total bacterial count used for the infection. The bar plot shows the mean values from three individual experiments and the error bars show the respective standard deviation. Statistical significance was assessed by two-tailed t-tests. A P-value < 0.05 was considered significant (***, $p < 0.005$).

Abstract ID: 3069 / Poster Number: 025.15P

The spectrum of biodestructive activity of strains in relation to nitroaromatic substances that are part of explosive compounds

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Co-authors: *Serhii Skrotskyi, Danguolė Montvydienė, Nataliia Matviienko*

Background: Water bodies of Ukraine are a significant source of biological resources that constantly ensure food security and livelihoods of the population, provide income and social development. But the military activities in Ukraine have resulted in severe pollution of ecosystems and the destruction of habitats, as well as a decrease in biodiversity, and ecosystem services. One of the environmental problems of Ukraine is the pollution of water bodies with explosive residues and related compounds, which include nitrate esters (GTN and PETN), nitroaromatic compounds (TNP and TNT), nitramines (RDX, HMX). It has been proven that these chemicals negatively affect the survival and health of various organisms and can have toxicity and high cumulative capacity, as well as mutagenic and carcinogenic effects.

Objectives: – was to screen for active strains that degrade nitroaromatic substances.

Materials and methods: Determination of the spectrum of biodestruction activity of strains in relation to nitroaromatic substances that are part of explosive compounds or are their derivatives was carried out on a liquid mineral medium. The studied compounds (0.05 g/l) were used as the only source of carbon and energy. 1,3-dinitrobenzene, 2-methyl-5-nitroaniline, 2,6-dinitrotoluene, 2,4-dinitrotoluene, 2-nitrotoluene. The growth of the cultures was assessed after 7 days of cultivation by changing the optical density of the culture liquid at a wavelength of 540 nm. A total of 54 strains of different taxonomic groups were studied in the work.

Findings: It was found that microorganisms best absorb 2-nitrotoluene, a smaller number of the studied strains are can to grow on a medium with 1,3-dinitrobenzene and 2-methyl-5-nitroaniline (76 and 79% of all studied strains). The worst microorganisms consumed 2,4-dinitrotoluene (54%). As a result of the study, the most active destructive strains of nitroaromatic compounds were selected (Dietzia maris, Rhodococcus erythropolis, Pseudomonas aureofaciens, and 3 strains without identification

Conclusion: The results of the research will be used for further studies of the decomposition of nitroaromatic substances and the degree of their destruction in model experiments.

Abstract ID: 2063 / Poster Number: 112.17P

The spread of a Panton-Valentine leucocidin-positive MRSA clone, ΨUSA300, with a mutation that causes the stabilisation of mobile genetic elements

Presenting author: Dr. Hiroshi Kaneko, Tokyo University of Pharmacy and Life Sciences, Hachioji, Japan

Co-authors: *Saki Higashitani, Masaki Mishima, Junzo Hisatsune, Motoyuki Sugai, Hidemasa Nakaminami*

Introduction: USA300 is a worldwide epidemic Panton-Valentine leucocidin-positive methicillin-resistant Staphylococcus aureus (MRSA) clone harbouring an arginine catabolic mobile element (ACME). ΨUSA300 is a variant of USA300 characterised by a deletion in the site-specific recombinase ccrB2 gene within the staphylococcal cassette chromosome mec (SCCmec) element. Recently, ΨUSA300 has accounted for approximately half of the MRSA isolates from community settings in Japan. However, the reasons behind this trend remain unclear.

Objective: In this study, we analysed the phylogenetic characteristics of ΨUSA300 and the effects of ccrB2 gene deletion.

Method: A total of 28 USA300 and 101 ΨUSA300 strains, isolated in Japan between 2013 and 2021, were analysed. Whole-genome sequencing was performed using a next-generation sequencer to construct a phylogenetic tree. The excision frequency of SCCmec elements and ACME was evaluated using real-time PCR. Mutants were constructed through plasmid-mediated recombination. Protein structure was predicted using AlphaFold2.

Findings: The phylogenetic tree indicated that ΨUSA300 emerged from USA300 around 2010 and subsequently disseminated rapidly throughout Japan. The excision frequency of SCCmec elements and ACME was significantly lower ($P < 0.01$) in the ΨUSA300 strains than in the USA300 strains. This excision frequency was significantly increased ($P < 0.01$) when the ccrB2 gene in ΨUSA300 was replaced with wild-type ccrB2 gene. Based on its structure, the deletion of CcrB2 protein was predicted to affect the DNA-binding ability of the protein.

Conclusion: The results of this study suggest that the stabilisation of mobile genetic elements has affected the spread of ΨUSA300.

Abstract ID: 1557 / Poster Number: 328.15P

The Y-complex initiates ribosome degradation in Bacillus subtilis

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Introduction: During nutrient starvation, the stringent response limits the synthesis and activity of new ribosomes by the activities of the second messenger (p)ppGpp. In *B. subtilis* cells, the coordinated action of (p)ppGpp with the stress transcription factor Spx can also downregulate translation to prevent the PQC system from becoming overloaded with newly synthesized proteins that are prone to misfold during proteotoxic stresses. Controlling translation is essential to adapt to stressful conditions.

Objectives: In this study, we aimed to understand which other genes are important for the heat shock response, with a special focus on the regulation of translation.

Method: We performed a genetic screening using the barcoded deletion library BKE from *B. subtilis* to identify mutants with altered heat shock resistance. These deletion mutants were further characterized using RNA-seq and proteomics

Findings: Our findings showed that the absence of genes responsible for regulating RNase Y activity, referred to as the Y-complex (*ymcA*, *ylbF*, *yaaT*), reduces fitness during heat shock. These deletion mutants also exhibit sensitivity to various stress conditions, including oxidative and osmotic stress, and display a mild growth defect when transitioning from the exponential to the stationary phase, indicating an impaired response to nutrient scarcity. By identifying RNA cleavage sites depending on *ymcA*, we determined that the Y-complex is crucial for the maturation, processing, and degradation of many RNA transcripts, including rRNA and mRNA of genes related to translation. Notably, this degradation of rRNA occurs at the end of the exponential phase, coinciding with nutrient scarcity in the media, suggesting that the Y-complex may play a role in ribosome degradation. We confirmed that rRNA degradation happens in mature ribosomes rather than in newly transcribed rRNA. The impairment of ribosome degradation due to the absence of *ymcA* leads to an accumulation of ribosomes during the transition to the stationary phase, as observed through mass spectrometry and the detection of hibernating 100S ribosomes. Furthermore, the Δ*ymcA* mutant also displays

increased ribosome levels during heat shock. Deleting *ymcA* in a genetic background that cannot synthesize (p)ppGpp inhibits the reduction of ribosome levels when transitioning to the stationary phase, accompanied by a significant growth defect during this phase, indicating that both (p)ppGpp and the Y-complex independently control ribosome downregulation in the transition phase and during proteotoxic stress conditions.

Conclusion: These results suggest that the Y-complex influences many RNA-dependent processes, including limiting active ribosome levels by triggering their degradation. In concert with other regulatory processes, cellular growth can be adjusted during the transition to the stationary phase. These mechanisms will concomitantly decrease translation, and are also harnessed to reduce cellular protein folding stress when facing proteotoxic stresses, such as elevated temperatures.

Abstract ID: 1156 / Poster Number: 258.16P

Therapeutic Rescue of Helicobacter pylori VacA-Induced Mitochondrial Damage in Gastric Pit Cells of the Antrum

Presenting author: Prof. Doc. Moo-Seung Lee, Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea; Korea University of Science and Technology, Daejeon, South Korea

Co-authors: *Hyun-Soo Cho, Tae-Su Han, Dae Soo Kim, Mi-Young Son*

Understanding the host cell specificity, pathogenicity, and molecular mechanisms of Vacuolating Cytotoxin A (VacA), a toxin secreted by Helicobacter pylori (Hp), is critical for developing innovative treatment approaches. Although VacA affects subcellular processes, particularly mitochondria, in a cell-type-specific manner, the absence of reliable models that replicate VacA-induced subcellular damage and enable the screening of novel drugs relevant to the human stomach has hindered our comprehension of mitochondrial networks in vivo. In this study, we employed human antrum gastric organoids (hAGOs) and tissue samples from Hp-infected patients to investigate the toxic effects of VacA-induced mitochondrial damage, specifically in mucus-producing gastric pit cells. Through transcriptional, translational, and functional analyses, we observed substantial mitochondrial fragmentation, reduced ATP production, and loss of mucosal barrier integrity in both VacA-intoxicated and Hp-infected hAGOs. Using these organoids, we screened clinically relevant small molecules and found that MLN8054, an Aurora kinase A inhibitor, could effectively reverse VacA-induced mitochondrial damage and restore gastric epithelium integrity. MLN8054 showed efficacy in both VacA-treated hAGOs and mice, highlighting the potential of hAGOs as a drug screening platform. These findings suggest that targeting mitochondrial quality control could offer a promising therapeutic strategy for mitigating VacA-mediated toxicity and disease progression associated with Hp infection.

Abstract ID: 1185 / Poster Number: 284.17P

Thermal Performance of Mycelium Composites Incorporating Vermiculite and Fomitella fraxinea

Presenting author: Prof. Hee Wook Ryu, Soongsil University, Seoul, South Korea

Co-authors: *Jayeon Yoo, Junsu Jang, Seung Yol Yoo, Kyung-Suk Cho, Jeonghee Yun*

Mycelium composites have garnered attention as eco-friendly alternatives to polystyrene products. Traditionally, these composites rely on organic matrices, but this study explores the use of an inorganic mineral, vermiculite, as a matrix material derived from forestry and agricultural by-products. The thermal properties of mycelium composites were evaluated under these novel conditions.

The fungal strain *Fomitella fraxinea* was used, with wheat flour serving as a nutrient source. The effects of nutrient concentration and moisture content on thermal conductivity were investigated, alongside a comparative analysis using oak sawdust in the matrix. Wheat flour was added at 10-30 wt% of vermiculite, and moisture content was adjusted to 40-70%. Sawdust was added at 0-100 wt%, and vermiculite particles with a size of 1.0-1.5 mm were used. Spawn cultivated on sawdust media was inoculated at 10 wt%. The prepared matrix was compacted into molds and incubated at 28°C for 7-10 days, followed by an additional 3 day incubation after demolding. Samples were dried at 80°C for 24 hours before analysis.

The results showed that *F. fraxinea* could grow effectively in both organic and inorganic matrices. The thermal conductivity of mycelium composites ranged between 0.05 and 0.07 W/m·K. Cone calorimeter tests indicated that total heat release was highest for sawdust-based mycelium composites (SBMC), followed by phenolic foam (PF), and vermiculite-based mycelium composites (VBMC). VBMC exhibited significantly lower total smoke release than the other materials.

This study highlights the potential of inorganic matrices like vermiculite in mycelium composite production, offering excellent thermal properties and versatile applications.

Abstract ID: 4128 / Poster Number: 400.17P

Thermophilic bacteria and their potential as biofertilizers under the climate change scenario

Presenting author: Dr. Carolina Yañez, Pontificia Universidad Católica de Valparaíso, Valparaiso, Chile

Co-authors: *Carolina Yáñez, Antonia Naciff, Fabian Cuadros, Mariam Charifeh, Martha Hengst*

Climate change negatively impacts agroindustry and food security. Between 2006 and 2016, rising temperatures led to a 23% loss in agricultural productivity. As a result, the use of chemical fertilizers has intensified, generating environmental and health risks. Biofertilizers based on plant growth-promoting (PGP) microorganisms have been used as an alternative; however, many of these microorganisms do not thrive under current temperature conditions. This study characterizes two thermophilic bacterial strains, Bacillus sp. LB7 and Bacillus sp. NP42, isolated from the Chilean Altiplano, as potential biofertilizers. Their PGP capabilities were evaluated by assessing siderophore production (CAS assay), phosphate solubilization (NBRIP agar), and indole-3-acetic acid synthesis (Salkowski reagent) at different temperatures. Additionally, their effects on seed germination and growth under moderate thermal stress (35°C) were compared with commercial biofertilizers. Results showed that thermophilic isolates produced 33% more siderophores and indole-3-acetic acid than the control PGP bacteria at 30ºC, and at 35°C, this difference increased to 50%. Moreover, at 40°C these isolates maintained their phosphate-solubilizing capacity, whereas the control showed no growth. Germination assays revealed no significant differences in fresh weight between seeds grown at 28°C and those stressed at 35°C when inoculated with the thermophilic bacteria, demonstrating their adaptability. Furthermore, greenhouse trials indicated that plants treated with thermophilic bacteria responded better to thermal stress, suggesting that these microorganisms could provide an effective solution for resilient agriculture under climate change.

Abstract ID: 3178 / Poster Number: 078.15P

Thinking outside the cell: A theoretical approach to understanding leakage and cross-feeding in bacteria

Presenting author: Mr. Abhishek Vaidyanathan, Humanitas University, Milano, Italy; IRCCS Humanitas Research Hospital, Milano, Italy

Co-authors: *Constantinos Xenophontos, Gabriele Micali*

Introduction: Most real microbial communities are complex and have many interacting players. This results in a rich diversity of ecological phenomena, but the fact that they are complex hinders our ability to have a mechanistic understanding of these interactions in different growth conditions. However, significant work has been done over the last two decades to have coarse-grained models for bacterial physiology (specifically, the Proteome Sector model), and we try to leverage this to make a mesoscopic model for ecological interactions.

Objective: This work aims to build a mathematical model that can be used to understand and predict the performance of different bacteria in simple microbial communities. We focus on one specific interaction: amino acid crossfeeding. We model prototrophs (amino acid self-sufficient bacteria) and auxotrophs (bacteria that rely on externally provided amino acids) and simulate how they perform in 2-player communities, where one or both the players have to leak amino acids to survive as a community.

Method: We used a mathematical modelling approach grounded in proteome sector theory to capture the interplay between bacterial physiology and nutrient availability. Monocultures and cocultures were simulated across different nutrient and antibiotic conditions to investigate growth outcomes and cross-feeding efficiencies. We have also tried to assume different models of leakage of amino acids.

Findings: Preliminary simulations show different leakage “regimes” (Fig 1.A), where the details of the regulation of amino acid synthesis influence the leakage in different nutrient conditions. Interestingly, translation-limiting antibiotics always seem to increase the leakage, which hints that antibiotic stress could amplify ecological interactions. On a community level, we can also propose a mechanism by which an Auxotroph-Auxotroph (Fig 1.B.1) system performs better than a Prototroph-Auxotroph (Fig 1.B.2) system through sloppy regulation of amino acid biosynthesis (this discrepancy is observed in some auxotroph-auxotroph systems).

Conclusion: Amino acid cross-feeding in cocultures has many intriguing results, but we don't have a mechanistic understanding of a good number of these phenomena. This work aims to propose a mechanistic model which predicts different leakage regimes in different nutrient conditions, as well as explains the better performance of some auxotroph-auxotroph systems as opposed to their prototroph-auxotroph counterparts.

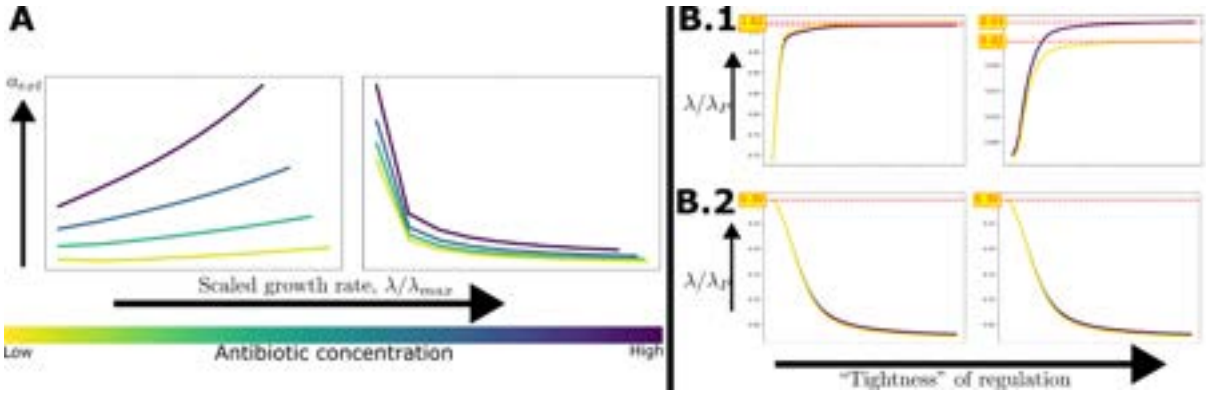


Figure 1: A: *Two possible leakage regimes, depending on the amino acid biosynthesis regulation.* ***B1:*** *A prototroph auxotroph system where the auxotroph (right) performs much worse compared to both the auxotrophs in* ***B2.***

Abstract ID: 2627 / Poster Number: 385.16P

Tissue-Specific Microbiomes in Blueberries: Insights into Microbial Composition, Stability, and the Role of Plant Genetics

Presenting author: Matteo Giese, PhD, Edmund mach foundation, Trento, Italy; Istituto universitario di studi superiori di Pavia, Pavia, Italy

Co-authors: *Claudia Coleine, Claudio Donati, Brian Farneti, Matteo Ajelli, Monica Cattani, Massimo Pindo, Simone Larger, Erika Stefani, Lara Giongo, Manuel Delgado Baquerizo*

Introduction: Fruits such as blueberries are vital for global food production and ecosystem sustainability, serving as essential resources for humans and animals. Plant microbial associated communities influence food quality and play key roles in plant physiology and development. However, the factors shaping blueberry fruit microbiomes and their stability remain poorly understood.

Objective: To investigate the taxonomic composition and biodiversity and factors shaping fungal and bacterial communities in different blueberry tissues.

Methods: Different tissues (leaf, fruit surface, rhizosphere and fruit pulp) of 10 blueberry cultivars were sampled. Microbial communities (fungi and bacteria) that colonize these tissues were analyzed using the metabarcoding technique.

Findings: The study revealed that the fruit pulp, fruit surface, rhizosphere and leaves harbor distinct microbiomes that remain stable over time. Plant genetics emerged as the primary factor influencing microbiome structure, followed by tissue type. A core microbiome was identified for each tissue, with dominant taxa (i.e. *Botrytis* or *Methylobacterium*) contributing significantly to the overall microbial composition. Some genera were shared across tissues, while others were tissue-specific, *Taphrina* for fungi and *Gaiella* for bacteria were found shared in the surface tissues of fruit and leaf. *Myrothecium* and *Micromonospora* were specific to the leaf surface and *Udeniomyces* and *Aridibacter* were specific to the fruit surface.

Conclusions: Blueberries exhibit tissue-specific microbiomes strongly associated with plant cultivar. These findings provide insights into the core microbial taxa of blueberry tissues, offering a foundation for microbiome-based strategies to improve plant health and productivity as global blueberry trade and cultivation expand.

Abstract ID: 3662 / Poster Number: 259.16P

Tn-seq analysis reveals genes encoded by vancomycin-resistant Enterococcus faecium for gut colonization

Presenting author: Gloria Carruana, FISABIO, Valencia, Spain

Co-authors: *Alejandra Flor-Duro, Javier Pons, Vincent De Maat, Willem Van Schaik, Carles Ubeda*

Multidrug-resistant bacteria, such as vancomycin-resistant *Enterococcus faecium* (VRE), are a major problem in hospitalized patients. VRE nosocomial infections usually start by the colonization of the gastrointestinal tract (GIT). However, little is known about the VRE encoded genes that are required for intestinal colonization. This knowledge could be crucial for developing new therapies to prevent colonization and subsequent infections caused by this pathogen. Here, we have generated a high coverage transposon mutant library of a vancomycin-resistant *Enterococcus faecium* (VRE) strain, with representative mutants for 85% of the encoded genes. Using this mutant library, we have performed *in vivo* experiments in mice following an antibiotic treatment model that mimics VRE gut colonization in hospitalized patients. Statistical analysis showed that the disruption of 9,7% of genes significantly and reproducible reduces gut colonization capacity of VRE. These include gene clusters potentially involved in mannose utilization, phosphate transport, galactose metabolism and polysaccharide formation. The effect of some of these genes was confirmed through targeted mutagenesis and *in vitro* and *in vivo* competition assays. These results increase our knowledge on the genetic requirements for gut colonization by VRE, which will facilitate the identification of novel targets for preventing VRE nosocomial infections.

Abstract ID: 2248 / Poster Number: 119.17P

Tn-seq identification of regulatory networks involved in cell wall biogenesis in Escherichia coli

Presenting author: Mrs. Lisa Bal, I2BC, Gif-sur-Yvette, France

Co-authors: *Delphine Patin, Thierry Touzé*

The biosynthesis of most bacterial cell wall polymers, such as peptidoglycan, relies on the lipid carrier undecaprenyl phosphate (C_{55}-P), for the translocation of their subunit across the plasma membrane. After the transfer of a subunit to a nascent polymer, the lipid is released as undecaprenyl pyrophosphate (C_{55}-PP) or C_{55}-P that must be recycled. As an essential process, it is of a great interest for the development of innovative antibacterial therapeutics. C_{55}-PP recycling therefore requires a dephosphorylation and the flip of lipid back to the inner side of the membrane. In most bacterial cells, both steps rely on apparent redundant proteins raising the question of the relevance of this plurality of proteins for a single function.

Our objective was to unveil the interplay and regulatory networks between multiple recycling proteins together with other proteins involved in cell envelope biogenesis or any other pathway in the bacterial model *Escherichia coli*. However, due to the redundancy of proteins, the discovery of such networks is intricate since none of the recycling protein, taken individually, is required to sustain growth or fitness. Then, we are applying a Tn-Seq approach on different mutant strains expressing a limited set of recycling proteins. The Tn-Seq allows us to identify genes, whose inactivation impacts the growth of the mutant strain as compared to the wild-type strain. Numerous genes within the peptidoglycan biosynthetic pathway or respiration were then highlighted and their link with C_{55}-P recycling was further investigated.

Abstract ID: 1052 / Poster Number: 402.17P

Towards a circular economy through microbial stability and in situ cultivation in a photobioreactor – chicken coop system

Presenting author: Ms. Marta Velaz Martín, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

Co-authors: *Laura Meisch, Anne-Kristin Kaster, Kersten S. Rabe, Christof M. Niemeyer*

Finding sustainable systems that minimize waste and maximize resource efficiency especially in agriculture is a major challenge for circular economy. In this context, biological systems such as photobioreactors offer an attractive way to convert under-utilized nutrient streams such as CO_2 into useful biomass products, such as photosynthetic microorganisms (PMOs) like *Spirulina* (Cyanobacteriota).

In this context, we investigated microbial interactions and community stability in an integrated photobioreactor-chicken coop system, where waste and nutrients were retained, to enable closed loop operations.

For this, samples taken from the photobioreactor at different timepoints were analyzed by metagenomics to evaluate the taxonomic composition and metabolic potential. First analysis demonstrated the impact of chicken house exhaust on the cyanobacterial culture via CO_2 fixation and provided insights into NH_3 metabolism. Taxonomic classification of the microbial community suspended in the photobioreactor revealed a predominance of bacterial organisms before aeration, with a notable shift to a more diverse community, including eukaryotic species, after exposure to chicken coop exhaust.

In perspective we integrated a novel macroporous material into the system to allow direct monitoring of microbial dynamics. This will provide further valuable insights into microbial interactions in this particular context and allow us to directly assess community stability. Understanding community stability is essential to run integrated, closed-loop biological production systems. It illustrates how microbiome research can contribute to circular economy and serves as a precursor for possible integration into biological systems for resource efficient and sustainable solutions.

Abstract ID: 4146

Towards biocomputing with synthetic oscillators

Presenting author: Yolanda Schaerli, Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland

Co-authors: *Jung Hun Park, Gàbor Holló, Hadiastri Kusumawardhani, Javier Santos-Moreno*

Oscillatory circuits exhibit periodic fluctuations in gene expression and are pervasive in living organisms, with examples in cell cycles, circadian rhythms, metabolic activities, brainwaves, and pattern formation during multicellular development.

The repressilator, a genetic circuit of three genes repressing each other to create oscillatory behaviour, was one of synthetic biology's earliest breakthroughs, showcasing the ability to engineer complex gene regulatory networks. Diverse applications of synthetic oscillators have already been proposed, ranging from fundamental research, bioproduction, biological computing and therapeutic applications to biological timers.

In this talk, I will present the "Optoscillator", an optogenetic implementation of the classic repressilator circuit. In growing bacterial colonies carrying this synthetic oscillator, temporal gene expression oscillations are translated into striking spatial ring patterns. We systematically analysed these patterns under various regimes of light exposure.

By integrating experimental data with mathematical modelling, we uncovered that this simple genetic circuit can display surprisingly rich dynamical behaviour when subjected to external periodic forcing, including synchronization, resonance, period doubling, period-4 dynamics, and even chaos.

I will also introduce our work on a CRISPR-based variant of the repressilator, the "CRISPRlator". I will conclude by highlighting our ongoing efforts to explore coupled synthetic oscillators for biocomputing applications and expand these systems into multicellular consortia.

Abstract ID: 2967 / Poster Number: 396.16P

Towards recombinant production of thiopeptides using Corynebacterium glutamicum

Presenting author: Mrs. Lara Zinnecker, Microbial Biotechnology, Department of Biology, Ulm University, Ulm, Germany

Co-authors: *Christian Riedel*

In light of the increasing problems with nosocomial infections by antibiotic-resistant pathogens such as *Staphylococcus aureus*, alternative or supplementary treatment options are urgently needed. Thiopeptides are a subclass of class I bacteriocins with antimicrobial activity against several Gram-positive bacteria. The recently discovered thiopeptide cutimycin was shown to be active against (antibiotic-resistant) *S. aureus* strains but is only produced by its natural producer *Cutibacterium acnes* at very low titers.

Since cutimycin production by *C. acnes* is limited and requires a long cultivation period, recombinant production of cutimycin might facilitate further research on the mechanisms and may be useful for biotechnological production. In recent years the biotechnological host *Corynebacterium glutamicum* was successfully established as producer for various bacteriocins. Preliminary experiments using supernatant from the natural producer showed that cutimycin does not inhibit growth of *C. glutamicum* making this biotechnological host a candidate for recombinant cutimycin production.

The genes for the thiopeptide itself as well as the enzymes for post-translational modifications are encoded in a 9.5 kb biosynthetic gene cluster (BGC) but genes for a dedicated transporter are missing. Here, we report on the construction of a recombinant *C. glutamicum* strain for production of cutimycin using a two-plasmid system.

Abstract ID: 2013 / Poster Number: 106.17P

Towards systematic antimicrobial resistance gene profiling in host-associated and environmental microbial communities

Presenting author: Dr. Marija Dmitrijeva, Institute of Microbiology, ETH Zurich, Zurich, Switzerland; Swiss Institute of Bioinformatics, ETH Zurich, Zurich, Switzerland

Co-authors: *Hans-Joachim Ruscheweyh, Andrew Abi Younes, Wolf-Dietrich Hardt, Daniel Mende, Georg Zeller, Shinichi Sunagawa*

Antimicrobial resistance (AMR) is a growing global health challenge. Routine monitoring of AMR distribution is predominantly based on phenotypic assessment in disease-causing pathogen culture or PCR-based analysis of known AMR genes within hospital or community healthcare settings. However, balancing and optimizing human, animal, and environmental health poses a need for methods that can evaluate AMR distribution in a species- and environment-agnostic manner. One such approach providing insights into AMR distribution across a wide diversity of microorganisms and across environments that include known reservoirs of AMR gene pools, such as wastewater and livestock farms, is high-throughput sequencing of microbial community DNA (*i.e.*, metagenomics). Unfortunately, a lack of standardized methodologies for the identification and quantification of antimicrobial resistance genes from metagenomics data currently impede cross-study comparisons, complicating systematic surveillance efforts and design of effective mitigation strategies.

Here, we present a metagenomic workflow that enables systematic, scalable AMR analyses across species and environments. By using the taxonomic profiling tool mOTUs in combination with a state-of-the-art ARG reference collection, we provide biologically meaningful estimates of mean AMR gene copy numbers per cell (*i.e.,* AMR density). After running the workflow on a systematically processed collection of 117K metagenomic samples from >50 distinct environments, we assessed cross-environmental and country-specific trends in AMR densities. The adoption of our method by researchers, clinicians, as well as policy makers could provide actionable insights that enhance our ability to track and respond to the AMR crisis.

Abstract ID: 2156 / Poster Number: 227.17F

Toxin/Antitoxin-system Transcriptomics in Colistin-Susceptible Carbapenem-Resistant Acinetobacter baumannii developing Persistence

Presenting author: Eleonora Chines, University of Pavia, Pavia, Italy

Co-authors: *Maria Lina Mezzatesta, Viviana Cafiso*

Carbapenem-Resistant *Acinetobacter baumannii* (CRAB) can respond to high antimicrobial-exposure entry into dormancy likely mediated by Toxin/Antitoxin (TA)-systems. TAs are stable toxins and unstable antitoxins previously implicated in persistence, tolerance, cellular-growth, and metabolism. Under stresses, changes in transcription and its rate result in antitoxin's degradation or inhibition, leading the toxin to trigger persisters.

We aimed to investigate the transcription of 8 shared ACICU-RefGenome-homologous TA-systems (AGP_acyltrn/panA, doc/NpoA, GNAT/relB, HicA/B, GNAT/HTH_31, Acetyltransf_4/HTH_24, SRPBCC_CalC_Aha1-like/vapB, cptA/B) in 2 clinical ST-2 colistin-susceptible CRAB having a dormant-state under high colistin-exposure.

RNA-seq was performed by Illumina-Short-Insert Library sequencing, transcriptome by Rockhopper tool, and functional analysis by KEGG, DAVID, and PANTHER in 2 ST-2 COL-S CRAB.

Findings: Transcripts were found in 4 TA-systems with expression only in toxin or antitoxin. Notably, **GNAT**/RelB toxin was an unusual histone acetyltransferase, that epigenetically acting as a virulence-factor, can mediate host-pathogen interaction, whilst **AGP_acyltrn**/PanA 1-acyl-sn-glycerol-3-phosphate acyltransferase toxin, catalyzing the acylation of lysophosphatidic acid, involved in membrane glycerophospholipid synthesis, can affect cell-growth. Antitoxin transcription was recovered in Doc/**NpoA,** a short-chain dehydrogenase responsible for the inhibition of the toxin-related phosphorylation of the Translation Elongation Factor EF-Tu, and SRPBCC_CalC_Aha1-like/**VapB** preventing RNA cleavage and translation, previously known to be involved in persistence. No TAs transcription was detected in hicA/B and cptA/B. small-antisense RNAs were found as Toxin-antisense in GNAT/RelB and HicA/B, along as Antitoxin-antisense in AHA_1/VapB.

Our findings revealed that 2 ST-2 COL-S CRAB are characterized by the transcription of TA-systems related in cellular-growth and virulence, putatively enhancing a prone population to develop persisters under stress condition.

Abstract ID: 4116 / Poster Number: 331.15P

Trace metals availability controls terminal electron acceptor utilization in *Escherichia coli*

Presenting author: Dr. Benoit De Pins, University of Naples Federico II, Naples, Italy

Co-authors: *Annarita Ricciardell, Jacopo Brusca, Monica Correggia, Luciano Di Iorio, Martina Cascone, Marco Giardina, Stefany Castaldi, Rachele Isticato, Roberta Iacono, Marco Moracci, Nunzia Nappi, Antonino Pollio, Costantino Vetriani, Serena Leone, Angelina Cordone, Donato Giovannelli*

Trace metals are essential to all living organisms, contributing to key physiological and ecological processes such as respiration, photosynthesis, and carbon and nitrogen fixation. Despite their fundamental role, the extent to which trace metal availability controls functional diversity and metabolic shifts remains unclear. Our work investigates how trace metal availability influences electron acceptor utilization in *Escherichia coli* and its broader implications for microbial evolution and competition.

Objective: To investigate how trace metal availability affects growth, electron acceptor utilization, and metabolic pathway usage in *E. coli*. (as modeled in Fig. 1).

Method: We assessed the physiological and proteomic responses of *E. coli* under metal-depleted conditions. We compared growth rates, metabolic shifts, and expression of metal homeostasis. We also supplemented metal-depleted cultures with specific trace metals, including molybdenum and iron, to observe the restoration of metabolic functions. Additionally, metagenome-assembled genomes (MAGs) from diverse ecosystems were analyzed to assess the potential prevalence of these metal-dependent metabolic shifts.

Findings: Trace metal depletion resulted in significantly reduced growth, premature fermentation onset, and increased energy expenditure for metal homeostasis, even when energetically favorable electron acceptors were available. Proteomic data revealed shifts in metabolic pathways, highlighting adaptive responses to trace metal scarcity. Similar results were observed in the Gram-positive *Bacillus subtilis* and the green algae *Stichococcus bacillaris*. MAGs analysis revealed many organisms encoding for multiple terminal oxidoreductases that rely on different metal cofactors, likely suggesting a widespread occurrence of this control across the microbial world.

Our findings challenge the traditional view that microbial metabolism is primarily shaped by substrate availability, highlighting instead the crucial role of micronutrient constraints.

Given the widespread reliance of biogeochemical oxidoreductases on metal cofactors, we propose that trace metal availability has played a pivotal role in shaping microbial evolution and ecosystem function throughout Earth's history.

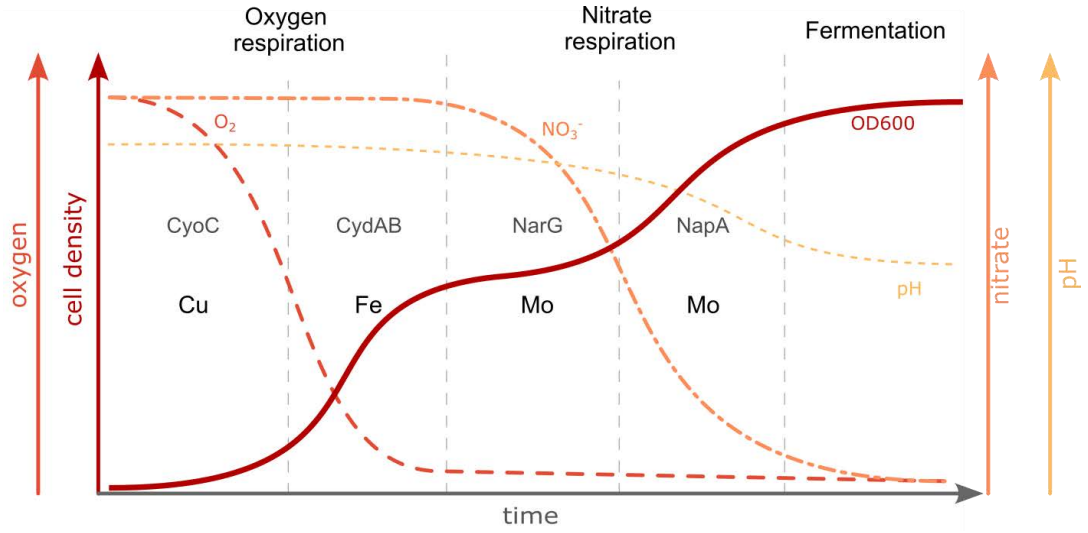


Figure 1: *Expected model of E. coli growth under oxygen and nitrate limiting conditions in the presence of the appropriate trace metals. A diauxic growth during the switch from aerobic respiration to denitrification is expected. Altering the availability of Cu, Fe and Mo will change the timing and type of metabolism utilized by the organism.*

Abstract ID: 2189 / Poster Number: 117.17P

Tracing the spread of AMR genes through Bacterial Membrane Vesicles

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Co-authors: *Melissa Berni, Andrea Zendrini, Sara Angelone, Chiara Bracchi, Laura Mazzera, Martina Tambassi, Marina Morganti, Luca Bolzoni, Erika Scaltriti, Stefano Pongolini, Ilaria Menozzi*

Introduction: *Klebsiella pneumoniae* (*Kp*) can produce Membrane Vesicles (MVs), also known for their role in horizontal transfer of Antimicrobial Resistance (AMR) genes between different bacteria species. *Kp* strains of our collection harboured threatening AMR genes (such as *blaOXA-181*) on plasmids that could be transfer to other Enterobacteriaceae species by MVs. Few sequencing data of MV-associated DNA are available and the presence of AMR plasmids with different copy-number makes bioinformatic analyses really challenging.

Objective: Development of a sequencing and data analysis protocol to analyse MV-associated DNA with the final aim to understand if *Kp* plasmids carrying interesting AMR genes could be transmitted through MVs.

Method: MVs isolated from *Kp* were treated obtaining unbroken and lysed MVs. Free DNA was removed from both samples with a DNase treatment. Remaining DNA was sequenced through Illumina platform and high-quality reads were mapped against reference genomes. Coverages of genomic and plasmid DNA of the two conditions were compared.

Findings: Analyses of high coverage regions obtained from sequencing data of unbroken and lysed MVs revealed differences in plasmids amount between the two conditions. Four of the six plasmids of our *Kp*104 tester strain resulted 1000-fold underrepresented in lysed MVs suggesting that these plasmids were included in MVs before the lysis. Following the same protocol, analysis of Kp strains with plasmids carrying *blaOXA-181* gene are ongoing.

Conclusion: We successfully identified the presence of plasmids in MV-associate DNA. Application of this protocol to other strains for tracing the spread of AMR genes through MVs is ongoing.

Abstract ID: 3927 / Poster Number: 198.17P

Tracking Multidrug-Resistant Escherichia coli in Bursitic Broilers: A One Health Perspective

Presenting author: Dr. Vanessa Silva, Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), Lisbon, Portugal

Co-authors: *Jessica Ribeiro, Pedro Pinto, Madalena Vieira-Pinto, Gilberto Igrejas, Sandrina A. Heleno, Filipa S. Reis, Patrícia Poeta*

Introduction: Sternal bursitis is an inflammatory condition in poultry that poses a significant challenge to animal and public health, particularly within the One Health framework, due to its impact on poultry welfare and the potential for zoonotic transmission.

Objectives: The aim of this study was to explore the prevalence of *Escherichia coli* in bursitic broilers, identify its antimicrobial resistance profiles, and assess its genetic characteristics, including resistance genes, integrases, virulence factors, and phylogenetic groups.

Methods: Forty pus samples from bursitic broilers at a Portuguese slaughterhouse were cultured on Chromocult® Coliform agar, with selective plating using cefotaxime (2 µg/mL). Antimicrobial susceptibility (CLSI, 2024) was assessed via disk diffusion (20 antibiotics). PCR identified resistance genes, integrases, virulence factors, and phylogenetic groups (A, B1, B2, D).

Findings: E. coli was isolated from 36 samples and one strain was resistant to cefotaxime. Five isolates were multi-resistant. Resistance profiles showed a predominance of resistance to ampicillin and tetracycline, and several resistance genes were identified. The most detected virulence factors were fimA (97%) and aer (47%). Integrons 1 and 2 were identified in three isolates and the predominant phylogroup was B1.

Conclusion: These findings highlight the need for surveillance and targeted interventions in poultry production. The detection of multidrug-resistant and virulent *E. coli* strains emphasizes the need for antimicrobial stewardship and biosecurity. Given the One Health implications, further studies are needed to explore zoonotic risks and bacterial dissemination pathways.

Abstract ID: 3416 / Poster Number: 280.15P

Tracking of Hexachlorocyclohexane (HCH) degrader Sphingobium indicum B90A in soil and engineering of Bacillus subtilis for HCH degradation

Presenting author: Prof. Charu Dogra Rawat, Ramjas College, University of Delhi, Delhi, India

The organochlorine pesticide, Hexacholrocyclohexane (HCH), is a global pollutant. Remediation field trial using Sphingobium indicum B90A (a potent HCH degrader) was conducted at Ummari village, Uttar Pradesh, India to decontaminate HCH dumpsite soils. Tracking B90A in soil was challenging due to other native sphingomonads with similar biochemical properties. An in-silico tracking strategy to identify unique marker genes of B90A is devised and evaluated. Whole-genome sequences of 120 species (Sphingobium, Novosphingobium, Sphingomonas, Sphingopyxis, and Sphingosinicella) were analysed. 45 genes unique to B90A were identified using bioinformatic tools. PCR amplification of a unique gene produced an 801bp amplicon in B90A-augmented test soil microcosms - in HCH-contaminated dumpsite soil microcosm B90A was tracked till day 6 post-inoculation. In field trial soil, B90A could be tracked till day 4 post-inoculation. The devised monitoring method, BioMarkTrack, can be employed for tracking bioinoculants during field trials. Another challenge faced during remediation was B90A survival, attributed to its slow growth, predation, or HCH availability/toxicity. Bacillus subtilis is a good model for genetic engineering. Genetically modified B. subtilis strains secreting HCH catabolic proteins (LinA/LinB) were produced. Bas-LinA degraded 96% of α-HCH and 76% of γ-HCH in 42 h in liquid medium (5ppm of each HCH isomer). Bas-LinB degraded 98% of the 5ppm β-HCH in 30h. Degradation of HCH to 52% (α-& γ-HCH) and 55% (β-HCH) in Bas-LinA and Bas-LinB augmented (@10ppm) HCH contaminated soil, respectively, were observed in 30 days. The results reveal potential of genetically modified B. subtilis strains for decontamination of HCH-polluted sites.

Abstract ID: 507 / Poster Number: 065.17P

Tracking the spread of multi-drug resistance vibrio species in coastal lake affected by climate change in Mediterranean, Italy

Presenting author: Ifra Ferheen, PhD, National Institute of Health, Rome, Rome, Italy

Co-authors: *Stefania Marcheggianii*

There is strong evidence from the literature that global warming is driving the emergence and spread of seafood-borne infectious diseases, including vibriosis. Tracking the rise of Vibrio-related illnesses in Europe due to climate change is crucial, along with their detection in aquatic ecosystems. Lake Paola is a coastal lake located in the Mediterranean region of Italy, which is under strong pressure due to climatic changes high temperatures and saline cone as well impacts from anthropogenic activities. This study was performed in the framework of EU-Blueadapt project, focusing on the detection of Vibrio species in Paola Lake during the bathing water season of 2023. Artificial plastic substrates were used as trap for colonization of Vibrio species and *Vibrio parahaemolyticus* (n=18), *Vibrio cholerae* (n=14), *Vibrio vulnificus* (n=16) were isolated and characterized for phenotypic resistant profiling, while 18 strains were selected for molecular identification and genotypic resistance profiling. Results indicate higher prevalence of different Vibrio species in hot season which positively correlates with temperature shifts and anthropogenic activities in summer around the lake. Moreover, these strains also demonstrate significant resistance to antibiotics belonging to different classes, including tetracyclines, cephalosporins, sulfonamides, and aminoglycosides. While genotypic resistance profiling reveals the positive detection of genes like *sul1*, *tetA*, *tetB*, *blaCTX-M*, and *cmlA1*, and two genes belonging to class 1 integrons. In nutshell, this study emphasizes the significant impact of climate-driven environmental changes on *Vibrio* spp. prevalence in aquatic ecosystem, highlighting the health risks posed by antibiotic-resistant strains in response to rising temperatures and fluctuating salinity levels.

Abstract ID: 3650 / Poster Number: 397.17P

Transcriptional adaptation of Listeria monocytogenes during growth in milk

Presenting author: Dr. Konstantinos Papadimitriou, Agricultural University of Athens, Athens, Greece

Co-authors: *Maria Gkerekou, Aleksandra Slavko, Konstantinos Valourdos, Eleftherios Drosinos, Marina Papadelli, John Kapolos, Panagiotis Skandamis*

Introduction: *Listeria monocytogenes* is a common foodborne pathogen causing outbreaks of listeriosis every year around the globe. It is a ubiquitous microorganism that can survive in many different foods, and its psychrotrophic nature allows it to grow even under refrigeration. Some of the most common contamination sources include milk and dairy products.

Objective: This study was performed to assess the molecular mechanisms underlying the ability of *L. monocytogenes* to grow in milk.

Method: The *L. monocytogenes* strain EGD-e was employed in RNA-seq experiments using Illumina sequencing technology. Subsequently, sequencing data was analyzed with appropriate bioinformatics tools to determine alterations in the transcriptional profile of the bacterium under the conditions tested.

Findings: Strain EGD-e was grown in synthetic medium at 30°C and in milk at 30°C or 7°C. RNA-seq experiments were performed with log-phase cells. Several hundred genes were found to be upregulated or downregulated during growth in milk at both temperatures compared to the control. Interestingly, a specific set of upregulated genes was common to the samples from the milk environment, independent of the temperature. Additionally, a distinct set of upregulated genes was identified as specific to 7°C when compared to samples from the synthetic medium or milk at 30°C. Functional analysis of these genes revealed a plethora of metabolic processes being activated, indicating a generalized adaptation process.

Conclusion: Our findings shed light on the molecular physiology of *L. monocytogenes* during growth in milk.

Abstract ID: 2656 / Poster Number: 322.15P

Transcriptional regulation of Vibrio cholerae hemagglutinin/protease HapA by TyrR

Presenting author: Dr. Vera Fengler, University of Graz, Graz, Austria

Co-authors: *Martina Woelflingseder, Joachim Reidl, Günther Koraimann, Amar Cosić*

Vibrio cholerae, the causative agent of the disease cholerae, causes fatal diarrhoea and mortality. It is a facultative pathogen and transits between two dissimilar habitats, the aquatic ecosystems and the human host. In the latter case, it colonizes epithelial cells in the small intestine. To successfully colonize the human host, *V. cholerae* needs to penetrate the intestinal mucus barrier to reach the epithelium, while at late stages of infection, it must detach from the intestinal mucus to re-enter aquatic ecosystems. One of the factors involved in both processes is the type II secreted metalloprotease hemagglutinin/protease (HA/protease), which is encoded by *hapA*. The expression of *hapA* is known to be induced at high cell density by the quorum sensing master-regulator HapR. Additionally, its expression is also dependent on the alternative sigma factor RpoS, which is crucial under starvation conditions. HapA is the major secreted protease of *V. cholerae* and is responsible for about 80-90% of the extracellular proteolytic activity [1, 2, 3].

We found that a *tyrR* deletion mutant exhibits almost no extracellular protease activity, comparable to the phenotype of a *hapA* deletion mutant. In Figure 1 a M9 skim milk agar plate is depicted, showing that the wild type *V. cholerae* strain CO986 produces a prominent halo resulting from proteolytic degradation of the skim milk, whereas a *tyrR* deletion mutant exhibits only a slight halo around the colonies. *hapR* and hence also *rpoS* deletion mutants do not form a halo at all. Based on our recent findings, evidenced by reporter gene fusions, we propose the need for another transcriptional activator: *V. cholerae*'s transcriptional regulator TyrR induces *hapA* expression, although both *hapR* and *rpoS* expression are unaffected. Furthermore, we show that, similar to *Escherichia coli* [4], TyrR is activated by the presence of aromatic amino acids, which increases its transcriptional regulatory activity. RNAseq studies revealed that while the expression of *hapA* appears to be activated by TyrR, another group of genes is repressed by the action of TyrR in a manner dependent on aromatic amino acids. These include a tyrosine-specific transport protein and a methyl-accepting chemotaxis protein.

In this respect, TyrR of *V. cholerae* regulates genes involved in the uptake and metabolism of aromatic amino acids, as known from *E. coli* [4], but additionally also genes necessary

for intestinal colonization and detachment as well as for chemotaxis. Physiological relevance of TyrR in *V. cholerae* appears to aim (i) at successful colonization of the human host by initial degradation of the intestinal mucus in order to reach the epithelium and (ii) at activation of mucosal detachment at late stages of infection. This indicates that TyrR may assist *V. cholerae* in environmental transitions, persistence in adverse environments, and escape from unfavourable niches.

References: [1] Häse et al., J Bacteriol., 1991, 173(11), 3311-7; [2] Silva et al., J Bacteriol., 2004, 186(19), 6374-82; [3] Wang et al., J Bacteriol., 2011, 193(23), 6529-38; [4] Pittard et al., Mol Micro., 2005, 55(1), 16–26.

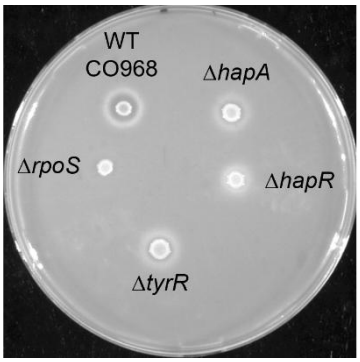


Figure 1: *Secreted protease activity on skim milk agar plates (M9 0.2% maltose 1% skim milk; 48 h at 37 °C) of CO968 wildtype, ΔhapA, ΔrpoS, ΔhapR, and ΔtyrR.*

Abstract ID: 1402 / Poster Number: 201.15P

Transcriptome analysis of changes in Microcystis aeruginosa growth under sericite treatment

Presenting author: Dr. So-Jeong Kim, Korea Institute of Geoscience and Mineral Resources, Daejeon, South Korea

Co-authors: *Hyeonjung Yu, Jungho Ryu, Dong-Wan Cho, In-Hyun Nam*

Microcystis aeruginosa (MA), a bloom-forming toxic cyanobacterium, produces secondary metabolites harmful to humans. While numerous studies have explored methods to control MA growth, the molecular mechanisms by which natural minerals influence its microbial activity remain underexplored. This study evaluated the potential of various minerals to inhibit harmful algal growth. Among five tested minerals, sericite demonstrated the most effective inhibition of Microcystis growth. As the concentration of sericite increased, photosynthetic activity gradually decreased. To uncover the inhibitory mechanisms, transcriptome analysis identified 88 differentially expressed genes. These genes were predominantly associated with inorganic ion transport, metabolism, and energy production, all up-regulated in the presence of sericite, indicating heightened cellular stress. The stress likely disrupts homeostasis and energy production, leading to growth inhibition. This study is the first to investigate the growth-inhibiting effects of sericite on Microcystis at the molecular level, suggesting that natural minerals like sericite could be viable options for managing harmful algal blooms in aquatic environments.

Abstract ID: 3067 / Poster Number: 388.17P

Transforming Carbon Dioxide Emissions into High-Value Pharmaceuticals: A Further Step Towards Sustainable Biotechnology

Presenting author: Dr. Sara Cantera, University of Valladolid, Valladolid, Spain

Co-authors: *Elisa Huang-Lin, Raquel Lebrero,*

The pharmaceutical industry faces growing demands to adopt sustainable practices that minimize environmental impact. This study presents an innovative bioproduction platform that transforms carbon dioxide, using renewable energy sources such as hydrogen or industrial by-products like thiosulfate into high-value pharmaceuticals, with a particular focus on ectoines. Ectoines, high-valued for their significant economic and societal benefits, are naturally produced by prokaryotes under high-salinity conditions and serve as essential ingredients in pharmaceuticals and cosmetics. Through a comprehensive genome mining analysis, 31 chemolithotrophic bacterial strains with the genetic capability for ectoine biosynthesis were identified. Among these, laboratory validation showed that *Hydrogenovibrio marinus* and *Guyparkeria halophila* were the most efficient strains, exhibiting the shortest doubling times and highest ectoine productivities when cultivated at optimum conditions. Using hydrogen as energy source, *H. marinus* achieved ectoine contents of 13.4% of the total biomass dry weight. Meanwhile, *G. halophila*, using thiosulfate, reached maximum ectoine contents of 40%, marking the highest levels reported for both autotrophs and most heterotrophs to date. Finally, continuous bioreactor optimization further enhanced ectoine productivities and biomass resilience, demonstrating the platform's scalability and robustness. These advancements not only show the first proof of utilizing underexplored bacteria for CO_2 valorization, but also establish a promising foundation for converting waste carbon emissions into high-value pharmaceuticals, paving the way for a greener and more sustainable pharmaceuticals manufacturing.

Abstract ID: 3311

Trans-kingdom conservation of structure and mechanism in defensin-like peptides

Presenting author: Dr. Ivan Sugrue, APC Microbiome Ireland, University College Cork, Cork, Ireland

Co-authors: *Carolin Ade, Paula M. O'Connor, Jan-Martin Daniel, Nathaniel Martin, Paolo Innocenti, Günther Weindl, Nico Kirsch, Colin Hill, Tanja Schneider, R. Paul Ross*

Introduction: Antimicrobial peptides are a ubiquitous host defence mechanism found across the super-kingdoms of life, eukarya, prokarya, and archaea, and represent valuable tools for developing novel antimicrobial therapies. One such peptide, the bacteriocin actifensin, is produced by *Actinomyces ruminicola* DPC7226 and exhibits potent activity against a broad range of gram-positive pathogens, including vancomycin-resistant *Enterococcus* (VRE) and *Clostridioides difficile*. Notably, actifensin shares structural similarities with the highly conserved cysteine-stabilized αβ-defensins produced by fungi, insects, and molluscs. This trans-kingdom conservation of structure and function could suggest a potential bacterial origin for eukaryotic defensins.

Objectives: The aim of this study was to advance the therapeutic potential of actifensin by conducting an in-depth biochemical characterization to elucidate its mechanism of action at the bacterial cell envelope and investigate its functional conservation with eukaryotic defensins. Additionally, we sought to assess therapeutic applicability of actifensin by evaluating its immunogenic, haemolytic, and cytotoxic effects. Lastly, we examined the structural conservation of actifensin with defensins from both closely and distantly related bacterial and eukaryotic species to explore the evolutionary origins of eukaryotic defensins.

Methods: The antimicrobial activity of actifensin was evaluated by determining its minimum inhibitory concentrations (MICs) against a panel of human pathogens. Mechanistic studies included β-galactosidase-based *Bacillus subtilis* bioreporter stress-response assays to identify cellular targets, followed by peptide-lipid complexation assays with peptidoglycan precursor components analysed using thin-layer chromatography. Complexation findings were validated with *Bacillus subtilis lia-lux* cell wall stress antagonization assays, and isothermal titration calorimetry experiments were performed to determine lipid binding constants using lipid vesicles. Membrane disruption was analysed via DiSC3(5) fluorescence, carboxyfluorescein-liposome assays, and the delocalization of GFP-tagged MinD, a polar protein responsive to membrane depolarization. Cell wall integrity, biofilm inhibition, and cytotoxicity were

assessed using phase-contrast microscopy, biofilm inhibition assays, and alamarBlue viability testing on Hep G2 mammalian cells, respectively.

Findings: Actifensin shares a mechanism of action with eukaryotic defensins, such as the fungal defensin plectasin, in that it inhibits bacterial cell wall synthesis through direct binding to lipid II, including the lipid II-D-lac variant responsible for vancomycin resistance in VRE. However, unlike plectasin and other defensins, actifensin binds lipid I with the same affinity and does not require UDP-GlcNAc for lipid II binding. Actifensin does not depolarize bacterial membranes directly; its antimicrobial activity instead results from disrupting peptidoglycan biosynthesis, weakening the cell wall and causing eventual cell death. Importantly, actifensin displayed low cytotoxicity and immunogenicity. Sequence-structural analysis revealed that actifensin belongs to a conserved trans-kingdom subfamily of CSαβ defensins, which we term GXGCP, conserved across bacteria, fungi, and ancient invertebrates. This subfamily may be ancestral to a separate subgroup, termed XTCD, found in more recently evolved insects.

Conclusion: The potent activity of actifensin against antimicrobial-resistant pathogens, combined with its low toxicity, highlights its promise as a candidate for therapeutic development. Furthermore, this study highlights the unexamined diversity of CSαβ defensins, including actifensin, and their structural and functional adaptations across kingdoms. These findings not only provide insights into the evolutionary history of antimicrobial peptides but also highlights opportunities for the development of novel antimicrobial strategies.

Abstract ID: 3732 / Poster Number: 303.16S

Transporter-minimal strains as platforms for optimization: rewiring transport for better bioconversions in E. coli

Presenting author: Mr. Liam Richard Jenkins Sánchez, Ghent University, Ghent, Belgium

Co-authors: *Inge Van Bogaert*

Introduction: Many things have to go right for cells factories to be truly effective. A crucial one is that feedstocks are quickly imported into the cell, and that the product of interest can be exported at high rates. Fortunately, this process can be engineered to bring cell factories to their optimal performance.

Metabolites almost never cross the membrane by passive, uncontrolled diffusion. Rather, an intricate collection of transporter proteins mediate these translocations, with embedded proteins forming 60% of the cell membrane. In *Escherichia coli*, the transportome is rich and highly redundant, with many transporters with overlapping affinities for most substrates. This is very convenient for the cell in its nutrient rich environment, but complicates transport optimization, uses huge amounts of energy, and can result in leaking valuable intermediates to the media, including core chemicals like glycerol.

Additionally, this makes it hard to study transport as a process, moreover due to a lack of reliable platforms that simulate *in vivo* conditions. There is a need for tools that enable us to select better transporters, while at the same time serving as starting points for tailored fermentations.

Objectives: We constructed a transporter minimal strain with minimal permeability to polyols, many of which are of high industrial or physiological interest. This platform is also used to individually screen transport proteins with improved characteristics in a high fidelity environment.

Method: CRISPR-Cas9 genetic engineering with recyclable markers was used to perform over 16 cumulative deletions in a single *E. coli* strain. This strain was tested for polyol permeability using a combination of growth assays in minimal media and transcriptional biosensor circuits for non-assimilable compounds, such as propanediols and sweeteners. Furthermore, various efficient transporter channels were tested in this strain, characterizing their function.

Findings: a transporter minimal strain lacking over 16 polyol transporters was constructed and showed minimal growth defects. Background permeability to a number

of linear polyols was established, and the involvement of efficient facilitators in polyol transport was confirmed.

Conclusion: bacterial membrane permeability is low even for small non-charged molecules. This work helps establish a platform for screening improved transporters with optimal characteristics for cell factories.

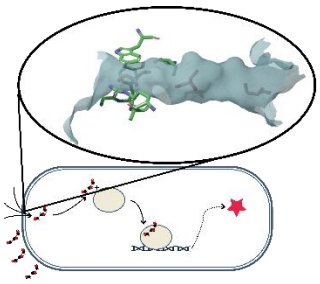


Figure 1: conceptual introduction of an improved transporter for polyols in *Escherichia coli*

Abstract ID: 1244 / Poster Number: 236.17F

Trimethoprim-resistant Salmonella Typhimurium strains isolated by adaptive evolution of reveal cross-protection to heat treatments

Presenting author: Mr. Ivo García-Penas, Instituto Agroalimentario de Aragón-IA2, Universidad de Zaragoza-CITA, Zaragoza, Spain

Co-authors: *Carlota Lahuerta, Raúl Campillo, Daniel Berdejo, Rafael Pagán, Diego García-Gonzalo*

Introduction: Antimicrobial resistance (AMR) is a major public health challenge, with *Salmonella* spp., a key foodborne pathogen, showing resistance to antibiotics like trimethoprim (TMP) used in human and veterinary medicine. The effects of AMR on the efficacy of food preservation methods, such as heat treatments, remain unclear.

Objective: The aim of this study was a) to isolate resistant variants (RVs) of *Salmonella enterica* Typhimurium LT2 (SeT) against TMP, b) to identify genetic determinants of resistance and c) to evaluate their cross-protection against heat treatments (HT).

Method: Adaptive evolution assays (ALE) were conducted at increasing concentrations of TMP, increasing 1.85-fold in concentration daily for 10 days. To assess the acquired resistance, broth microdilution was employed to determine the minimum inhibitory concentrations (MICs) against TMP for each RV. RVs were exposed to lethal heat treatments (54 ºC, 30 min) and compared with SeT. Whole genome sequencing (WGS) was conducted to identify mutations involved in the AMR of the RVs.

Findings: Five resistant variants (RVs) of *Salmonella* Typhimurium evolved with trimethoprim (TMP) showed MICs 8–16 times higher (32–64 µg/mL) than SeT, (4 µg/mL). While SeT showed around 5 log cycles of inactivation under heat treatment, three RVs showed a decrease in their inactivations of around 1 log cycle. WGS identified mutations in AMR-related genes, notably *ygdP*, linked to alarmone cleavage and possibly to heat tolerance.

Conclusions: AMR bacteria, such as TMP-resistant *Salmonella* Typhimurium LT2, can compromise the effectiveness of food preservation treatments, raising concerns about their impact on food safety.

Abstract ID: 359 / Poster Number: 085.15P

Tuning Biofilm Mechanics in Pantoea sp. YR343: Unlocking the Secrets of Soil-Friendly Bacteria

Presenting author: Prof. Jinju Chen, Loughborough University, Loughborough, United Kingdom

Co-authors: *Sam Charlton, Saikat Jana*

Introduction: Pantoea sp. YR343 is a plant-associated bacterium with significant agricultural potential due to its ability to promote plant health and growth. This bacterium forms biofilms that may enhance soil aggregation and moisture retention through the production of exopolysaccharides [1]. Understanding the rheological properties of these biofilms is crucial for their applications in sustainable agriculture.

Objectives: 1) Examine the impact of UDP-sugar biosynthesis gene deletion on biofilm structure; 2) Clarify the link between biofilm microstructure and viscoelasticity.

Method: *Pantoea sp. YR343* was isolated from the rhizosphere of *Populus deltoides* [2]. Transposon mutagenesis was performed on Pantoea sp. YR343 to generate its mutant ΔUDP, which exhibited altered exopolysaccharide synthesis. 1: 0, 1: 10, 1: 50, 1: 250, 0: 1 ratios of WT to ΔUDP were mixed by vortexing. These cultures were then transferred on sterile agar plates, incubated at 24 °C for 48 h. Biofilm microstructure and rheological properties were characterised using confocal microscopy and rheometer, respectively.

Findings: We found that packing density increases with the ratio of ΔUDP (Fig. 1), which correlates with reduced water content. Our results demonstrate a progressive change in viscoelastic behavior, transitioning from a weak gel to a strong gel, and ultimately to a glass-like state, driven by variations in biofilm composition and structure (Fig. 1). Additionally, we identified the Payne effect, a nonlinear viscoelastic phenomenon previously observed in polymer colloidal systems [3].

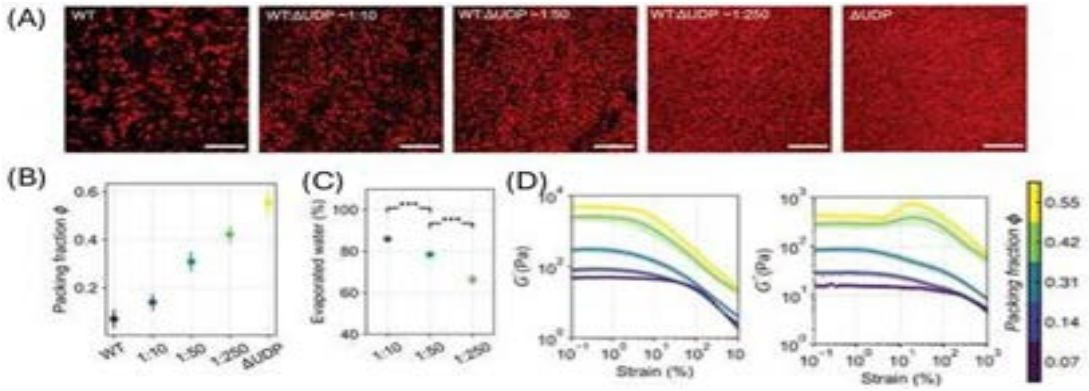


Figure 1: (A) Confocal images of biofilms (B) Packing density (C) Evaporated water content (D) Rheological properties of biofilms.

Conclusions: The presented results demonstrated that exopolysaccharide production influenced the micro-scale structure, water content and rheology in bacterial biofilms. These insights advance our understanding of how biofilm mechanical properties may be tailored for agricultural applications.

Abstract ID: 1761 / Poster Number: 024.17F

Twisting the Narrative: Picobirnavirus' Homomeric 'Churro' Protein Points to Bacteriophage Identity

Presenting author: Ms. Abbey Hutton, Flinders University, Adelaide, Australia

Co-authors: *Susanna Grigson, Robert Edwards*

Picobirnavirus (PBV) is an RNA virus, traditionally associated with diarrheal illness, however, it has yet to be formally affiliated with a specific disease. The true biological nature of PBV remains unknown, as high levels of genetic diversity within and between sequences, as well as in the genetic codes used for translation, leading to speculation that the virus may infect prokaryotes. Recent studies suggest PBV possesses structural mechanisms that are bacteriophage associated, such as bacterial lysis, which could redefine PBVs role in microbial ecosystems. Our objective was to investigate the structural characteristics of PBVs open reading frame 1 (ORF1) gene and determine its potential as evidence for PBVs being bacteriophages.

Due to the unculturable nature of viruses like PBV, we used computational modelling tools, AlphaFold and AlphaFold-multimer to predict the multimeric state and protein structures of unidentified PBV genes. We validate these predictions using protein separation and characterisation techniques such as native and SDS PAGE gel electrophoresis as well as transmission electron microscopy images.

We show that the uncharacterised ORF1 gene of the PBV genome forms a homomeric, coiled coil with 10 subunits. Structural features shared significant similarities with the pilot protein found in *PhiX174*, which is involved in genome transfer into the host. The discovery of 'churro-like' homomeric proteins provides compelling evidence that PBV may infect prokaryotes and could be used to predict the viruses host. These findings challenge the primary classifications of PBV as a eukaryotic virus, requiring further investigation into their functional and ecological significance as potential bacteriophage.

Abstract ID: 732 / Poster Number: 420.17P

Two Novel Aldehyde Dehydrogenases (DHY-SC-VUT5 and DHY-G-VUT7) Mined from Metagenome of Hydrocarbon Contaminated Soils

Presenting author: Dr. Cindy Baburam, Vaal University of Technology, Vanderbijlpark, South Africa

Introduction: Hydrocarbon pollution is becoming a growing environmental concern globally due to the increase in anthropogenic activity. This inadvertently supports the need to identify enzymes for their targeted degradation. The search for novel biocatalysts such as monooxygenases, alcohol dehydrogenases and aldehyde dehydrogenases, have relied on conventional culture-based techniques but this allows sourcing of the biomolecules from only 1-10 % of the microbial population leaving most of the biomolecules unaccounted for in 90-99 % of the microbial community. The implementation of a metagenomics approach, a culture-independent technique, ensures that more or less than 100 % of the microbial community is assessed. Aldehyde dehydrogenases are vital for aerobic hydrocarbon degradation and is involved in the last step of catalysing the oxidation of aldehydes to carboxylic acids. With the global increase in hydrocarbon pollution of different environments, this enzyme has the potential to be used in enzymatic bioremediation applications.

Objectives:

1. To isolate total metagenomic DNA from hydrocarbon contaminated soil samples.
2. To construct a Fosmid library using metagenomic DNA from soil samples.
3. To conduct function driven analysis using specific hydrocarbon substrates.
4. To purify, characterise and assess enzyme activity.

Methods: Fosmid clones with hydrocarbon degrading potential were identified from a library constructed from the metagenome of hydrocarbon-contaminated soil. These candidates were functionally screened to identify dehydrogenase enzymes. Accordingly, the fosmid insert of the positive clones were sequenced using the PacBio next generation sequencing platform and *de novo* assembled using CLC Genomic Work Bench. The codon adjusted sequences were synthesised and cloned into pET30a(+) expression vector and transformed and expressed in *E. coli* BL21 (DE3). The enzymes were purified, characterized and their activity assessed.

Findings: The 1233 bp long open reading frame (ORF) for DHY-SC-VUT5 was found to share a protein sequence similarity of 96.7 % to short-chain dehydrogenase from *E. coli*. The 1470 bp long ORF for DHY-G-VUT7 was found to share a nucleotide sequence similarity of 23.9 % to glycine dehydrogenase (decarboxylating) (EC 1.4.4.2) from *Caulobacter vibrioides* (strain NA1000 / CB15N) (*Caulobacter crescentus*). The in-silico analyses and blast against UNIPROT protein database with the stated similarity show that the two dehydrogenases are novel. Biochemical characterization revealed that the highest relative activity was observed at substrate concentrations of 150 mM and 50 mM for DHY-SC-VUT5 and DHY-G-VUT7, respectively. The Km values were found to be 13.77 mM with a Vmax of 0.009135 µmol/min and 2.832 mM with a Vmax of 0.005886 µmol/min for DHY-SC-VUT5 and DHY-G-VUT7 respectively, thus a potent and efficient enzyme for alkyl aldehyde conversion to carboxylic acid.

Conclusion: The novel identified aldehyde dehydrogenases in this study are currently being used to make up enzyme cocktails for biodegradation of alkanes. Moreover, since the discovered enzymes are novel, it would be interesting to solve their structures by crystallography and explore the downstream applications.

Abstract ID: 903 / Poster Number: 403.17P

Two-step fermentation process of second cheese whey using white and selected LED lights in a 5-L bioreactor

Presenting author: Dr. Luca Bernabò, University of Florence, Florence, Italy

Co-authors: *Giulia Daly, Viola Galli, Alessandra Adessi, Lisa Granchi*

Introduction: Second cheese whey (SCW) poses environmental challenges due to its high lactose content. It can be valorized through a two-stage fermentation process. In the first stage, lactic acid bacteria (LAB) perform dark fermentation (DF), converting lactose into lactic acid. In the second stage, purple non-sulfur bacteria (PNSB) drive photofermentation (PF), using light and lactate to produce biohydrogen (Bio-H_2) and poly-β-hydroxybutyrate (PHB), two high-value molecules. PNSB bacteriochlorophylls (BChls) optimally absorb light at 590 nm and 800–880 nm, indicating that tailoring light conditions to these wavelengths could enhance the production of these valuable bioproducts.

Objectives: This study aims to valorize SCW by producing Bio-H_2 and PHB through photofermentation using a 5-L photobioreactor. Two lighting conditions were tested to assess how light quality influences the production of Bio-H_2 and PHB in PNSB: white LEDs (WL) and selected LEDs (SL, 593 and 860 nm).

Methods: The process included two stages: (1) DF of SCW with a co-inoculum of *Lactococcus lactis* MK L84 and *Lacticaseibacillus paracasei* MK L49 under different pH ranges to enhance lactate production; (2) photo-fermentation of fermented SCW using *Rhodopseudomonas palustris* 42OL under WL and SL conditions. Trials were conducted in duplicate.

Findings: During DF, optimal lactic acid production was achieved at pH 4.5–5.5. Under SL conditions *R. palustris* 42OL showed enhanced yields, producing 0.47 L H_2/L substrate and 1.66 % wPHB/wCDW.

Conclusions: The integration of dark and photofermentation efficiently transformed SCW into Bio-H_2 and PHB, with selected LED lights enhancing Bio-H_2 and PHB production in *R. palustris* 42OL.

Abstract ID: 4010 / Poster Number: 305.15P

Type I and Type II Methanotrophs Employ Different Strategies for Phosphate Assimilation from Metal-Phosphate Minerals

Presenting author: Dr. Tra Thi Huong Nguyen, Chungbuk National University, Cheongju, South Korea

Co-authors: *Yongman Kim, Min-Seo Kang, Joo-Han Gwak, Sung-Keun Rhee*

Flooded rice paddies are a significant source of anthropogenic methane emissions, contributing approximately 10% of global methane (CH_4) emissions (1). Aerobic methane oxidation by methanotrophs plays a crucial role in mitigating these emissions by converting CH_4 into carbon dioxide (CO_2) (2). Phosphorus is a key limiting factor for methanotroph growth in rice paddies (3). However, the mechanisms by which methanotrophs acquire phosphate from metal-phosphate minerals, a dominant form of phosphorus in these environments, remain poorly understood. This study investigated the growth of ten type I and type II methanotroph strains in media using insoluble $FePO_4$ mineral as the sole source of iron and phosphorus. Type II methanotrophs demonstrated more efficient phosphate utilization from $FePO_4$, with higher growth yields and rates compared to type I strains. Notably, iron hydroxide precipitates were observed in prolonged cultures of type II methanotrophs. Further analysis of strains BLU1 (type I) and MS2 (type II) revealed that the presence of complexed iron (Fe^{3+}-EDTA) significantly inhibited growth of BLU1 from $FePO_4$, while slightly retarding MS2 growth without affecting yield. Neither strain grew in the presence of insoluble iron hydroxide, indicating a tight link between the iron cycle and phosphate availability. These findings suggest that methanotrophs possess systems for utilizing phosphate from $FePO_4$ minerals, with type II methanotrophs potentially employing more efficient distinct strategies. Comparative transcriptomic analyses will provide deeper insights into the mechanisms by which methanotrophs acquire phosphate from $FePO_4$ minerals.

Abstract ID: 4150

Type III CRISPR-Cas Effectors: Diverse Enzymatic Arms of Bacterial Immunity

Presenting author: Gintautas Tamulaitis, Life Sciences Center, Institute of Biotechnology, Vilnius University, Vilnius, Lithuania

Type III CRISPR-Cas systems provide prokaryotic immunity by detecting foreign RNA and initiating a cyclic oligoadenylate (cOA)-mediated signaling cascade. Upon RNA target recognition, the Csm/Cmr effector complexes synthesize cOA molecules from ATP, which activate auxiliary defense proteins containing CARF or SAVED domains fused to various effector domains, such as nucleases, proteases, deaminases or membrane-associated modules. These effectors degrade nucleic acids or regulate gene expression to suppress viral propagation and may induce programmed cell death. While several auxiliary nucleases have been well characterized, many CARF/SAVED-associated effectors remain functionally unexplored.

In this study, we investigated novel type III CRISPR-associated effectors. Bioinformatic analyses identified a widespread protein family containing a CARF domain, a winged helix-turn-helix (wHTH) domain, and a RelE-like toxin domain. Structural and biochemical characterization revealed that this cOA-activated CARF-wHTH-RelE effector cleaves mRNA in the ribosomal A-site, effectively halting translation and contributing to antiviral defense.

We also characterized the tripartite CalpL-CalpT-CalpS effector system from Candidatus C. acidaminovorans, comprising a cOA-sensing SAVED-Lon protease (CalpL), an anti-σ factor (CalpT), and a σ factor (CalpS). Upon cOA_4 binding, CalpL forms filaments that position its protease domain to cleave CalpT within the CalpT-CalpS complex. The degraded CalpT releases CalpS, enabling transcriptional reprogramming in response to infection.

Finally, we demonstrated that type III CRISPR-Cas systems possess self-limiting mechanisms to degrade cOA signals. This can occur through dedicated ring nucleases or intrinsic catalytic activity of CARF or SAVED domains in the effectors themselves, providing a feedback loop to terminate signaling and prevent excessive activation that could harm the host cell.

This work received funding from the Research Council of Lithuania (agreement S-LL-24-6).

Abstract ID: 2303 / Poster Number: 386.15P

Tyrosine aminotransferase (TyrB)-dependent aspartate synthesis requires salvage of a specific B6 vitamer in Salmonella enterica

Presenting author: Ms. Brandi Buckner, University of Georgia, Athens, United States

Co-authors: *Diana Downs*

Introduction: The active cofactor form of vitamin B_6 is pyridoxal-5'-phosphate (PLP). In *Salmonella enterica,* two PLP-dependent enzymes can generate aspartate: aspartate aminotransferase (AspC) and tyrosine aminotransferase (TyrB). When AspC is not present, a secondary activity of TyrB can supply *S. enterica* with sufficient aspartate for growth. The ability of TyrB to compensate for AspC is lost when the PLP Homeostasis Protein (PLPHP) homolog, YggS, is absent.

Objective: YggS and its homologs modulate B_6 vitamer homeostasis by an unknown mechanism. TyrB is a PLP-dependent enzyme, which makes the synthetic aspartate auxotrophy of an *aspC yggS* mutant a promising model system for defining the function of YggS at a molecular level.

Method: The synthetic aspartate auxotrophy of *aspC yggS S. enterica* mutants was interrogated with genetic analyses. Various genes encoding the B_6 vitamer homeostasis pathway were disrupted. A variety of mutant strains were assayed for growth in the absence of aspartate and presence of various B_6 vitamers.

Findings: Loss of pyridox(am)ine oxidase (PdxH) results in a B_6 auxotroph that can be satisfied with B_6 vitamers, pyridoxal or pyridoxamine. However, TyrB-dependent growth of an *aspC yggS pdxH* strain requires supplementation with pyridoxamine. The common B_6 vitamer, pyridoxal, fails to allow TyrB-dependent growth of the *aspC yggS pdxH* mutant.

Conclusions: The ability of pyridoxamine, but not pyridoxal, to rescue *pdxH aspC yggS* strains suggests a role for YggS in utilization of pyridoxal, but not pyridoxamine, by TyrB. A working model for the role of YggS in this system is discussed.

Abstract ID: 3036 / Poster Number: 258.17P

Ultrahigh-throughput discovery of novel ketoreductases using droplet microfluidics and in vitro expression

Presenting author: Mrs. Laura Blas, Centro de Biología Molecular Severo Ochoa – UAM, Madrid, Spain

KREDs are nicotinamide-dependent enzymes that convert ketones to secondary chiral alcohols and vice versa to provide enantiopure building blocks from prochiral ketones for the pharma and fine chemical industry for a multitude of applications including in consumer products.

Screening for KREDs and other relevant enzymes for biocatalysis in the microbial diversity can be carried out 1000x faster and cheaper compared to conventional high-throughput screening by means droplet microfluidics. Therefore, the aim of this work was to develop and use an ultrahigh-throughput screening (uHTS) workflow for the discovery of novel KREDs in the natural genetic diversity.

To that end, an existing KRED assay in microtiter plate format was adapted to uHTS format using a modified substrate with reduced leakage. Recombinant expression was carried out *in vivo*, encapsulating single-cells or *in vitro*, encapsulating single plasmid molecules in droplets. Then, a metagenomic library from a garden soil sample (1.5 million-clone library, average insert size of 3 kbp) was constructed in a pBluescript SK vector. Individual clones of the metagenomic library were then encapsulated together with a fluorogenic substrate in 20 µm diameter "water-in-oil" (w/o) microdroplets acting as "test tubes" and screened, selecting the most fluorescent individuals of the population.

Abstract ID: 806 / Poster Number: 189.15P

Ultraviolet treated-mutant of Enterococcus mundtii revealed the change in gene expression associated with oxidative stress response leading high-carotenoid production under low oxygen condition

Presenting author: Tatsuro Hagi, National Agriculture and Food Research Organization, Tsukuba, Japan

Co-authors: *Sora Hayashida, Miho Kobayashi, Masaru Nomura*

Introduction: Several lactic acid bacteria(LAB) can produce carotenoid, known as diaponeurosporene, with an antioxidant and immunomodulatory effect. So high-carotenoid producing LAB is expected as a potential probiotics. On the other hand, our previous study demonstrated carotenoid production was upregulated in only aerobic condition causing oxidative stress. This result implied that carotenoid production in LAB can be restricted under low oxygen condition like gut.

Objectives: This study aimed to improve carotenoid production of LAB under low oxygen condition by UV-treatment and decipher the change in metabolism of the UV-treated mutant.

Method: *Enterococcus mundtii* was treated with ultraviolet, and mutant strain with an ability to produce carotenoid under low oxygen condition was selected. Comparative transcriptome analysis between wild type (WT) strain and mutant strain was performed. In addition, carotenoid production and antibacterial activity of mutant strain were compared with WT strain.

Findings: Mutant strain which can produce carotenoid under low oxygen condition (approximately 0.5% oxygen) was obtained. WT strain couldn't produce carotenoid under the same condition. The amount of carotenoid production of mutant strain under aerobic condition was also much higher than that of WT strain. Transcriptome analysis showed that genes associated with oxidative response genes including carotenoid biosynthesis were upregulated in mutant strain. The upregulation of bacteriocin biosynthesis genes leading higher-antibacterial activity was also observed.

Conclusion: Carotenoid production can be improved under low oxygen condition in mutant strain. Mutant strain may be able to produce carotenoid and reveal effective immunomodulatory effect in the gut.

Abstract ID: 1846 / Poster Number: 222.15P

Uncovering a Vital Role of Membrane Protein VvMP in Vibrio vulnificus Pathogenesis

Presenting author: Dr. Kohei Yamazaki, Kitasato University, Towada, Aomori, Japan

Co-authors: *Takashige Kashimoto*

Introduction: *Vibrio vulnificus* is a marine pathogen responsible for severe wound infections and septicemia, often resulting in high mortality. It is also a causative agent of necrotizing soft tissue infection (NSTI). Rapid proliferation and immune evasion mechanisms drive its virulence; however, the molecular factors underlying these processes remain unclear. In this study, we investigate a newly identified membrane protein, VvMP, to elucidate how this lethal bacterium establishes infection and evades host defenses.

Objective: This study aimed to clarify the role of the membrane protein VvMP in the pathogenicity of *V. vulnificus*.

Method: A VvMP deletion strain (ΔVvMP) and a complementary strain (pVvMP) were constructed. These strains were used to evaluate multiple aspects of *V. vulnificus* pathogenicity. First, we assessed the growth rates of these strains in vitro to ensure that VvMP deletion did not impact basic bacterial proliferation. Second, we investigated their lethality in a murine wound infection model, along with bacterial dissemination using in vivo imaging systems (IVIS). Third, we examined the strains' resistance to neutrophil killing by utilizing neutropenic mice and HL-60 cells, which serve as a model for neutrophil-like immune responses.

Findings: Although ΔVvMP retained normal growth in vitro, it demonstrated reduced lethality and limited dissemination in immunocompetent mice. Bacterial loads in muscle tissue were significantly lower compared to the wild-type strain. In addition, HL-60 cell assays revealed that ΔVvMP exhibited decreased resistance to neutrophil-like cells. In contrast, when neutropenic mice were used, ΔVvMP caused mortality comparable to the wild-type strain. These results suggest that VvMP plays a critical role in immune evasion and survival in vivo.

Conclusion: VvMP is indispensable for efficient in vivo proliferation and pathogenicity of *V. vulnificus*. Targeting this membrane protein could present new therapeutic opportunities for managing severe *V. vulnificus* infections.

Abstract ID: 1927 / Poster Number: 322.16F

Uncovering polyhydroxyalkanoates (PHAs) biosynthetic potential in marine host-associated bacteria: identifying novel candidates for sustainable bioplastics production

Presenting author: Ms. Ana S. Fernandes, IBB- Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Lisbon, Portugal; i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon, Portugal

Co-authors: *Maria Teresa Cesário, Rodrigo Costa*

As concerns over climate change-related emergencies increase, so do efforts to mitigate the impact of human activity on the environment. Thus, replacing petroleum-based plastics with biodegradable, bio-based materials is key to combat current climate change. Polyhydroxyalkanoates (PHAs) are biodegradable, microbially produced bioplastics, accumulated by bacteria as carbon reservoirs in surplus carbon conditions. Although the production of PHAs has been widely studied, bioprocess engineering costs and limited knowledge of the wealth of PHA-producing bacteria in nature still hamper profitable industrial-scale production.

This study uncovers PHA biosynthesis potential among marine, host-associated bacteria in pursuit of novel microorganisms that can contribute to lowering costs and enhancing efficiencies of production. Seventy-nine genomes from bacteria associated with marine sponges, corals, and fish – spanning Alphaproteobacteria, Gammaproteobacteria, Flavobacteriia, Actinomycetes, Bacilli, and Cytophagia -, were screened for the presence of PHA synthase, PhaC, and its subunits. Genotypically-positive isolates underwent phenotypic validation through Nile Red staining and epifluorescence microscopy.

Fifty genomes from Alphaproteobacteria and Gammaproteobacteria contained, at least, one PhaC-encoding sequence. Phylogenetic inference of the 68 detected sequences placed 52 within class I, 4 in class II and 2 in class III subunits, and indicated possible horizontal gene transfer within class I. Staining showed accumulation of PHA granules in 37 of these isolates, comprising 21 of 39 bacterial genera assessed genotypically. Notably, PHA production is reported for the first time in the genera Grimontia, Shimia, Pseudophaeobacter, and Marinicauda.

This study uncovers genotypically diverse and novel PHA-producing marine bacteria, offering candidates of interest for the bioplastics sector.

Abstract ID: 3301 / Poster Number: 332.17P

Uncovering the Genetic Basis of Microbiome Recruitment in Grapevine Using QTL Analysis: Association with Fermentative and Pathogenic Taxa

Presenting author: Ms. Lena Flörl, ETH Zürich, Zürich, Switzerland

Co-authors: *Jerry Lin, Dario Cantu, Nicholas Bokulich*

Different grapevine cultivars harbor distinct microbial communities which are expected to influence plant health, berry yield and resulting wine characteristics. Yet the underlying genetic mechanisms in microbiome recruitment have not been studied in grapevines. In this project we identified for the first time specific genetic loci and their putative functions which are associated with microbial symbionts in *Vitis vinifera* (grapevine).

Using QTL mapping in a population of 140 F1-progeny grapevine genotypes, we investigated how the host genome influences grape berry-associated bacterial and fungal communities. We identify robust associations between various microorganisms and the grape genome, including pathogenic fungi such as *Botrytis spp.* and fermentative yeasts such as *Saccharomyces cerevisiae*. Many of these taxa map to the same genetic loci linked to plant immune response, suggesting that specific genetic loci broadly influence microbial community assembly on fruit surfaces.

Our findings demonstrate that grapevine genetics significantly shape the microbiome, even under varying environmental conditions; moreover, our results suggest that broad, rather than symbiont-specific mechanisms control microbial colonization of fruit, revealing an emergent “domino” effects with implications for plant-fungal-bacterial interactions.

This study provides a framework for understanding genotype-microbiome interactions in perennial plants, enabling targeted experiments to establish causal relationships in microbiome recruitment. It also offers insights for breeding programs to enhance disease resistance and fermentation quality, advancing sustainable viticulture.

Abstract ID: 2882 / Poster Number: 235.17F

Uncovering the Genetic Basis of Staphylococcus aureus in Livestock: Insights into MGEs, Virulence, and Antimicrobial Resistance

Presenting author: Mrs. Jose Francisco Díaz Méndez, Universidad Cardenal Herrera-CEU, CEU Universities, Valencia, Spain

Co-authors: *Patricia Máscaros Núñez, Carmen Martínez Seijas, Alberto Arnau Bonachera, Laura Selva Martínez, Juan Manuel Corpa Arenas, David Viana Martín*

Introduction: *Staphylococcus aureus* is one of the most important livestock pathogens, associated with severe diseases that cause significant economic and productivity losses. Its adaptability to hosts, driven by mobile genetic elements (MGEs) and virulence factors, along with the rising antimicrobial resistance (AMR), highlights the importance of genomic studies.

Objective: To comprehend host-specific adaptations, this work evaluates the phylogenetic connections, MGEs, pangenome diversity, virulence, and AMR of *S. aureus* strains across livestock hosts.

Method: A total of 1,889 *S. aureus* strains from six livestock species were analysed, with reads trimmed, assembled and annotated with Fastp, Shovill and Bakta. AMR and virulence genes were screened using AMRFinder, pangenome analysed with Panaroo, and phylogeny constructed with IQ-TREE. MGEs were detected using Platon and PhiSpy.

Findings: A total of 105 *S. aureus* Sequence Types (STs) were identified, with predominant STs varying by species: ST352 (bovine), ST133 (goat and sheep), ST398 (pig), ST121 (rabbit), and ST5 (chicken). Pangenome analysis revealed 2,038 core genes shared by all strains. Thirteen core AMR genes and 25 core virulence genes were present across all hosts, indicating shared resistance and virulence factors. Rabbits had the highest AMR gene count, while bovine and rabbits had the most virulence genes counts.

Predominant plasmids, such as *pUSA02* in pigs, *pSAP082A* in rabbits and *pRJ80* in bovines, highlight the role of MGEs, in spreading AMR genes.

Conclusion: This study reveals genetic diversity in *S. aureus* across livestock and highlights the public health risk posed by MGEs carrying AMR genes.

Abstract ID: 3071 / Poster Number: 026.15P

Uncovering the methane sink potential and habitat preferences of methanotrophs across 10,000 Danish metagenomes

Presenting author: Ms. Kalinka Sand Knudsen, Aalborg University, Aalborg, Denmark

Co-authors: *Thomas Jensen, Mantas Sereika, Per Nielsen, Mads Albertsen, Caitlin Singleton, Francesco Delogu*

Methane emissions need to be reduced if we are to keep global surface temperatures below the critical threshold of 1.5-2 °C. To address this issue, it is crucial to understand the diversity and biogeography of methanotrophs, the only biological sink of methane. Recent studies in functional gene phylogeny and diversity have shown that many putative methanotrophs remain to be discovered. Historically, methanotrophs have been identified by the marker genes *pmoA* and *mmoX*, encoding two isoforms of methane monooxygenase. However, *pmoA* and *mmoX* share sequence similarity to monooxygenases oxidising non-methane compounds. This complicates identification based solely on marker genes and requires detailed information on gene phylogeny and downstream metabolic potential. Here, we screened 10,000 Danish metagenomes for methanotrophy marker genes to identify the best samples for long-read sequencing and genome recovery. We successfully expanded poorly described groups, such as *Rhodomicrobium* (recovered 9 medium quality (MQ) and 4 high quality (HQ) genomes) and the potential atmospheric methane oxidizer USCγ (recovered 7 MQ and 3 HQ genomes), both previously characterised by only a single MQ genome. Using our updated genome database, we linked the distribution of novel methanotrophs to their metabolic potential across a variety of Danish habitats, including fields, sediments, forests, grasslands, greenspaces and peatlands. Most methanotrophs were unique to a few habitats, while specific groups of potential atmospheric methane oxidisers were widespread across the country.

Abstract ID: 3312

Uncovering the Resistome of Urban Water Systems: A Multi-Omics Analysis of Plasmid-Driven Antibiotic Resistance Gene Dissemination

Presenting author: Prof. Søren Sørensen, University of Copenhagen, Copenhagen, Denmark

Co-authors: *Wanli He, Joseph Nesme*

Urban water systems (UWSs) act as reservoirs for antibiotic resistance genes (ARGs), and as an ideal environment for spreading ARGs by horizontal gene transfer via plasmids. However, the details of the dynamics of the plasmid encoded ARGs in the UWSs` resistome are not well understood.

In this study, we captured more of UWSs` plasmid and antibiotic resistome across three European countries by combining direct plasmidomic sequencing, metagenomic sequencing, and whole-genome sequencing of plasmid isolates, than that of each individual method.

We found a critical role for plasmids in carrying and spreading ARGs, especially clinically relevant beta-lactamases resistance genes, and identified plasmids' significant role in promoting UWSs and microbiota`s resistome. Interestingly, ARGs, at same time, also boost plasmid prevalence, broaden host range, and increase plasmid transfer frequency. Importantly, we identified different wastewater treatment processes that had distinct effect on plasmids and their associated resistome, offering insights into optimizing treatments to limit ARG spread.

In conclusion, this work provides the first attempt at a holistic description of the UWSs` resistome, its structure and dynamic significantly expanding the current knowledge.

Abstract ID: 2188 / Poster Number: 471.15S

Understanding Bacillus spore deposition on textiles

Presenting author: Ms. Rebecca Pearman, Northumbria University, Newcastle Upon Tyne, United Kingdom

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Laundering seeks to restore textiles to their original condition by removing both visible stains (e.g., grass, oil, make-up) and invisible residues (e.g., bacterial metabolites, sweat, sebum). However, the laundering process is a significant contributor to water and energy consumption. While washing temperatures in Europe have gradually decreased from 60°C to approximately 40°C (1, 2), there remains a pressing need for advanced biotechnologies capable of delivering effective cleaning at even lower temperatures, such as 30°C and below. For instance, biotics such as *Bacillus* which are currently used as finishes in non-washables (e.g. mattresses) have potential for use in fabric care products.

Conceptually, *Bacillus* spores will deposit onto textiles during the wash cycle, then during wear the spores will subsequently germinate into vegetative cells in response to soils (sweat, sebum, stains) and produce extracellular enzymes for soil degradation – thereby initiating the cleaning process before the textiles enter the next wash cycle. To ensure appropriate efficacy of the biotechnology, adequate spore deposition on the textiles during the wash and appropriate germination and enzyme production must be determined.

This project aims to mechanistically understand *Bacillus* spore interactions on specific textiles.

A *Bacillus* consortium was isolated into individual strains (strain A, B, C) and sporulated using sporulation media at 37°C. Subsequently, the spores were characterised for surface hydrophobicity (microbial adhesion to hydrocarbons assay)), surface charge via zeta potential (zetasizer) and morphology (phase-contrast microscopy). The spores were then dyed with a far-red fluorescent dye (Alexa Fluor 647) and deposited onto knitted cotton fibres; using a method to mimic washing machine cycling, dried and analysed by confocal microscopy. Percentage deposition of the spores onto the cotton fibres was conducted by Image analysis in ImageJ (Figure 1).

Surface hydrophobicity varied among the strains, with spores of strain A exhibiting hydrophilic properties, while spores of strains B and C were hydrophobic. Zeta potential measurements revealed that all spores were negatively charged. The spores also differed

in morphology: spores of strains A and B formed aggregates, whereas strain C remained as planktonic spores. Initial deposition experiments on clean knitted cotton fibres showed that spores of strain C had the highest deposition, followed by spores of strain B, with spores of strain A showing the least deposition (Figure 1).

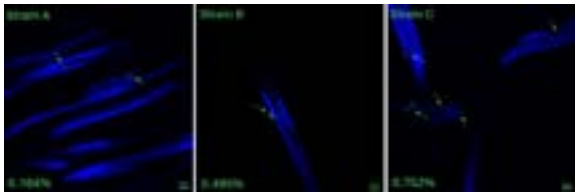


Figure 1: Deposition of Bacillus spores of different strains (A, B, C) on knitted cotton fibres visualized by confocal laser scanning microscopy. Spores are depicted in green with knitted cotton fibres shown in blue. Percentage deposition was calculated from ImageJ analysis and is depicted on each image. Bar = 10µm.

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Abstract ID: 180 / Poster Number: 164.16P

Understanding phage-bacteria interaction differences in Pseudomonas aeruginosa and their impact on phage efficiency and therapy

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The recent resurgence of phage therapy as an alternative treatment approach for multidrug-resistant bacterial infections, including those caused by Pseudomonas aeruginosa, has encountered several challenges, particularly in understanding the biological mechanisms of phage-host interactions. In this study, we isolated and characterized the novel lytic P. aeruginosa phage HMGUpa1 from sewage water. Transmission electron microscopy (TEM) revealed that HMGUpa1 belongs to the Siphovirus morphotype. The host range of HMGUpa1 was screened and five P. aeruginosa strains – NMI 2427/06 (Pa3), NMI 2409/06 (Pa4), NMI 1010/06 (Pa8), NMI 1334/14 (Pa13) and DSM 50071 (50071) – were identified as susceptible hosts. Notably, HMGUpa1 displayed variable efficiency across these host strains, with 50071 exhibiting the highest susceptibility (phage killing effficiency ~90% 15 hours post-infection). To investigate the underlying causes of the differences, we examined phage infection properties, including the adsorption rate, latency period, and burst size, alongside genomic variations in the bacterial hosts. Comparative genomic analyses revealed distinct gene profiles among the host strains, which may contribute to the observed susceptibility differences. Transcriptomic analysis of phage-infected P. aeruginosa strains revealed a significant downregulation of phage defense mechanisms in strain 50071, in contrast to the upregulation of defense systems in other host strains. These findings highlight the complexity and dynamics of phage-host interactions, which play a key role in the outcome of phage-based treatments. Understanding these interactions is essential for successful phage therapy against multidrug-resistant bacteria.

Abstract ID: 2811 / Poster Number: 124.15F

Understanding Recent Oropouche Genome Evolution for Enhancing Early Warning Infectious Disease System

Presenting author: Prof. Doc. Rania Siam, Ross University School of Medicine, Saint Michael, Barbados

Co-authors: *Ali AH Elbehery, Sanjay Pooran,*

Oropouche virus (OROV) disease, primarily problematic to South America and the Caribbean, is a febrile illness transmitted by infected arthropods. Over 10,000 cases were reported in 2024, including in areas previously unaffected, causing rare fatalities in healthy adults and potential adverse pregnancy outcomes.

OROV is a bunyavirus with a trisegmented (-) RNA genome, where the large (L) segment encodes the viral polymerase, the medium (M) segment encodes surface glycoproteins (Gn and Gc) and the non-structural protein NSm, and the small (S) segment codes for the nucleocapsid protein (N) and the non-structural protein NSs.

This study seeks to investigate the recent evolution of the OROV genome to support the development of an early warning system (EWS) for infectious diseases.

Genomic segments of Oropouche virus (OROV) were retrieved from GenBank based on ≥90% BLASTN coverage to reference sequences (NC_005776.1, NC_005775.1, NC_005777.1). A total of 586 L, 563 M, and 490 S segments were aligned using MAFFT, and phylogenetic trees were constructed with IQ-TREE. Metadata, including collection date and location, were extracted, and the trees were visualized and annotated with iTOL. The divergence of genome segments, specific genes, and key point mutations will be presented.

Our analysis demonstrates the evolutionary trajectory of recent OROV sequences, highlighting significant genetic divergence from earlier strains. We will discuss how incorporating these recent genomic changes into an updated diagnostic approach can improve the responsiveness of EWS.

As the virus continues to evolve, surveillance and diagnostic strategies will be crucial in improving public health responses.

Abstract ID: 886 / Poster Number: 421.17P

Understanding the Enhanced SMX removal by Iron-Carbon and Activated Carbon Biofilms and the Microbial Response Mechanism

Presenting author: Dr. MOHAMMAD EBRAHIM AZARI NAJAF ABAD, Karlsruher Institut für Technologie, Karlsruhe, Germany

Co-authors: *Min Bolin, Li Xie*

Antibiotics have garnered substantial global attention, due to their potential hazards to human health and ecosystems. Biofilm processes have demonstrated considerable promise in biodegrading antibiotics and are widely employed in studies on antibiotic removal. Carriers play a crucial role in biofilm systems by facilitating antibiotic removal through the combination of physicochemical processes and biodegradation. The unique microhabitats provided by carriers result in biofilm formation with diverse characteristics, which is critical for effective antibiotic degradation. However, the microbial mechanisms to degrade the antibiotics across different carrier biofilm systems remain incompletely understood. To investigate the underlying mechanisms, two commonly used carriers, iron-carbon (Fe@C) and granular activated carbon (AC), and control (CTL, no carrier), were selected for constructing the integrated fixed-film activated sludge systems, under exposure to 1 mg/L sulfamethoxazole (SMX).

As shown in Fig. 1a, both AC and Fe@C carriers greatly facilitated the SMX removal. The AC group exhibited superior performance, achieving complete SMX removal (100%). This was followed by the Fe@C group, which achieved a removal rate of 64.8% ± 9.8%, both significantly exceeding CTL (21.5% ± 13.1%) groups ($p < 0.05$). Notably, microbial degradation accounted for 83.7% of SMX removal in the AC group, followed by the Fe@C group at 63.3%, according to the SMX mass balance. The improvement can be attributed to the adsorption of SMX within the carrier, which promoted the contact between SMX the and biofilm on the carrier, thereby enhancing SMX degradation. Furthermore, biofilms on the AC and Fe@C carriers exhibited heightened activity, characterized by higher levels of extracellular polysaccharides (PS, 55%~63%) and proteins (PN, 15%~20%). These substances facilitated SMX degradation through biofilm formation and signal transmission (Fig. 1b).

To reveal the SMX removal mechanisms, metagenomics sequencing was applied to analyze the microbial communities and their function. As presented in Fig. 1c, the functional bacteria enriched on the carrier contributed to SMX biodegradation. The dominant bacteria, including *Thauera, Rubrivivax,* and *Hydrogenophaga*, were supported

on the carrier, *which* is capable of degrading SMX through co-metabolism. Additionally, the AC group exhibited a notable increase in signal transduction within biological pathways. The two-component system serves as the primary signal transduction mechanism for responding to environmental cues, promoting biofilm formation and antibiotic biodegradation by regulating gene transcription and expression. Conversely, Fe@C carriers elevated ARG levels to bolster microbial survival under SMX stress while the AC carrier effectively reduced ARG accumulation (Fig.1d). The reduction of ARGs may be related to the complex ecological niche of the AC carrier. The distribution of microbial colonization and SMX adsorption across distinct pores of AC, with indirect interaction facilitated by extracellular enzyme diffusion. Meanwhile, the microelectrolysis environment caused by Fe@C may induce stress oxygen species response, thus aggravating the spread of ARGs.

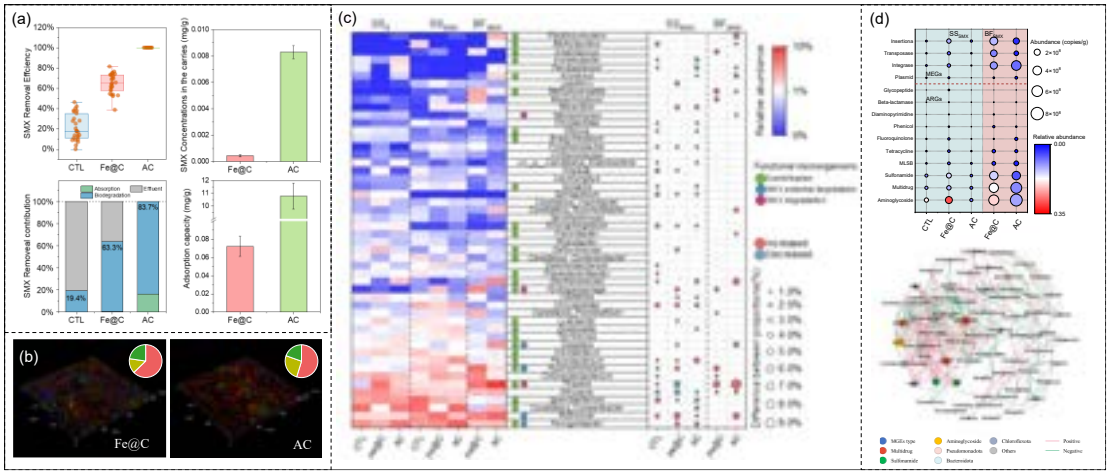


Fig. 1 SMX removal performance and mass balance (a), CLSM images of biofilm (b) (polysaccharide, red; lipid, yellow; protein, green), microbial community structure (c), and ARGs distributions (d).

Abstract ID: 1656 / Poster Number: 080.15P

Understanding the Role of Temperature in the Survival of Carbapenemase-Producing E. coli in Coastal Ecosystems

Presenting author: Ms. Niamh Cahill, University of Galway, Galway, Ireland

Co-authors: *Dearbháile Morris*

Introduction/Objective: Coastal ecosystems are increasingly vulnerable to microbial contamination driven by anthropogenic activities, including wastewater discharge and agricultural runoff. These contaminants often introduce harmful pathogens and antimicrobial-resistant organisms (AROs) into marine environments, posing potential public health risks in recreational waters. Climate change, particularly rising temperatures, may further alter the behaviour and survival of these microorganisms in coastal waters. This study explored how temperature changes influence the survival of clinically significant carbapenemase-producing *E. coli* (CPE) in an artificial seawater environment.

Methods: Artificial seawater, supplemented with 7.5% OECD synthetic sewage as a nutrient source, was spiked with 10^5 CFU/100 mL of NDM-5 and OXA-48 producing *E. coli*. Samples were incubated at temperatures ranging from 11.0°C-29.8°C, representing predicted median and maximum temperatures for current (2020–2030) and future (2089–2099) timepoints across Northern and Southern Europe. To assess survival over time, the concentration of *E. coli* was determined at 0, 24, 48, and 72 hours using the Colilert®-18 system (IDEXX).

Findings: The results demonstrated a temperature-dependent decline in survival for both CPE strains. At cooler temperatures (11.0°C–14.8°C), survival was prolonged, with approximately 13–15% of NDM-5 and 11–13% of OXA-48-producing *E. coli* remaining viable by 72 hours. While at higher temperatures (26.0°C–29.8°C), survival decreased rapidly, with only 0.6% of NDM-5 and 0.7% of OXA-48-producing *E. coli* remaining by 72 hours when incubated at 29.8°C.

Conclusion: These findings highlight the intricate relationship between microbial survival and environmental temperature, highlighting the potential for climate change to shape the persistence of AROs in coastal ecosystems. Cooler climates may inadvertently provide conditions for prolonged survival of harmful pathogens, increasing the potential risks to public health. This study emphasises the need for targeted management strategies to address microbial contamination in light of changing climatic conditions,

supporting the development of policies that protect both environmental and human health.

Abstract ID: 1844 / Poster Number: 420.16F

Understanding the water kefir ecosystem through (meta)genomics and metatranscriptomics

Presenting author: Dr. Marko Verce, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

Co-authors: *Quentin Vanderauwera, Stefan Weckx, Ines Mandić-Mulec*

Introduction: Water kefir (WK) is a fermented beverage obtained by fermenting a sugary solution with dried fruits using water kefir grains (WKGs). WKGs are dextran granules harbouring a fermentative microbial ecosystem, whose composition varies depending on origin, although the key ecosystem members are always lactic acid bacteria and yeasts. So far, only interactions between certain pairs of WK lactic acid bacteria and yeast species have been studied, even though WKGs host many species, often from other microbial groups.

Objective: The objective of this study was a global analysis of the taxonomic composition, functioning, and interactions of the whole ecosystem using a combined approach including genomics of microbial isolates, long-read metagenomics of WK, and metatranscriptomics of the WKGs at 12 h of fermentation.

Findings: Among the bacteria, isolates of *Lacticaseibacillus paracasei*, four *Liquorilactobacillus* species, *Schleiferilactobacillus harbinensis*, *Bifidobacterium psychraerophilum*, and two *Acetobacter* species have been obtained from the WK. Among the yeasts, isolates of *Brettanomyces bruxellensis, Hanseniaspora valbyensis, Zygotorulaspora florentina*, and [Candida] *boidinii* have been obtained. Additionally, metagenomics revealed the presence of *Lentilactobacillus hilgardii*, *Oenococcus sicerae*, and *Zymomonas mobilis*. Initial metatranscriptomic analysis suggested that *Liquorilactobacillus nagelii* and *Zygotorulaspora florentina* were the most transcriptionally active bacterial and yeast species, respectively.

Conclusion: The data set will enable further gene expression analysis in the context of taxonomically characterised WKGs at least in *Liq. nagelii* and *Z. florentina* to reveal which genes are the most important for them in WKGs, presumably reflecting the most important functions for nutrient assimilation and for microbial interactions in this ecosystem.

Abstract ID: 761

Unfolding the collective functional potential of a synergistic multispecies community through genotypic and phenotypic analyses

Presenting author: Ms. Dana Ronin, University of Copenhagen, Copenhagen, Denmark

Co-authors: *Mads Frederik Hansen, Maximilian Lukas Flaig, Mette Burmølle*

Introduction: The properties observed in multispecies biofilms differ from those in their monospecies counterparts. In this study, we focus on a well described, model system composed of four bacterial species: *Paenibacillus amylolyticus*, *Microbacterium oxydans*, *Stenotrophomonas rhizophila* and *Stenotrophomonas maltophilia*. This community has previously demonstrated synergistic biofilm formation capabilities, as well as enhanced keratin degradation, protection from grazers, and enhanced drought tolerance. Such properties have a range of potential industrial and agricultural applications. Past studies have investigated the behaviors of this community in various experimental designs. The throughput of such studies is limited given the resources spent on trial and error to identify a condition of interest to test.

Objectives: In this study, we aimed to develop a bioinformatically guided approach to selecting phenotypic assays relevant for experimental testing. In doing so, we increase the efficiency as well as couple genotypic properties with phenotypic validation.

Methods: First, a complete genome was assembled for each strain, using both long- and short-read sequencing data. We conducted an in-depth bioinformatic analysis, identifying biofilm matrix genes of interest, cooperative genes, secondary metabolite gene clusters, antibiotic resistance genes, enzymatic activity genes and metabolic degradation pathways.

The genomic analysis was then complemented with phenotypic assays; we tested for the antibiotic resistance profiles on surfaces, enzymatic activity, carbon source utilization, and xenobiotic degradation.

Findings: We identified genes in all four strains related to interspecies communication, biofilm formation, secondary metabolite production, antibiotic resistance, enzymatic activity, and xenobiotic degradation. Specifically, degradation pathways for bisphenol A and lindane – compounds derived from plastic or industrial waste, respectively – were identified. Given that metabolism was the biggest category of gene functions, we conducted a thorough substrate utilization assay, where we identified 20 different

substrates with higher growth as a four-species community compared to any of the individual species alone. We also observed cellulase activity solely in the 4-mix community, even though cellulases were identified in all four of the genomes.

Conclusion: By using a bioinformatically guided approach to pinpoint functional assays of relevance, we identified several emergent community properties in this four species community, including enhanced metabolism of 20 substrates and cellulase activity.

Abstract ID: 3221

Unique microbial communities at Arctic cold seep ice-water interfaces: insights into seasonal and spatial dynamics

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Co-authors: *Gabrielle Kleber, Andrew Hodson, Christine Dupuy, Dimitri Kalenitchenko*

Methane serves as electron donor for methanotrophic microorganisms, initiating energy flow into trophic chains in light-deprived ecosystems[1]. In the Arctic, methane stored below the permafrost surfaces via groundwater[2]. A recent study[3] demonstrated that the ice-water interface that forms every winter above a methane-saturated Arctic seepage (Seep 1) hosts an abundant sulfur-oxidizing microbial community at the ice-water interface, fueled by energy from microbial anaerobic oxidation of methane. This revealed that ice provides a habitat with suitable electron acceptors to chemoautotrophic microorganisms[3]. Inspired by this discovery, our study tests the hypotheses that (1) this microbial community is a unique feature of the winter ice-water interface, establishing depending on environmental factors and ice growth rate, (2) ice cores preserve biochemical and genetic signals of microbial activity at the ice-water interface, and (3) ice-associated microbial communities distinct from their source are widespread across similar environments. We performed shotgun metagenomics to compare summer and winter metagenomes from Seep 1, and conducted 16S rRNA gene sequencing on winter time-series samples at Seep 1, and at ice-water interfaces from nine additional sites. Metagenomics identified distinct gene pools confirming that the microbial chemosynthetic potential in bottom ice is absent in summer oxygenated waters and winter anoxic waters (**Fig. 1A**), highlighting the uniqueness of bottom ice microbiota. The time series showed two distinct ice-water interface microbial communities: (1) anaerobic methanotrophs and sulfate-reducing bacteria resembling water communities (**Fig. 1B**, T1-T3) and (2) sulfur-oxidizers and aerobic methanotrophs absent from water (**Fig. 1B**, T4). Variations in the latter community's distribution in the core suggest sporadic establishment on the bottom ice throughout winter, and alternating capture of the water or bottom ice-associated community by the ice core. Among nine other sites, three exhibited high dissimilarity between bottom ice and water microbial communities, indicating this phenomenon extends beyond Seep 1 (**Fig. 1B**). Together, our findings underscore the ecological significance of under-ice microbial processes, defending the need to incorporate ice microbiomes into microbiological studies of extreme aquatic systems, particularly those involving seasonal ice-water interfaces. They also emphasize

the vulnerability of such communities to ice growth perturbations, urging greater attention as polar seasons face increasing disruptions.

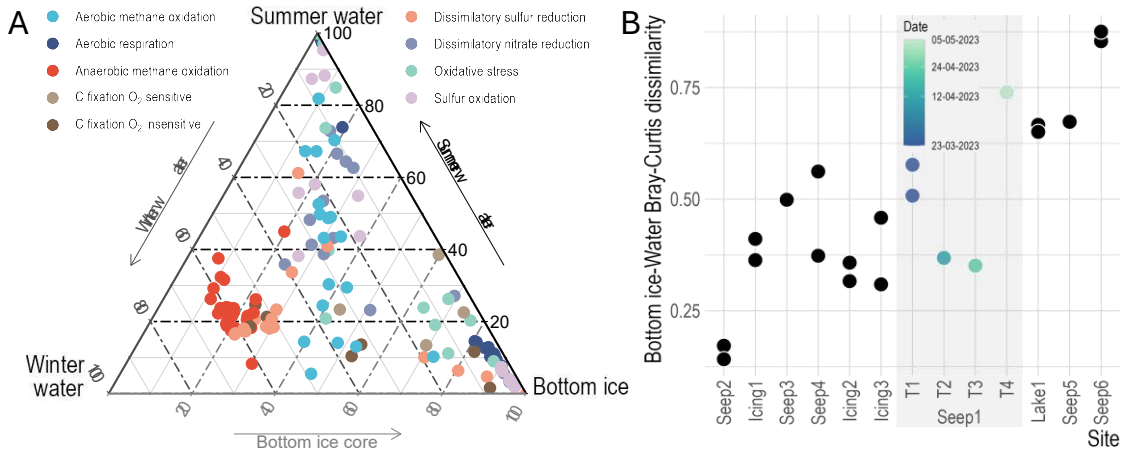


Figure 1: (A) Ternary plot showing the proximity between summer water, winter water and winter bottom ice selected microbial metabolic potential at Seep 1. Each point represents a gene, the color its metabolic pathway. Gene abundances add up to 100%, split between the three samples. (B) Bray-Curtis distances (y axis) between microbial community composition of bottom ice samples and the water at the same site (x axis), computed from 16S rRNA gene amplicon sequence variant relative abundances. Each dot represents a bottom ice sample.

Abstract ID: 2275

Unlocking Genetic Engineering in Difficult-to-Engineer Bacteria

Presenting author: Dr. Craig Woods, Forge Genetics, Nottingham, United Kingdom; University of Nottingham, Nottingham, United Kingdom

Introduction: Microbial genetic engineering has enabled significant advancements in biotechnology; however, many bacterial species remain difficult to manipulate genetically. Current tools such as CRISPR-Cas systems often fall short in these organisms due to low DNA transfer rates and the inherent toxicity of Cas proteins which makes plasmid transfer difficult or impossible. If plasmid transfer is achieved CRISPR-Cas approaches rely on identifying rare double crossover mutants from a population which inevitably also contains “escapees” in which the Cas nuclease counter-selection was not effective. These are especially common because of the selection pressure for defunct Cas nucleases during the initial plasmid transfer. The end result is laborious screening and repeated frustrated attempts to obtain mutants.

We have developed a highly-efficient genetic system with broad applicability across bacterial systems and beyond which is especially well suited to engineering genetically recalcitrant species. The system utilises a targetable Group II intron which inserts a meganuclease recognition site, followed by counter-selection of that site with a meganuclease while providing a homology cassette to select for the desired mutant. This system benefits from simple programmability similar to CRISPR while offering a more robust mutant generation procedure. We have termed this technique "Forge Editing".

Objectives: The primary objectives of this research were to:

1. Develop a versatile gene-editing tool for clean deletions and insertions with simple reprogrammability and adaptability across biology.
2. Demonstrate Forge Editing’s effectiveness in species where CRISPR systems failed or were inefficient.

Method: Edited cells were acquired in a two step procedure. Step 1 involved the insertion of a targetable group II intron at the site of the intended edit. Intron-containing cells were selected on the basis of a retrotransposition-activated marker (RAM).

In step 2 intron-containing cells were transformed with plasmids harbouring a homology cassette and a meganuclease. The homology cassette created the desired clean edit via

double homologous recombination, and the meganuclease counter-selected unedited cells.

Findings:

- Forge Editing consistently achieved high editing efficiencies in species with low DNA transfer rates where CRISPR-Cas systems showed little to no success.
- The smaller plasmids used in Forge Editing were readily taken up by challenging bacterial species, reducing transformation barriers.
- Unlike Cas proteins, which exhibited deleterious effects in many species, Forge Editing plasmids were non-toxic and maintained high cell viability.
- Successful editing was demonstrated in several non-model bacteria, including *Clostridium autoethanogenum* and *C. sporogenes*.

Conclusion: Forge Editing provides a solution for genetic engineering in bacteria traditionally considered intractable. By eliminating toxic elements and reducing plasmid size, it addressed the limitations of CRISPR-Cas systems, particularly in species with low DNA transfer rates. Its successful application in difficult-to-work-with bacteria highlighted its potential to unlock new avenues in healthcare and industrial microbiology. Future work will focus on expanding Forge Editing's applicability to additional bacterial species and collaborating with research groups working on difficult to engineer species.

Abstract ID: 3601 / Poster Number: 295.16S

Unlocking siderophore production potential: Comparative heterologous production and enzyme activity of BibA

Presenting author: Ms. Shalini Singh, Helmholtz Zentrum Dresden Rossendorf, Dresden, Germany

Co-authors: *Nora Schoenberger, Rohan Jain, Katrin Pollmann*

Introduction: Bisucaberin, a tetradentate siderophore is a potential candidate for siderophore-assisted metal recovery technologies. Siderophore-assisted metal recovery technology is considered as sustainable, effective, and energy efficient approach in metal recovery industry. The sensitivity and selectivity of different hydroxamate siderophores such as bisucaberin towards target metals such as lanthanides gives the scope of expansion of this technology. However, cost effective large-scale production of siderophore is a foreseen challenge for siderophore-assisted technologies. We aim to overcome this challenge using biological production of target siderophore in industrial production bacterial strains. Bisucaberin, a hydroxamate siderophore, produced by *Aliivibrio salmonicida* share cognate gene cluster (~5kb) comprising of three genes. Generally, the flux of intermediates determines the type of hydroxamate siderophore biosynthesized by the organism. As the flux of these intermediates is differently regulated in each organism, hence the type of siderophore produced is dependent on hosts. Optimal host and determination of flux using the enzyme activity of each enzyme are therefore two key steps for enhancing the target hydroxamate siderophore production.

Objective: This paper aims to establish a strategy for cost-effective bisucaberin production through two main objectives. Firstly, to identify a suitable host for enhanced production of the first enzyme, lysine decarboxylase, involved in bisucaberin synthesis. Secondly, to optimize the unlabeled enzyme activity method in comparison with the labeled enzyme activity assay. This project (Fig1) serves as a foundation for future research in enzyme kinetics for siderophore-related enzymes, significantly contributing to the large-scale economical production of target siderophores.

Methods and Findings: IPTG induction and pET vector system with Strep-tag are used for production and purification of BibA in *E.coli* (T7 Express) in 5L batch reactor. The unlabeled enzyme activity method, using Isothermal Titration Calorimetry (ITC) with a single injection system, was successfully employed to determine enzyme activity. Enzyme activity was also assessed by the labeled method, with HPLC serving as the reference method. *V.natriegens* (Vmax X2) is closely related to *A.salmonicida* and is expected to have similar flux balance of intermediates. This suggests that Vmax can serve

as better host for target production of bisucaberin. Therefore, similar approach is being used for production and assessment of BibA activity in *V.natriegens* (Vmax X2).

Conclusion: Heterologous production of individual enzymes such as BibA of cognate gene cluster bibABC in different hosts is a crucial step to assess suitable host for bisucaberin production. Unlabelled enzyme activity method such as ITC is a useful tool for flux related studies of BibA. This strategy can be used to achieve targeted cost-effective hydroxamate siderophore production in the best-suited host.

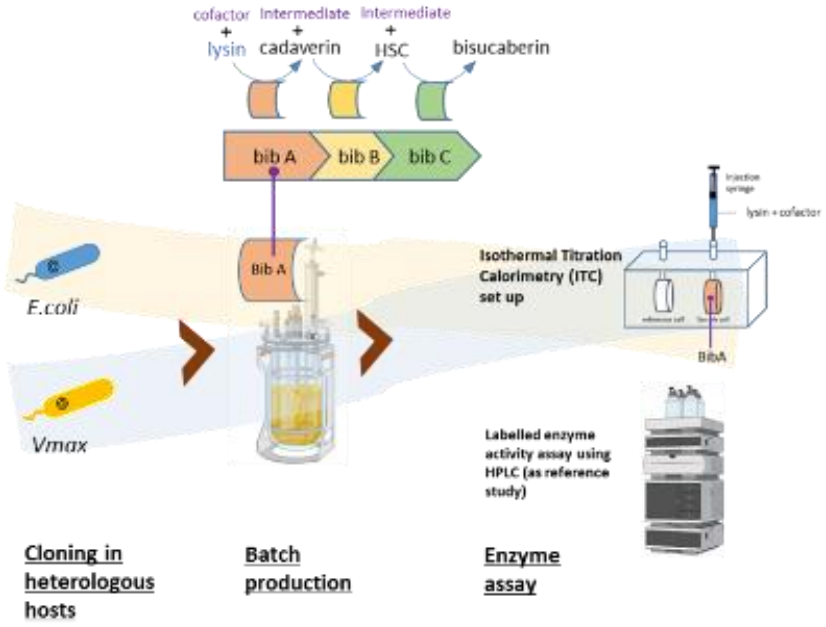


Figure 1: Comparative heterologous production and enzyme activity of BibA.

Abstract ID: 656

Unlocking the potential of accessory xylanolytic enzymes from marine Bacillus sp. NIORKP76 for enhanced saccharification of various agro-residues

Presenting author: Dr. Pankaj Parab, Government College of Arts, Science and Commerce, Khandola, Goa, India; CSIR-National Institute of Oceanography, Donapaula, Goa, India

Co-authors: *Rakhee Khandeparker*

The effective disintegration of lignocellulosic materials requires a consortium of enzymes due to their exorbitant complexity. Accessory xylanolytic enzymes, such as arabinofuranosidase (AFase) and acetyl-xylan esterase (AXE), are decisive enzymes which have proven their critical role in effective degradation of agro-residues in amalgamation with xylanases. The fattened demand for these enzymes, coupled with their limited supply, has made them valuable biomolecules in various industries. In current study, under optimized physio-chemical conditions, Bacillus sp. NIORKP76 was found to naturally produce xylanase and two accessory xylanolytic enzymes, AFase and AXE, when grown on inexpensive agro-industrial waste (wheat bran) under solid-state fermentation. Preliminary characterization of AFase and AXE showed molecular weights of approximately 66 kDa and 45 kDa, respectively. Both enzymes exhibited optimal pH at 6.0 and retained 100% residual activity over a wide pH range (5.0 to 8.0) even after 24 hours of incubation. The maximum activity for both AFase and AXE was observed at 60°C, with AFase retaining 100% and AXE 80% of its activity when incubated at temperatures 30°C to 40°C respectively for 24 hours. The xylanolytic enzymes produced by Bacillus sp. NIORKP76 exhibited key properties necessary for various industrial applications, including bio-bleaching of kraft pulp, fruit juice processing, and animal feed production. These enzymes hold significant promise for enhancing the efficiency of agro-residue utilization in diverse sectors.

Abstract ID: 2384 / Poster Number: 472.15S

Unlocking the Potential of Antarctic Marine Bacteria: Advancing Recombinant Protein Production for Human Terapeutic Applications

Presenting author: Mr. Andrea Coletti, University of Naples Federico II, Napoli, Italy

Co-authors: *Concetta Lauro, Marzia Calvanese, Angelica Severino, Gennaro Apuzzo, Maria Luisa Tutino, Ermenegilda Parrilli*

Introduction: Psychrophilic bacterial cell factories present a promising solution for producing difficult-to-express proteins. Among these, *Pseudoalteromonas haloplanktis* TAC125 (*Ph*TAC125) stands out as a particularly effective system. *Ph*TAC125 combines unique biological and physiological traits with extensive genetic optimization, making it a powerful platform for synthesising human proteins. A notable application of this cell factory is the scalable recombinant production of human CDKL5 (*h*CDKL5), a protein kinase that is highly prone to forming insoluble and inactive aggregates and undergoing proteolysis in conventional hosts.

Objective: The importance of this work is underscored by two factors: first, the growing global demand for *h*CDKL5 for health-related applications and physiological studies; and second, the lack of a dedicated cell factory for scalable, industrial-grade recombinant human protein production. Therefore, this study focuses on developing tailored and flexible *h*CDKL5 production conditions in *Ph*TAC125. Additionally, to assess the quality and quantity of the heterologous product obtained is crucial to establish dedicated analytical tools, such as quantitative ELISA.

Methodology

To bridge the gap between laboratory-scale research and industrial applications, a Process Analytical Technology (PAT) approach was applied to optimize production conditions and enhance *Ph*TAC125 performance as a cell factory. For this purpose, a rich medium and synthetic medium developed using a *Ph*TAC125 metabolic model were tested. Furthermore, the effect on the recombinant production of different additives, identified by the means of the metabolic model, was screened. Hence, to measure the full-length, soluble *h*CDKL5 recovered in bacterial lysates, a tailored sandwich ELISA assay was designed to quantify the soluble recombinant product synthesised by the psychrophilic expression system. Additionally, specific protein kinase assays evaluated

the activity of the recombinant product. These analytical tools were exploited to assess *Ph*TAC125 performances in terms of yields and quality in different production conditions.

Findings and conclusions: Variations in the quantity and quality of recovered *h*CDKL5 were observed based on media composition, affecting protein phosphorylation status, stability, and activity. The rich medium yielded the highest protein recovery, quality and process efficiency. Hence, large-scale production was implemented using the rich medium, as it proved to be the optimal production condition for recombinant *h*CDKL5. On the other hand, the synthetic medium significantly underperformed in terms of product yields compared to the rich medium. Yet, obtaining a medium tailored to the specific metabolic needs of the recombinant host represents a significant opportunity to set up high-performance bioreactors and improve industrial applicability. So, to improve *Ph*TAC125 performances in the synthetic medium, the addition of specific additives to its composition was tested. Hence, it was observed that process yields improved by a three-fold factor. Consequently, the DoE approach was employed to further enhance this production condition. In conclusion, *Ph*TAC125 demonstrated remarkable flexibility as a cell factory, thriving under various production conditions. This adaptability, combined with customized optimization strategies, underscores its potential for scalable and efficient industrial applications.

Abstract ID: 789

Unlocking the Potential of Patagonian Yeasts for Novel Lager Hybrids

Presenting author: Assoc. Prof. Francisco Cubillos, Universidad de Santiago de Chile, Santiago, Chile

Co-authors: *Jennifer Molinet, Vasni Zavaleta, Rike Stelkens,*

The growing demand for novel and diverse beer profiles stresses the need for innovative biotechnological approaches leveraging unique yeast strains. The recent discovery of genetically diverse *Saccharomyces eubayanus* lineages in Patagonia offers an exceptional opportunity to expand the repertoire of lager yeasts, which have traditionally been limited to a few strains worldwide. By harnessing this untapped genetic diversity, we aim to generate new *S. cerevisiae* × *S. eubayanus* hybrids with unique brewing traits.

In this study, we employed experimental evolution, hybridization, and mitochondrial selection to develop robust yeast hybrids with enhanced fermentation profiles and distinctive aromatic characteristics. Hybridization success varied across parental lineages, with certain combinations, such as those involving Beer *S. cerevisiae* and PB-2 *S. eubayanus* lineages, yielding high rates of polyploid hybrid formation. These hybrids demonstrated phenotypic variability, driven by genetic rearrangements and selective pressures, enabling high ethanol tolerance, improved maltotriose consumption, and unique aroma profiles. Remarkably, ploidy did not influence differences in the hybrids' fermentative capacity. However, we identified differences depending on the mitochondria, where hybrids with Patagonian *S. eubayanus* mitochondria exhibited superior fitness and greater evolutionary potential, comparable to other species and strains.

Genomic and transcriptomic analyses revealed key genetic modifications during the hybrid's adaptation to beer fermentative conditions. These included variations in *IRA2* and copy number variation for *IMA1*, and *MALX* genes, which enhanced sugar metabolism and glycolytic flux. Additionally, the hybrids' volatile compound profiles were significantly influenced by the dominant inheritance of aroma-related genes from the *S. eubayanus* parent, offering diverse brewing possibilities. Our work highlights the potential of Patagonian yeast diversity to drive innovations in beer biotechnology, creating novel brewing strains with improved fermentation efficiency and unique sensory attributes.

Our findings underscore the value of hybridization and experimental evolution as strategies for developing next-generation lager yeasts design to meet the demands of a dynamic brewing industry.

Abstract ID: 3906 / Poster Number: 009.17S

Unlocking the Taggiasca olive orchard microbiota: metabarcoding approach to reveal the bacterial and fungal above- and below-ground communities

Presenting author: Mrs. Chiara Traina, Università degli Studi di Torino, Grugliasco, Italy

Co-authors: *Livio Antonielli, Tanja Kostic, Luca Cocolin*

The olive tree (*Olea europaea* L.) is one of the most important crops in the Mediterranean region, essential for in terms of economic and agricultural significance. In Liguria, North-West Italy, the Taggiasca cultivar is grown in a distinctive landscape characterized by steep hills and terraced slopes. This region produces high-quality Taggiasca extra-virgin olive oil (bearing the P.D.O mark) and fermented olives, both renowned for their superior quality. In our study, the main objective was to unravel the bacterial and fungal communities of above- (barks, leaves, olive fruits) and below-ground (rhizospheres, soils) compartments with the use of a metabarcoding approach for a better understanding of the plant-associated niches and secondly, to identify the core microbiota within compartments. 24 trees were sampled across three different areas of the orchard according to an altitude gradient to monitor the potential microbiota shifts. Overall, relative abundance of the fungal and bacterial populations showed that the most represented phyla were *Ascomycota* and *Firmicutes* respectively, with the main fungal genera being *Aureobasidium* and *Cladosporium* and the most abundant bacterial ones represented by *Levilactobacillus* and *Sphingomonas*. Beta-diversity based on Bray-Curtis dissimilarity showed significant differences among compartments, with olives and leaves samples being mostly characterized by the genera *Aureobasidium* and *Cladosporium* and *Levilactobacillus* and *Pediococcus,* for fungi and bacteria respectively, with no significant difference among areas. Barks and rhizospheres were mainly characterized by the presence of *Pseudophaemoniella*, *Penicillium* and *Nocardioides, Actinomycetospora* for fungi and bacteria respectively, with no significant difference among areas. To determine the core microbiota, Venn diagrams were generated based on 70% shared ASVs. Comparing all areas, some of the ASVs common to all compartments were Aureobasidium *pullulans*, *Veronaea botryose*, *Cladosporium herbarum* and *Blastococcus*, *Solirubrobacter*, *Streptomyces* for fungi and bacteria at the lowest taxonomic level, respectively. This study represents an effort towards a better understanding of the complex microbial consortia that characterizes the Taggiasca olive tree microbiota. It also represents a focus into the investigation of the variability among

endophytic and epiphytic populations of the different compartments, as well as their connections with the fermented fruits, given the relevance of the previously mentioned microorganisms in the fermentative process.

Abstract ID: 114 / Poster Number: 406.17P

Unraveling genome plasticity and acquisition of hyaluronic acid capsule trait in environmental GRAS isolate -Bacillus subtilis K3C- through whole genome de novo sequence analysis

Presenting author: Ms. Simran Gagneja, Panjab university, Chandigarh, India

Co-authors: *Neena Capalash, Prince Sharma*

Background: Genome plasticity is central to adaptation under selective pressure conditions and has attracted a considerable interest over the years. The expression of capsule trait is often associated with virulence and extended environmental breadth. Virulent Streptococcus spp. are known to express hyaluronic acid as capsular material that has commercial utility. In our study, we have identified an environmental GRAS strain producing hyaluronic acid as capsular material and evidences suggesting the gene cluster acquisition and expression were collected using whole genome sequence analysis.

Objective: This study aimed at investigating the evolutionary origin of has gene cluster acquired by the environmental isolate B. subtilis K3C. Our study aimed to analyze the niche adaptability of strain under environmentally stressed condition through acquisition of diverse phenotypic traits from available gene pool.

Method: 146 isolates were screened for hyaluronic acid production using hyaluronidase enzyme assay developed in our lab. The strain positive for hyaluronic acid production was sequenced using illumina sequencing platform. Genome plasticity was analyzed through assessment of horizontal gene transfer hotspots in the strain, pan-genome analysis, identification of orthologous gene clusters and presence of phage regions, mobile genetic elements and plasmid in the strain. The bacterial surface morphology was studied using FE-SEM and structural characterization of the purified capsular material was performed using H1NMR.

Findings: Our findings suggest that the strain K3C has acquired different phenotypic traits through transformation-mediated HGT process. Intact prophage regions were identified in the genome, however, has gene cluster was not integrated into the prophage regions, rather genes were integrated in different chromosomal regions. The reticulogram inferences revealed that has gene cluster acquired by the strain was phylogenetically closer to the has operon present in Streptococcus equi and S. pyogenes. The

phyloproteome analysis of hyaluronan synthase gene suggests that it has a common firmicutes ancestry. The prevalence of has operon has been reported across different Bacillus species. has operon was acquired by B. anthracis, B. cereus and B. tropicus through plasmid-mediated HGT. Overall, our findings shed light on genome plasticity observed in strain evolution. The surface morphology analysis and structural characterization of purified capsular material also validated our data.

Conclusion: Whole genome sequence analysis revealed high degree of plasticity, gene multiplicity and acquisition of complete has gene cluster through transformation-mediated HGT by the strain K3C.

Significance: Since, in our study we have highlighted acquisition of hyaluronic acid capsule trait in an environmental strain which does not harbor genes for toxins and virulence factors, suggests that alternative technologies could be developed for the commercial production of hyaluronic acid.

Abstract ID: 4011 / Poster Number: 117.16P

Unraveling Microbial Dysbiosis and Antibiotic Resistance in Chronic Pouchitis

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Inflammatory bowel diseases (IBDs) are chronic conditions that severely impact quality of life. Ulcerative colitis (UC), the most common IBD, causes chronic inflammation of the colon and rectum, requiring surgery in 20-30% of cases due to medical intractability, dysplasia, or colorectal cancer. The preferred surgical treatment is total restorative proctocolectomy with ileal pouch–anal anastomosis, removing the colon and rectum and creating a pouch by attaching the folded ileum to the anus. Although the surgery aims to remove inflammation, 80% of patients develop pouchitis, with 15-20% experiencing chronic pouchitis within the first five years post-surgery.

Antibiotics are the first-line treatment for pouchitis. In chronic pouchitis, prolonged antibiotic use increases antimicrobial resistance susceptibility in the pouch microbiome. Furthermore, pouchitis is linked to gut microbiome dysbiosis, marked by reduced diversity and shifts in specific bacterial taxa. Antibiotic exposure can augment the dysbiosis, increasing the relative abundance of Enterobacteriaceae and reducing protective bacteria. Fecal microbiota transplantation (FMT) has been proposed to restore microbiome balance. However, our placebo-controlled study found that while FMT altered the microbiome, it had no clinical benefit over the placebo. In a follow-up study (unpublished), assessing the long-term effect of FMT on the pouch microbiome, we find no significant improvement of the gut microbiome, as it quickly returns to baseline composition characteristics. As FMT from normal gut donors proved ineffective, it led to an investigation into the factors that define a healthy pouch microbiome, and the impact of the high antibiotic consumption in the patient group compared to that of a healthy pouch microbiome and normal gut microbiome.

To do this, the gut microbiome of three study cohorts, termed sick pouch, healthy pouch (asymptomatic patients with a pouch), and normal gut, were sequenced, and metagenome assembled genomes (MAGs) were recovered. We find that for many of the patients, their pouch microbiome is dominated by *E. coli*. Due to the potential implications associated with a high relative abundance of *E. coli*, we assess the antibiotic resistance gene (ARG) profile, and the plasmid content of the *E. coli* MAGs. We find that

the *E. coli* MAGs from the sick pouch patients have a significantly higher occurrence of single nucleotide polymorphisms conferring resistance specifically to fluoroquinolones (an antibiotic consumed by all patients in the sick pouch group), compared to the healthy pouch and normal gut cohorts. We also find that the sick pouch *E. coli* MAGs have an increased number of associated plasmids, and an elevated presence of ARGs on the plasmids conferring resistance to antibiotics consumed by the patient group compared to the healthy pouch and normal gut cohorts.

These findings prove that the pouch microbiome composition, sick pouch and healthy pouch microbiomes, significantly differs from gut microbiome of individuals with a normal gut physiology, suggesting that the healthy pouch microbiome could serve a better choice for baseline comparisons compared to the normal gut microbiome. We also show that there is an increased selection pressure on ARG carrying *E. coli* directly corresponding to the consumed antibiotics.

Abstract ID: 590 / Poster Number: 034.16P

Unraveling the Role of the HrpA/HrpB Secretion System in Neisseria meningitidis-Induced Pyroptosis during Meningitis

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Introduction: Neisseria meningitidis is a leading cause of bacterial meningitis, where its HrpA/HrpB two-partner secretion system (TPS) plays a critical role in immune evasion and triggering pyroptosis. However, the specific contribution of HrpA/HrpB to pyroptosis and disease progression in vivo and in immune cells is not fully understood.

Objective: This study aimed to investigate the role of the HrpA/HrpB TPS in activating pyroptotic pathways in a mouse model of meningitis.

Method: Using a reference serogroup C strain (93/4286) and an isogenic hrpB knockout mutant, we evaluated bacterial replication, systemic spread, and pyroptosis activation in the brains of infected mice, as well as in THP-1 macrophages. Pyroptotic markers, including caspase-1, caspase-11, gasdermin-D, and the release of IL-1β and IL-18, were analyzed via western blotting and cytokine assays. In THP-1 cells, the intracellular survival of the hrpB-deficient strain was also assessed through gentamicin protection assays.

Findings: Mice infected with the hrpB-deficient strain exhibited increased survival, reduced bacterial loads in the brain, and diminished activation of caspase-1, caspase-11, and gasdermin-D compared to those infected with the wild-type strain (Fig. 1). In THP-1 cells, the hrpB-deficient strain showed a significantly reduced intracellular replication and pyroptotic response, with lower IL-1β production and impaired gasdermin-D activation.

Conclusion: The HrpA/HrpB TPS plays a pivotal role in Neisseria meningitidis-induced pyroptosis in both brain tissues and immune cells, promoting bacterial survival and inflammation. Targeting this pathway could represent a promising strategy for treating invasive meningococcal disease.

Abstract ID: 809

Unravelling Autotransporter Mechanisms: A Path to New Anti-Virulence Therapies

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Introduction: Antimicrobial resistance (AMR) is a critical global health challenge, demanding innovative strategies to combat bacterial infections. Autotransporters, secreted through the Type V secretion system, represent a major class of virulence factors in Gram-negative bacteria[1]. These proteins are pivotal in pathogenic processes, including bacterial aggregation, biofilm formation, host cell adherence, and cytotoxicity.

Objective: Our research aims to elucidate the structures and mechanisms underlying autotransporter function, investigate regulatory pathways influencing their activity, and develop targeted inhibitors to mitigate bacterial virulence.

Method: We employed a multidisciplinary approach combining X-ray crystallography, biophysical assays, cellular assays, and phenotypic studies to unravel the mode of action of bacterial autotransporters. High-resolution structures and comprehensive characterisation of key autotransporter proteins, including adhesins such as Ag43 and TibA, and toxins like EspC and Ssp, have provided critical insights into their functions. Additionally, we explored the regulatory role of glycosylation in modulating autotransporter-mediated virulence and biofilm dynamics.

Findings: Our studies revealed crucial structural and functional insights into self-associating autotransporters (SAATs) and autotransporter toxins, underscoring their roles in bacterial pathogenesis. Ag43 and TibA, prominent SAAT adhesins, were shown to drive bacterial aggregation and biofilm formation through a conserved self-association mechanism[2,3]. Glycosylation by TibC was found to inhibit TibA self-association, uncovering a novel regulatory pathway for biofilm modulation. Moreover, we elucidated the structural mechanisms of cytotoxins Ssp and EspC, highlighting their roles in host cell invasion and cytotoxic damage[4]. Leveraging these findings, we developed inhibitors targeting Ag43 and EspC, demonstrating promising strategies to disrupt biofilm formation and reduce bacterial toxicity[5].

Conclusion: Our work provides critical insights into the structure, regulation, and inhibition of bacterial autotransporters, paving the way for innovative antimicrobial strategies that "disarm rather than kill" pathogens. This approach offers a promising

avenue to combat infections and address antimicrobial resistance by minimising the selective pressure for resistance development.

Abstract ID: 1552 / Poster Number: 392.15P

Unravelling the Core and Accessory Genome Dynamics of Enterobacteriaceae in Carbon Metabolism

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Enterobacteriaceae are commonly colonizing the animal gut and impact public health. While strain-specific metabolic features can promote gut colonization, we lack systematic knowledge regarding the core metabolism shared among *Enterobacteriaceae*.

To address this gap, we have developed a bioinformatics pipeline to analyse the pan-genome of nearly 20,000 genomes across 16 *Enterobacteriaceae* genera (80 species). Our study sheds light on the organization and function of the core and accessory genome, with particular focus on energy metabolism. We found that for *Enterobacteriaceae*, as facultative anaerobes, genes necessary for monosaccharide-fuelled mixed acid fermentation are part of the core genome. In contrast, most genes for anaerobic respiration and most carbohydrate utilization genes belong to the accessory genome, likely reflecting their dependence on environmental availability of the respective substrates. Our analysis also reveals a high variability in carbohydrate utilization systems ranging from just 3 in a symbiotic *Serratia* species to 40 in an *Escherichia coli* strain, providing a framework for understanding why different *Enterobacteriaceae* strains can co-exist in the same intestine.

Understanding *Enterobacteriaceae's* metabolic capacity helps clarify the distinction of nutrients consumed by all *Enterobacteriaceae*, and niche-defining nutrient sources, which are strain- or lineage-specific. Our results shed light on bacterial nutrient exploitation during gut colonization in health and disease, aiding in the development of targeted interventions for microbiome research and infectious disease control. The theoretical framework described here could be adapted to analyse core physiological characteristics of other important microbial taxa from diverse environments in the future.

Abstract ID: 4012 / Poster Number: 425.16F

Unravelling the crosstalk between phosphate starvation response and immunity during legume-pathogen interactions

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Phosphate (Pi) is an essential macronutrient that plays a crucial role in plant growth and development. However, Pi availability to plants is rarely sufficient to support crop yield. Pi-induced stress has been shown to positively or negatively interact with pathogen-induced immune responses, and this crosstalk between phosphate homeostasis and immune signaling is relatively unexplored, especially in legumes. Here, we aim to elucidate the crosstalk between Pi stress and immune signaling and its impact on disease resistance in *Medicago truncatula* infected with the powdery mildew pathogen *Erysiphe pisi*. We assessed plant growth and physiological traits in hydroponically grown *M. truncatula* plants exposed to Low Pi (LP) stress and Sufficient Pi (SP) levels. We found that the LP treatment negatively affects the plant growth traits such as shoot and root length and whole plant fresh weight. Further, we found a significantly higher root Pi content in the SP treatment compared to the LP treatment. Also, low phosphate induced the expression of phosphate starvation response (PSR) marker genes, including *SPX1*, *SPX3*, *MT4*, *PT6* and *PHR2*. A correlation between plant growth and expression levels of the PSR genes was observed in plants treated with low phosphate. Overall, the highest fold changes were observed for *SPX1* and *MT4*, suggesting their pivotal roles in Pi stress response. *PHR2*, a transcription factor, showed intermediate upregulation, consistent with its regulatory role in coordinating responses to Pi stress. We are currently investigating the level of disease resistance and the expression of defence marker genes in LP and SP-treated plants infected with *E. pisi*. We expect that PSR genes might be involved in moderating the defence against *E. pisi*. Overall, this study will reveal how plant immunity is regulated by the phosphorus status of the plant, providing novel insights into the fundamental processes underlying plant disease resistance.

Abstract ID: 3413

Unravelling the divergent effects of endocrine disrupting chemicals on human gut bacterial physiology and microbiome dynamics: driving properties for toxicity and detoxification strategies

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Chronic exposure to endocrine-disrupting chemicals (EDCs) adversely affects environmental ecosystems and human health, yet their interactions with the human gut microbiome remain largely unexplored. Xenobiotics may disturb gut microbiome, with some bacteria capable of metabolizing them and mitigating their toxicity. Our objectives were to (i) investigate the ecotoxicity of various EDCs on gut bacteria; (ii) examine how EDC-tolerant species may protect EDC-sensitive species; and (iii) evaluate EDC-induced shifts in gut microbial community dynamics. We selected 38 EDCs (13 phenols, 9 phthalates, 8 per- and poly-fluoroalkyl substances (PFAS), 8 pesticides) based on exposure patterns from the French couple-child cohort (SEPAGES) and epidemiological data (Fig.1A) and evaluated the effects of EDCs (30µM to 1000µM) on the growth of 40 commensal and pathogenic gut bacteria. The potential protective effects of EDC-tolerant bacteria on sensitive bacteria were tested by feeding their metabolites or co-culturing them using the Cerillo Duet System. Finally, we exposed faeces-derived microbial communities (microcosms) to 22 EDCs and characterized microbial diversity and composition using 16S rRNA gene sequencing and Quantitative Insights Into Microbial Ecology 2 suite. Monoculture assays revealed that PFAS or phenols reduced the growth of 20 species by at least 25% whereas phthalates affected 10 species. The toxicity of parabens and PFAS depended on chain length (the longer, the more toxic) while phthalates' toxicity on molecular weight (the lighter, the more toxic). The response towards EDCs were bacterial phylogeny and physiology dependent: Gram-negative, non-motile or obligate anaerobes were more vulnerable to EDCs than Gram-positive, motile, or microaerophilic (Fig.1B). Phylogenetically, Pseudomonadota was the most resistant phylum, while Bacteroidiota was the most sensitive. When co-cultured with 3 sensitive bacteria (e.g., *Bacteroides ovatus*), resistant strains either reduced (*Lactobacillus reuteri*) or exacerbated (*Streptococcus salivarius*) EDC toxicity (Fig.1C). In microcosms, triclosan, triclocarban, bisphenol B, butylparaben, and perfluorodecanoic acid significantly altered community structure (Fig.1D). The relative abundance of Bacillota

and Pseudomonadota species were significantly higher in EDC-exposed microcosms (Fig.1E). Microbial metabolism of butylparaben and diethyl phthalate reduced their bioavailability, resulting in a reduction in concentration from 25% to 75% depending on the microcosm. (Fig.1F). Persistent EDC exposure disrupts microbial ecology, underscoring the urgency of understanding adaptation mechanisms.

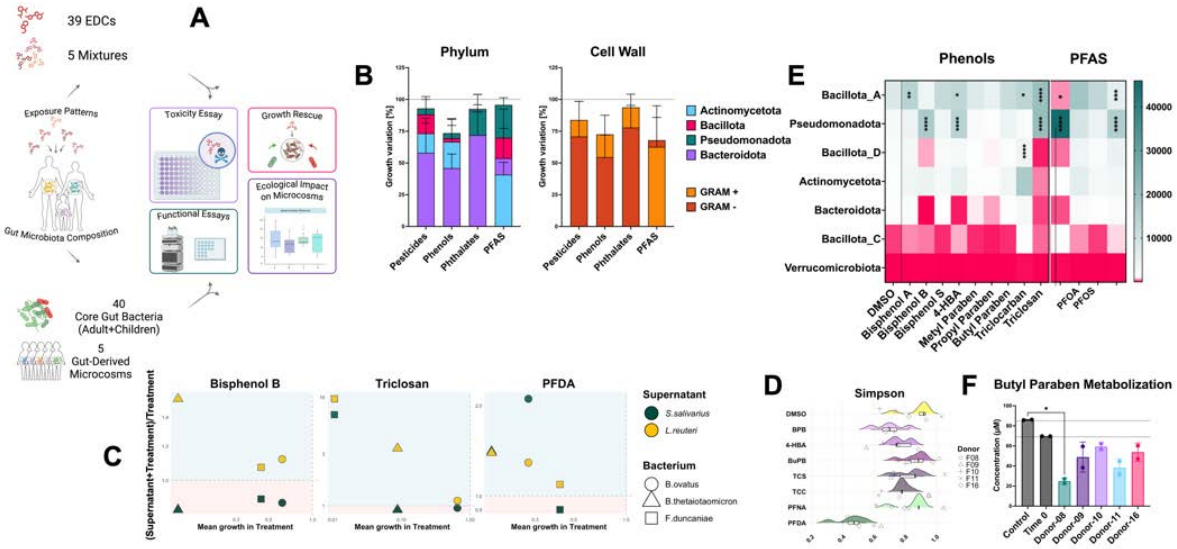


Figure 1: (A) An overview of the experimental design; (B) Effect of different EDC classes on the growth of different categories of gut bacteria; (C) Effect on growth rescue of co-culture with resistant strains; (D) Reduction of alpha diversity of human microcosms after EDC exposure; (E) Microbial signatures of exposure to EDCs; (F) Metabolization of butylparaben by human-derived microcosms.

Abstract ID: 2681

Unravelling the Microbial Community Shifts and Interactions in Polluted Coastal Sediments

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Coastal zones are highly vulnerable ecosystems, continuously exposed to pollution, particularly from anthropogenic activities, leading to the accumulation of pollutants in marine sediments. While microorganisms are known to rapidly respond to environmental changes, sediment microbial communities remain less explored than aquatic communities, especially in the Adriatic Sea. In stable marine ecosystems, microbial communities engage in complex interactions that support the functioning of the microbial food web.

This study aimed to: (1) examine alterations in microbial communities within polluted coastal sediments; (2) identify the key drivers shaping these microbial communities; and (3) evaluate potential disruptions of microbial interactions in the presence of pollutants.

We analysed 67 sediment samples collected from seven ports and bays along the Croatian coastline. Comprehensive characterization, including chemical and toxicity profiling, was conducted, and k-means clustering was used to categorize sediment samples into five levels of anthropogenic disturbance: low, mild, medium, high, and extreme. Total DNA was successfully extracted from 55 samples and amplicon sequencing targeted three microbial communities – prokaryotes, protists and fungi. Bioinformatic analyses were performed to determine alpha and beta diversity patterns, identify the effects of individual factors on microbial communities, and investigate interaction changes under heavy-metal pollution (Bi, Cd, Cu, Hg, Zn).

Our findings revealed that the abundance of specific microbial families changed in response to the level of anthropogenic disturbance, though these changes did not follow a straightforward trend. Biogeographic factors – particularly location – dominantly influenced all three microbial communities. Furthermore, the presence of heavy metals weakened interactions between microbial communities, particularly between prokaryotes and protists. Since these interactions are essential for the functioning of the microbial food web, their disruption could destabilize food web dynamics.

These findings represent a crucial step toward understanding the mechanisms underlying microbial community assembly, especially in benthic communities of coastal zones exposed to ongoing anthropogenic pressures.

Abstract ID: 2246 / Poster Number: 231.15P

Unravelling the structure-function of a tandem pair of glycoside transporters in the marine flavobacterium Zobellia galactanivorans DsijT

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Introduction: Members of the *Bacteroidota* phylum have colonized all types of habitats and substrate niches. A unique feature of *Bacteroidota* genomes is the presence of **Polysaccharide Utilization Loci** (PUL) which are clusters of co-regulated genes encoding a complement of cell surface glycan-binding proteins, **TonB-dependent transporters (SusCD)**, carbohydrate active enzymes and carbohydrate sensors/ transcriptional regulators. While the SusCD transporters are considered as the hallmark of PULs, only three of them have been structurally characterized so far[1,2,3].

Objective: The aim of the study is to deepen the understanding of the **SusCD transport system** in glycan uptake.

Methods and Findings: We work on the marine flavobacterium ***Zobellia galactanivorans*** **Dsij^T** which is a model organism for the bioconversion of algal polysaccharides[4]. We have identified a PUL which contains an adjacent pair of SusCD genes, defined as **tandem repeat SusCD** (trSusCD). A recent study suggests that they provide a selective advantage in environments where diverse dominant species compete for similar metabolic niches[5]. Nevertheless, the function and structure of trSusCD proteins have never been investigated so far. Transcriptomic data suggest that the two adjacent SusCD could target different substrates, as the PUL is upregulated in presence of laminarin, mixed-linked glucan and xylan[6]. This substrate versatility would expand our perceptions regarding PUL machineries, which so far have demonstrated gene organization that suggests one cognate PUL for each substrate type.

Conclusion: Investigating the structure-function relationships of these proteins by combining different approaches (genetics, biochemistry, crystallography/CryoEM) will provide novelty and a better understanding of PULs functionality.

Abstract ID: 2066

Unveiling Anaerobic Life: The Hidden Drivers of Biogeochemical Cycles in Supraglacial Cryoconite Ecosystems

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Introduction: Glacier environments, particularly cryoconite holes, represent unique ecosystems where microbial communities thrive under extreme conditions. These microhabitats play a crucial role in global biogeochemical cycles. While aerobic microorganisms have been extensively studied in cryoconite, recent evidence suggests that anaerobic microorganisms constitute a significant yet poorly understood component of these environments. Despite their potential contributions to carbon, nitrogen, and sulfur cycling, anaerobes have remained largely overlooked, partly due to methodological biases in microbial community studies.

Objectives: This study aims to address the knowledge gap surrounding anaerobic microorganisms in supraglacial environments by:

- Exploring the diversity and metabolic roles of anaerobic bacteria in cryoconite holes on an Alpine glacier.
- Highlighting their ecological significance and contributions to biogeochemical processes.
- Demonstrating the complex interactions within anaerobic microbial networks in these environments.

Methods: Cryoconite sediment samples were collected from four cryoconite holes at multiple time points during a summer day. The study employed 16S rRNA gene and transcript analyses to characterize microbial diversity and activity. Shotgun metatranscriptomics further identified key metabolic pathways, focusing on anaerobic processes such as denitrification, fermentation, and syntrophy. Diversity indices, metabolic profiles, and community structures were analyzed to reveal the contributions of anaerobic taxa.

Findings: Anaerobic microorganisms were found to constitute a significant and active fraction of the microbial communities in cryoconite holes, contradicting earlier

assumptions about these environments being predominantly oxygen-rich. Genera such as *Clostridium*, *Propionibacterium* and *Syntrophobacter*, were identified, representing diverse metabolic capabilities, including fermentation, sulfate reduction, and syntrophic hydrogen metabolism.

The findings suggest that cryoconite holes harbour stratified redox conditions, with anaerobic niches supporting unique microbial interactions. Syntrophic relationships among fermentative bacteria, hydrogenotrophs, and methanogens highlight the ecological complexity of these habitats. These processes contribute to the cycling of organic matter and other critical biogeochemical functions, showcasing the significant yet underappreciated roles of anaerobic communities in supraglacial ecosystems.

Conclusion: This study provides new insights into the overlooked diversity and ecological roles of anaerobic microorganisms in cryoconite environments. The findings emphasize the importance of these taxa in biogeochemical cycling and call for a broader focus on anaerobic processes in future studies of supraglacial ecosystems. As glaciers continue to recede due to climate change, understanding the dynamics of these ecosystems is critical for assessing their global impact.

Abstract ID: 2563 / Poster Number: 248.15P

Unveiling Hub Protein Properties in Eubacteria

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Background

Hub proteins play a pivotal role in cellular regulation by interacting with numerous other proteins. Understanding the unique properties that enable these proteins to bind multiple partners is crucial for elucidating their functional roles.

Objectives: This study investigates whether bacterial hub proteins share common features that predispose them to interact with many binding partners.

Methods: Hub and non-hub proteins were extracted from three interactome databases, covering a diverse range of evolutionarily distant bacterial species. Subsequent analyses focused on determining the enrichment of Gene Ontology (GO) terms, levels of intrinsic disorder, and amino acid composition.

Results: The analysed hub proteins participate in various cellular processes, spanning a wide array of gene ontology terms. This suggests that their hub properties may be attributed to intrinsic protein features. The results revealed that intrinsically disordered regions are one of the main determinants of whether a protein functions as a hub. Across all studied proteomes, hub proteins showed a significantly higher prevalence of both short and long disordered regions compared to the overall proteome and non-hub proteins. These regions often corresponded to interactive loops or extended unstructured domains at the N- and C-termini. The extent of disorder was also found to be highly dependent on bacterial GC content and proteome size. Additionally, amino acid analysis of these disordered regions revealed a significant enrichment of lysine and arginine residues, which likely contribute to hub proteins' ability to interact with multiple partners and regulate diverse biological processes.

Conclusions: This study highlights the distinctive characteristics of bacterial hub proteins, particularly the prevalence of intrinsically disordered regions and specific amino acids, as critical factors enabling their functional versatility in cellular regulation.

Abstract ID: 3308 / Poster Number: 174.17P

Unveiling the Dynamics of Antibiotic Resistance in Clinical Enterococcus: Insights into Species and Infection-specific Patterns

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Co-authors: *Joana Monteiro Marques, Mariana Coelho, Cinthia Alves-Barroco, Maria Teresa Barreto-Crespo, Teresa Semedo-Lemsaddek*

Enterococcus spp. are bacteria present in the gastrointestinal tract of humans and animals, the environment, and are emerging pathogens causing serious healthcare-associated infections. *Enterococcus faecalis* and *E. faecium* are most clinically significant, with vancomycin-resistant *E. faecium* posing a high-priority threat due to limited treatments. This study aimed to characterize *Enterococcus* spp. from clinical infections and evaluate antibiotic resistance phenotypes in relation to species and infection sites.

Over five months, 148 enterococci were collected from six healthcare facilities in Portugal, covering diverse clinical sources, including urinary/genital tract infections, purulent infections, systemic infections, and other sources. Following presumptive genus confirmation on selective media, 85 genetically distinct isolates (urine and vaginal swab n=45, pus n=25, blood n=8, others n=7) were selected using RAPD-PCR and identified by species-specific multiplex PCR. Antibiotic susceptibility to 11 clinically relevant antibiotics was assessed following CLSI guidelines.

Among the identified isolates, 86% were *E. faecalis*, 9% *E. faecium* and 5% *E. durans*. High levels of resistance were observed, particularly to quinupristin-dalfopristin (84%, of which 92% are *E. faecalis*; >75% across infection sites) and erythromycin (reaching 60% in enterococci from purulent infections). Vancomycin resistance was detected in 27% of isolates (50% in *E. durans*). Resistance to β-lactams, oxazolidinones, quinolones, tetracyclines, and aminoglycosides was also identified. Species-specific resistance patterns identified *E. faecalis, E. faecium*, and *E. durans* as critical contributors to multidrug resistance.

This research underscores the importance of ongoing surveillance of antibiotic resistance in *Enterococcus*, opening new insights on the role of species and infection sites in shaping resistance phenotypes.

Abstract ID: 1434 / Poster Number: 290.17P

Unveiling the Fatty Acid and Carotenoid Potential of Antarctic Snow Algae

Presenting author: Dr. Sung Mi Cho, Korea Polar Research Institute, INCHEON, South Korea; University of Science and Technology, INCHEON, South Korea

Co-authors: *Jeongha So, Jong Hee Kim, Namju Lee, Sanghee Kim, Kitae Kim*

Colored snow patches represent a fascinating phenomenon observed in polar and alpine regions, where microalgae known as snow algae proliferate. These organisms play a crucial role as primary producers, significantly influencing nutrient and carbon cycling within both snow and terrestrial ecosystems. This study analyzes the fatty acid and carotenoid profiles of five snow algae isolated from snow patches in the Barton Peninsula of Antarctica: *Micractinium simplicissimum*, *Raphidonema nivale*, and three candidate species from the genera *Sphaerocystis*, *Chloromonas*, and *Tritostichococcus*. Optimal growth temperature analyses revealed that *R. nivale* and *Tritostichococcus* sp. are psychrophilic, while *M. simplicissimum*, *Sphaerocystis* sp., and *Chloromonas* sp. are psychro-tolerant, thriving at temperatures of up to 25°C. The two psychrophilic species contained long-chain polyunsaturated fatty acids (LC-PUFAs), including C20:4 (arachidonic acid, AA) and C20:5 (eicosapentaenoic acid, EPA). In contrast, the psychro-tolerant species exhibited high PUFA content, comprising 69% to 73% of their total fatty acids. Metabolomic analysis identified astaxanthin, a characteristic pigment of red snow algae, along with diadinoxanthin and dopaxanthin, carotenoids typically found in diatoms and plants. Based on these findings, *R. nivale* appears to be the most promising strain for the production of PUFAs and carotenoids (astaxanthin and diadinoxanthin) under low-temperature conditions. .

Abstract ID: 2707 / Poster Number: 252.15P

Unveiling the genetic mechanisms of bioluminescence in Omphalotus guepiniiformis: Insight into stress-dependent light production of fungi

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Co-authors: *Eung-Jun Park, Yeongseon Jang, Kang-Hyeon Ka, Rhim Ryoo*

Bioluminescence refers to the production of light in living organisms that results from the luciferase-catalyzed oxidation reaction of luciferin. Recent studies in molecular biology and comparative genomics have shed light on the genes associated with fungal bioluminescence, as well as the evolutionary trajectories of their clusters. Nonetheless, most studies on fungal bioluminescence have primarily focused on quantifying light intensity under diverse conditions. To gain insights into the molecular mechanisms underlying the bioluminescent responses of *Omphalotus guepiniiformis* under environmental fluctuations, we conducted the cloning and sequencing of the genes encoding hispidin synthase, hispidin-3-hydroxylase, and luciferase enzymes, all of which play critical roles in the bioluminescence pathway of fungi. Each gene exhibited a high sequence similarity to those found in other luminescent fungal species. Next, the transcriptional alterations of these genes in response to abiotic stresses were investigated. Wound stress significantly increased bioluminescence intensity by upregulating the expression of genes associated with the bioluminescence pathway, whereas temperature stress resulted in a reduction of bioluminescence intensity through a non-transcriptional mechanism. These results indicate that *O. guepiniiformis* modulates its bioluminescent response in a stress-dependent manner. To the best of our knowledge, this study is the first investigation into fungal bioluminescence at the level of gene expression. Considering the available genome information and artificial cultivation method, *O. guepiniiformis* could serve as a model species for further studies on the biological and ecological meaning of fungal bioluminescence.

Abstract ID: 3589 / Poster Number: 452.15P

Unveiling the genomic diversity and ecological distribution of Spiribacter in hypersaline environments

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Co-authors: *Maria Jose Leon, Blanca Vera-Gargallo, Rafael R. De la Haba, Cristina Sanchez-Porro,*

Introduction: The genus *Spiribacter*, within the family *Ectothiorhodospiraceae* and class *Gammaproteobacteria*, includes halophilic bacteria widely distributed in hypersaline environments. These microorganisms are characterized by reduced genomes (1.7–2.2 Mb) with a single ribosomal operon, simplified metabolic pathways, and a G+C content of 62.7-66.0 mol%. Despite their relevant role in saline habitats, cultivation challenges have limited our understanding of their diversity and ecological function.

Objective: This study aims to expand the knowledge of the genomic diversity, ecological significance, and adaptive mechanisms of *Spiribacter* species through a combination of culture- and genomic-based approaches.

Methods: Twelve new halophilic strains isolated from solar salterns in Spain were recently isolated and described as three novel species. A comprehensive genomic diversity analysis of the genus was performed, integrating data from isolates and metagenome-assembled genomes (MAGs) available from public databases. Additionally, 16S rRNA gene amplicons and shotgun metagenomes were analyzed to investigate the environmental distribution and ecological role of *Spiribacter*.

Findings: Genomic analyses revealed that all members of the genus *Spiribacter* have streamlined genomes, optimized for survival and adaptation to hypersaline conditions, with genes supporting osmoprotective mechanisms and metabolism of carbon and sulphur compounds. This study highlights the ecological role of members of *Spiribacter* in hypersaline environments and their genomic adaptations. Although originally isolated from Spanish salterns, their biogeographical distribution is quite wide, with special occurrence in intermediate saline habitats.

Conclusion: This research provides significant insights into the genomic diversity and ecological role of *Spiribacter*, advancing the knowledge of their adaptive mechanisms in hypersaline ecosystems.

Abstract ID: 2278 / Poster Number: 419.16F

Unveiling the Tripartite Symbiosis between Arbuscular Mycorrhizal Fungi, Associated Bacteria, and Maize Using an Innovative In Vitro Whole Plant System

Presenting author: Mrs. Varsha ., The Energy Resource Institute, Gurugram, India; Deakin University, Geelong, Australia

Co-authors: *David Cahill, Lambert Brau, Mandira Kochar, Leena Johny*

Introduction: In natural ecosystems, bacterial biofilms play an essential role in interacting with surfaces, while arbuscular mycorrhizal fungi (AMF) significantly contribute to subsoil microbial biomass. The interaction between AMF and AMF-associated bacteria (AABs) presents a fascinating avenue for understanding plant growth and stress resilience.

Objective: This study aims to investigate the complex tripartite symbiosis between AMF, AABs, and maize plants using an innovative in vitro whole plant assay system to evaluate plant growth, root colonization, and biofilm formation.

Method: A novel two-compartment Petri plate system was developed to simulate the rhizosphere and track interactions between AMF (Rhizophagus irregularis), AABs (Pseudomonas myrsinacearum and (Priestia filamentosa), and maize. Plant growth parameters, root colonization, sporulation, and biofilm formation were monitored over a 30-day period.

Findings: The tripartite interaction significantly enhanced plant growth, with plants showing greater shoot and root lengths, thicker stems, and more root tips compared to controls. Notably, plants co-inoculated with R. irregularis and P. myrsinacearum exhibited superior growth compared to those with P. filamentosus. AMF colonization was highest with P. myrsinacearum (81.33%), and biofilm formation was observed on AMF hyphae and maize roots. Scanning electron microscopy revealed bacterial biofilms on AMF structures, confirming their role in the tripartite interaction.

Conclusion: This study presents a novel in vitro approach to explore the intricate dynamics of AMF, AAB, and plant interactions, highlighting their potential to enhance plant growth and ecological sustainability. The system offers valuable insights into the complex symbiosis that could aid future agricultural strategies.

Abstract ID: 3074 / Poster Number: 448.15P

Unveiling type II toxin-antitoxin systems in hybrid Escherichia coli strains: an in silico approach

Presenting author: Ms. Jessika Silva, Butantan Institute, São Paulo, Brazil

Co-authors: *Eneas Carvalho, Alejandra Del Carpio, Waldir Elias, Danielle Munhoz, Roxane Piazza*

Toxin-antitoxin (TA) systems are intricate genetic modules that play pivotal roles in various bacterial processes, including cell formation, persistence, biofilm formation, and stress responses. These systems comprise toxins that inhibit essential physiological processes and antitoxins that protect the cell. Despite their importance, the functions of TA systems in bacterial chromosomes remain poorly understood, emphasizing the need for further characterization.

This study aimed to investigate the prevalence of TA systems in *Escherichia coli* (*E. coli*) genomes, with a focus on a hybrid strain of atypical enteropathogenic and extraintestinal *E. coli* (BA1250). Utilizing *in silico* analysis tools, BLAST, TADB database, and TAfinder 3.0, we identified 26 TA loci in BA1250, with only one locus, SrnC-SrnB, located on a plasmid.

Expanding our analysis to 53 genomes from various *E. coli* pathotypes, including enteropathogenic *E. coli* (EPEC), extraintestinal *E. coli* (ExPEC), and hybrid strains, revealed a significant presence of TA systems. Notably, TA systems such as CcdB-CcdA, YhaV-PrlF, MazE-MazF, YoeB-YefM, and PasT-PasI were prevalent in hybrid strains, appearing in 69.2% of them.

Phylogenetic analysis demonstrated that BA1250 and most hybrid strains belonged to phylogroup B2, while others were distributed among phylogroups B1, F, and A. Interestingly, hybrid strains exhibited a higher frequency of TA systems compared to EPEC, suggesting a potential adaptive advantage under stress conditions.

These findings highlight the diversity and significant presence of TA systems in pathogenic *E. coli*, particularly in hybrid strains. Our study provides valuable insights into the genetic adaptability and potential roles of TA systems in bacterial pathogenesis.

Abstract ID: 487

Upcycling PBAT-PLA Mulch Films: Harnessing Rhodococcus opacus for converting Bioplastic into Triacylglycerols

Presenting author: Dr. Upasana Pal, RWTH Aachen University, Aachen, Germany

Co-authors: *Denise Urban, Lars Blank, Sihyun An*

Introduction: The accumulation of biodegradable PBAT-PLA mulch films in agricultural fields can pose significant environmental challenges, these films often persist in soils. Furthermore, the carbon loss might in the future not be acceptable, undermining also their intended sustainability, hence asking for innovative waste management solutions. This study addresses this gap by exploring a biotechnological strategy to enzymatically hydrolyze PBAT-PLA mulch films and upcycle the resulting monomers into high-value bioproducts, such as triacylglycerols (TAGs), using the oleogenic bacterial strain *Rhodococcus opacus* DSM 43250. This approach integrates enzymatic and microbial processes to promote sustainable waste valorization.

- **Objectives:** Hydrolyze PBAT-PLA mulch films into monomeric components using the PETase enzyme.
- Investigate the metabolic capacity of *R. opacus* to utilize these monomers as substrates.
- Enhance the strain's substrate utilization through Adaptive Laboratory Evolution (ALE).
- Demonstrate the conversion of monomers into TAGs as value-added bio-based products.

Methods: PBAT-PLA mulch films were enzymatically hydrolyzed using PETase to produce monomers, including 1,4-butanediol (BDO), adipic acid (AA), terephthalic acid (TA), and lactic acid (LA). The hydrolysate was supplemented with Delft Minimal Medium, maintaining monomer concentrations at 14.5 mM, 10.4 mM, 8.7 mM, and 4.1 mM for BDO, AA, TA, and LA, respectively. The growth and metabolic activity of the wild-type (WT) *R. opacus* and its ALE-derived strains variants were assessed. Physiological parameters, including growth rates and substrate utilization profiles, were monitored. Nitrogen-limited conditions were used to evaluate TAG production, with product characterization performed via high-performance liquid chromatography.

Findings: *R. opacus* demonstrated efficient metabolism of the enzymatically hydrolyzed monomers, exhibiting simultaneous utilization of all four substrates. The WT strain achieved growth rates of 0.18 h^{-1} (BDO), 0.48 h^{-1} (AA), 0.14 h^{-1} (TA), and 0.27 h^{-1} (LA). ALE strategies significantly enhanced substrate utilization, yielding strains with almost doubled growth rates of 0.32 h^{-1} on TA and 0.46 h^{-1} on LA. Under nitrogen-limited conditions, the strains produced diverse TAGs with chain lengths ranging from $C_{14\text{-}20}$ and varying degrees of saturation. These findings highlight the feasibility of the approach, with implications for plastic waste valorization and bio-based product synthesis.

Conclusion: This study demonstrates a novel biotechnological approach to addressing the environmental challenges posed by PBAT-PLA mulch films. By combining enzymatic hydrolysis with microbial metabolism, *R. opacus* was successfully leveraged to upcycle plastic-derived monomers into TAGs. This work contributes to sustainable waste management by offering a pathway for converting plastic waste into high-value bio-based products. The strategy aligns with global efforts to promote circular bioeconomy principles, providing benefits for agriculture, biotechnology, and waste management sectors.

Abstract ID: 4081 / Poster Number: 426.16F

URMICRO – A Brazilian Microbial Resource Unit for Biodiversity Preservation

Presenting author: Prof. Luis Batista, Federal University of Lavras, Lavras, Brazil

Co-authors: *Harisson Souza, Miriam Santos, Kelly Pereira, Fabiana Passamani*

The Microorganism Culture Collection of the Department of Food Science (CCDCA) at the federal University of Lavras (UFLA) was stablished in 2010 to preserve representatives of Brazilian microbial biodiversity. Due to the growing demand for fungal, yeast, and bacterial isolates, the collection underwent restructuring in 2020, leading to the implementation of the Microbial Resource Unit (URMICRO) under the CNPq-funded Taxonomy Training Program. Located at the Biodiversity and Genetic Resources Center (CEBioMinas) and affiliated with the Institute of Natural Sciences (ICN/UFLA), URMICRO is accredited as a Trustworthy Depository by the National Council for Genetic Heritage (CGEN) and registered in the National System for Genetic Heritage Management (SisGen, C356129). URMICRO currently maintains approximately 1650 filamentous fungi and 250 yeast isolates from diverse substrates, including native soils (Atlantic Forest, Cerrado, and Caatinga), agricultural soils (coffee and grapevines), and food products (cheese, cured meats, grapes, nuts, and grains). The collection includes genera such as *Aspergillus, Penicillium, Talaromyces, Cladosporium, Mucor, Geotrichum, Paecilomyces, Candida, Kluyveromyces, Yarrowia, Debaryomyces, Trichosporon*, and *Pichia*. Preserving microbial diversity is more important than ever, as climate change and environmental shifts threaten microbiota stability. By maintaining these microorganisms, URMICRO ensures the availability of essential strains for biotechnology, agriculture, food safety, and sustainability. Beyond preservation, it fosters innovation and scientific discovery, contributing to sustainable solutions for global challenges. Providing access to well-preserved microbial cultures is vital for advancing research, strengthening biotechnology, and promoting environmental resilience.

Abstract ID: 1697 / Poster Number: 178.16P

Use of circulating host miRNAs as biomarkers for plague diagnosis

Presenting author: Mrs. Emelyne Bougit, Institut Pasteur, PARIS, France

Co-authors: *Pierre Lê-Bury, Olivier Dussurget, Javier Pizarro-Cerdà*

Yersinia pestis is the etiological agent of plague, a vector-borne zoonotic disease, which caused massive epidemics throughout history and remains a global health concern. Left untreated plague can lead to 100% mortality in 48 hours. Therefore, early diagnosis is a major stake for clinicians. However, current diagnostic tests targeting bacteria have major limitations. MicroRNAs (miRNAs) have emerged as promising candidates for diagnostic tests due to their role in infectious diseases and stability in biofluids. Thus, our study was designed to identify circulating host miRNAs as early biomarkers of plague.

We screened 172 systemic miRNAs with RT-qPCR panels during murine plague, identifying 8 promising biomarkers. However, this targeted approach based on commercial panels was non exhaustive and biased. To establish an unbiased and comprehensive host miRNAs signature during plague progression, we implemented miRNA-sequencing for discovery of circulating miRNA and specific RT-qPCR for validation of their levels in blood. The miRNA profiling is being performed in murine models recapitulating bubonic, pneumonic and septicemic plague at early and late timepoints. The selected miRNAs will be used to develop a simple, robust and reliable tool for point-of-care diagnosis in remote areas. Additionally, the functional role of the selected miRNAs will be investigated by silencing specific miRNAs *in vitro* and *in vivo* and by monitoring host response during infection. Our study represents a first step towards the development of innovative diagnostics and therapies, such as immunomodulatory agents, to improve patient care.

Abstract ID: 1815 / Poster Number: 158.16P

Use of Treponema pallidum molecular diagnostics to assess oropharyngeal lesions in patients with clinically unsuspected diagnosis of syphilis

Presenting author: Ivva Philipova, PhD, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

Co-authors: *Venelina Milanova, Elena Birindjieva, Viktoryia Levterova*

Introduction: Syphilis is a sexually transmitted infection caused by *Treponema pallidum* and the incident rate has been rising in Europe during the past years. The diagnosis of syphilis can be challenging due to the various clinical manifestations at different stages and atypical lesions can be mistaken for symptoms of other diseases.

Objective: To report our experience with three cases with oropharyngeal lesions, clinically thought to have oral cavity cancer, in which an unsuspected diagnosis of syphilis was made.

Method: Clinical samples from oropharyngeal lesions were sent for laboratory testing as part of the differential diagnostic workup for suspected oral cavity cancer patients. According to history molecular diagnostics of sexually transmitted genital ulcer disease (GUD) was performed.

Findings: Molecular diagnostics revealed the presence of *T. pallidum* in three cases with severe sore throat, difficulty swallowing, swollen lymph nodes in the neck, and ulcerative lesions in the oral cavity. Subsequently, a diagnosis of syphilis was confirmed clinically and by serology. After adequate antimicrobial treatment, the complaints were resolved within several weeks.

Conclusion: Syphilis is still a relevant differential diagnosis of oropharyngeal lesions, but it is clinically often not suspected. Therefore, the presence of oropharyngeal lesions and compatible patient history should alert the physician, and samples from suspicious lesions should also be examined for syphilis alongside histopathology.

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Abstract ID: 1646 / Poster Number: 095.17P

Using an "artificial fitness gradient" to unravel the effects of population-wide fitness variation on the cost of antibiotic resistance

Presenting author: Mr. Jack Duxbury Barber, University of Aberdeen, Aberdeen, United Kingdom

Co-authors: *Tom Vogwill*

Understanding microbial evolutionary dynamics is key to anticipating and countering the emergence of antimicrobial resistance (AMR). However, the disconnect between in vivo observations of the fitness cost bacteria pay to maintain resistance in the absence of antibiotics and the cost observed during in vitro evolutionary experiments remains a longstanding challenge in AMR research. One potential explanatory factor for this discrepancy is the wide variation in fitness within "real-world" bacterial populations (which could produce a corresponding variation in the cost of antibiotic resistance), contrasting the lack of variation in fitness within clonal lab populations of bacteria. We aim to investigate the effect of fitness variation within bacterial populations on the cost of antibiotic resistance; to do this, we have created an "artificial fitness gradient" using classical experimental evolution techniques. By isolating populations of *E. coli* at regular time-points over a period of adaptation to unfavourable growth conditions, multiple sub-populations with variations in fitness have been generated. Each of these sub-populations will be made antibiotic-resistant, and the fitness cost of antibiotic resistance for the different sub-populations will then be quantified. We anticipate that fitter sub-populations will pay a higher cost to evolve and maintain antibiotic resistance, and less-fit sub-populations will pay a comparatively lower cost. Any such variation across the artificial fitness gradient will confirm the hypothesis that variations in fitness within bacterial populations affect the cost of antibiotic resistance and will help to unravel why in vitro experimental evolution work fails to capture the true fitness cost of antibiotic resistance.

Abstract ID: 2702 / Poster Number: 070.16P

Using functional metaproteomics to identify novel SCFA ester-degrading enzymes associated with gut microbiota and neuroimmune diseases

Presenting author: Ms. Marina Prisacar, Ruhr-Universität Bochum, Bochum, Germany

Co-authors: *Lars I. Leichert*

Introduction: Growing evidence suggests that reduced amounts of short-chain fatty acids (SCFAs) are associated with neuroimmune diseases like multiple sclerosis. Investigating gut microbiota-derived SCFA-degrading enzymes could enhance our understanding of the gut microbiome's role in these pathologies. To discover such enzymes, we apply a functional metaproteomics approach. Here, all proteins expressed in a microbial community are screened for a desired activity using in-gel zymography, and then selectively analyzed by mass spectrometry, using metagenomic data for protein identification. This workflow requires a reliable in-gel zymography assay that detects lipolytic enzymes that hydrolytically cleave SCFA esters.

Objective: Establishing and benchmarking an in-gel zymography-based assay for identifying gut microbiota-derived enzymes that hydrolyze SCFA esters, enabling a functional metaproteomics workflow.

Method: SCFA ester-hydrolyzing enzymes were visualized using the substrates 4-methylumbelliferyl butyrate (4-MUB) and 4-methylumbelliferyl propionate (4-MUP). A commercial lipase from *Aspergillus oryzae* served as positive control, and protein extracts from gut microbiota samples were screened for activity.

Findings: This assay demonstrated high sensitivity, reproducibility, and efficiency, detecting enzyme amounts below 0.1 µg and quantifying activity over three orders of magnitude. SCFA ester-degrading enzymes were detected in gut microbiota samples.

Conclusion: A robust in-gel zymography assay was successfully established to detect SCFA ester-hydrolyzing enzymes in gut microbiota protein extracts, providing a crucial prerequisite for the application of the functional metaproteomics workflow to investigate patient samples.

Abstract ID: 4151

Using genomics and systems biology for the design of the next generation of yeast probiotics

Presenting author: Duccio Cavalieri, Department of Biology, University of Florence, Florence, Italy

Session Advances in Yeast Biotechnology.

In this presentation we will summarise recent findings on the role of *Saccharomyces cerevisiae* in probiotic theraphy.

The yeast *Saccharomyces cerevisiae* is a microorganisms generally recognized as safe that has been used for centuries in the production of fermented products such as bread, beer, wine and cheeses like Kefir. A number of *S.cerevisiae* strains have been proposed as domesticated, due to their association with human industries, while others have been isolated from natural sources.

A strain belonging to the Saccharomyces cerevisiae genus, *Saccharomyces boulardii* first isolated in 1923 by French scientist Henri Boulard from lychee and mangosteen fruit, is one of the few probiotic with claims recognized from EFSA. This Saccharomyces cerevisiae strain has specific traits associated to the survival in the human host. S.*boulardi* has been shown to survive the passage through the stomach and the gastrointestinal tract, to grow at 37°C, to be resistant to bile acids, proteolytic degradation, antibacterial and antibiotic treatments, and to be able to treat travelers Diarrhea. In this presentation we show that adaptation to the human host is common to several *S.cerevisiae* strains. *S.cerevisiae* is associated to the consumption of fermented foods or fruits, following ingestions *S.cerevisiae* persists in the host's gut for Metagenomic analyses indicate that *S.cerevisiae* is found at a higher frequence in individuals from Africa and regions where the consumption of fermented foods is more frequent, in Crohn's patients, in the gut of divoltine insects such as Crabro wasps and social wasps, and that It is able to use the host immune system to fight battles with potentially pathogenic bacteria. This happens both trough production of acetic acid and also trough the induction of innate immune responses, trained immunity. Yet while the ability to survive the host gut is an essential trait for a probiotic, the ability to colonize the host can potentially be a threat in immunocompromised hosts. Finally we propose that several strains of *S.cerevisiae* have the potential to act as probiotics, and that an approach combining genomics, high throughput phenotyping, and the analysis of the strains immunogenic potential is a powerful strategy for the development of the next generation of probiotics.

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Abstract ID: 974 / Poster Number: 133.15P

Utilization of Alkaliphilic Bacteria in Mass Reduction and Degradation of Moromi-Kasu(Soy Sauce Lees) and Evaluation of Their Effects

Presenting author: Mr. Shunki Ota, Toyo Unversity, Asaka, Saitama, Japan

Co-authors: *Masahiro Ito*

Moromi-Kasu(soy sauce lees) is a byproduct of the manufacturing process of soy sauce, a traditional Japanese seasoning. Approximately 60,000 tons of Moromi-Kasu are produced annually (1). Although often reused as livestock feed, its high salt content and roughly 25% crude fiber composition make its reuse challenging.

This study aimed to reduce the volume of Moromi-Kasu through a biorefinery approach and investigate the effective utilization of the residue after degradation by alkaliphilic bacteria. A total of 21 strains of alkaliphilic bacteria were isolated from soil samples using an alkaline medium containing Moromi-Kasu supernatant. To evaluate their ability to degrade Moromi-Kasu, powdered Moromi-Kasu was added to the medium, and degradation process was visually compared to a sterile control.

The residual rate of Moromi-Kasu in the culture liquid was measured every three days using a membrane filter. Among the 23 strains tested, which including two known alkaliphilic bacteria, strain A5 demonstrated the highest degradation performance,achieving a residual rate of 20% after eight days. The culture liquid obtained after the degradation process by strain A5 was filtered and sterilized,resulting in a 36% residual rate when added to fresh medium containing Moromi-Kasu. Enzyme activity tests revealed that strain A5 exhibited stable protease and amylase activity over time.

In conclusion, strain A5 effectively degrades Moromi-Kasu, indicating its potential for biorefinery applications.

Abstract ID: 973 / Poster Number: 390.16P

Utilization of Moromi-Kasu (Soy Sauce Lees) for Cost-Effective Polyhydroxyalkanoate (PHA) Production Using Alkaliphilic Bacteria

Presenting author: Ms. Jiarui Wang, Toyo university, Asaka, Saitama, Japan

Co-authors: *Ito Masahiro*

Polyhydroxyalkanoates (PHA) have attracted significant attention as alternative materials to petroleum-based plastics due to their excellent biodegradability, diverse structures, and physical properties like conventional plastics (1). However, the high production cost of PHA remains a challenge (2), and developing methods that utilize more cost-effective substrates has become an urgent priority.

Moromi-Kasu (soy sauce lees), a by-product generated during soy sauce production, is produced at a volume of approximately 60,000 tons annually in Japan (3). Due to its high salt and moisture content, its disposal poses a significant challenge. This study aimed to utilize Moromi-Kasu, an underutilized resource, as a nutrient medium for alkaliphilic bacteria to achieve efficient PHA production under highly alkaline conditions. Specifically, the supernatant of Moromi-Kasu, adjusted to high alkalinity, was used as the culture medium.

PHA accumulation was visualized by staining with Nile Red dye (4), and fluorescent microscopy confirmed the presence of red granules (PHA) within the cells. Furthermore, the PHA content was quantitatively analyzed using NaClO (5). As a result, the selected bacterial strain exhibited robust growth in the alkaline Moromi-Kasu medium. NaClO analysis revealed the maximum PHA accumulation and the optimal cultivation time, demonstrating the feasibility of efficient PHA production under these conditions.

This study highlights the potential of Moromi-Kasu (soy sauce lees) as a viable substrate for PHA production using alkaliphilic bacteria. The findings represent a significant step toward the development of cost-effective and sustainable PHA production technologies.

Abstract ID: 3569 / Poster Number: 066.15P

Utilizing Schizophyllum commune to Enhance Substrate Quality and Reduce Peat Usage in Sustainable Horticultural Practices

Presenting author: Ms. Rhedia Tehrin Proma, Erfurt University of Applied Sciences, Research Centre for Horticultural Crops, Erfurt, Germany

Co-authors: *Katja Burow, Philipp Franken, Erika Kothe*

Peat is a popular key component of growing media in conventional horticulture because of its high pH tolerance and ability to enhance water retention. However, the limited availability of peat and the environmental concerns surrounding its extraction necessitate the development of sustainable alternatives. This study investigated the use of *Schizophyllum commune*, a wood-decaying fungus, as a putative stabilizing agent to enhance substrate quality and reduce peat usage.

A substrate containing 50% peat, 25% each of green compost, and digestate was used to assess the impact on *Petunia hybrida* cv. "Mitchell" growth. *S. commune* was inoculated to both steamed and non-steamed substrates and plant growth was monitored until six weeks.

Various plant growth parameters, including biomass accumulation, shoot and leaf development, were similar between the two treatments, indicating that both substrate mixtures positively supported plant development. However, the analysis of nitrogen content in dry biomass revealed significant differences between the two treatments. This suggests that *S. commune* can influence nutrient dynamics within the substrate, potentially promoting plant growth independent of sterilization.

While further investigation is needed to fully understand these differences, the findings suggest the potential of *S. commune* as a sustainable and effective alternative for improving horticultural substrates' quality and reducing peat dependence. It can be a remarkable move towards more environmentally friendly and resource-efficient horticultural practices.

Abstract ID: 3868 / Poster Number: 399.17P

Valorising volatile fatty acids to produce carotenoids in purple non-sulphur bacteria

Presenting author: Simone Krings, PhD, University of Mons, Mons, Belgium

Co-authors: *Ruddy Wattiez, Baptiste Leroy*

Introduction: Anaerobic digestion (AD) has become a frequently used method to manage organic waste, producing biogas, containing around 50% methane and 50% CO_2, and digestate which contains ammonium and volatile fatty acids (VFA). Nevertheless, arresting the AD before methanogenesis, results in digestates with increased levels of VFA, which could enable their valorisation using purple non-sulphur bacteria (PNSB). The versatile metabolism of PNSB allows the production of microbial proteins, polyhydroxyalkanoates (PHA), hydrogen or carotenoids.

Objectives: First, the ammonium resistance of purple non-sulphur bacteria needed to be assessed, followed by the assimilation of VFA. Finally, the carotenoids produced were characterised.

Method: *Rhodospirillum rubrum* was cultured in a MELiSSA medium containing synthetic digestate, composed of 40% acetate, 20% propionate, 15% butyrate, 5% isobutyrate, 10% valerate and 10% isovalerate as carbon sources, while the ammonium concentrations were standard (35 mM NH_4Cl) or higher (70, 140, 210, 350 mM NH_4Cl). Classical microbiology techniques (including spectrophotometry and spread plate techniques) were used to determine the growth of the bacteria. Mass spectrometry (Sciex ZenoTOF 7600) was used to determine the assimilation of VFA and to characterise the carotenoids. The carotenoids produced were assessed for their antioxidant properties.

Findings: In the standard synthetic digestate medium, *Rs. rubrum* assimilated acetate and propionate first, (iso)butanoate and valerate next and isovaleric acid last (Fig. 1A). Compared to the standard medium, in synthetic digestate media containing 70 mM or 140 mM of NH_4Cl, the growth of *Rs. rubrum* was not hindered. However, statistically significant reduction of growth of *Rs. rubrum* was observed in culture media containing 210 mM or 350 mM NH_4Cl (Fig. 1B). After methanol extraction removing the bacteriochlorophyll, the main carotenoid spirilloxanthin (spx) was extracted from the bacteria using hexane. The MS/MS spectra of spx revealed fragments at m/z = 295.19 and 597.44.

Conclusion: *Rs. rubrum* was able to survive to up to 140 mM NH_4Cl without any reduction in growth. The preferential utilisation of certain VFA compared to others will be investigated using proteomic analyses. Following the optimisation of the methods, digestate from an anaerobic digestion using kitchen waste will be added to the bacterial cultures to assess its effects on the production of carotenoids.

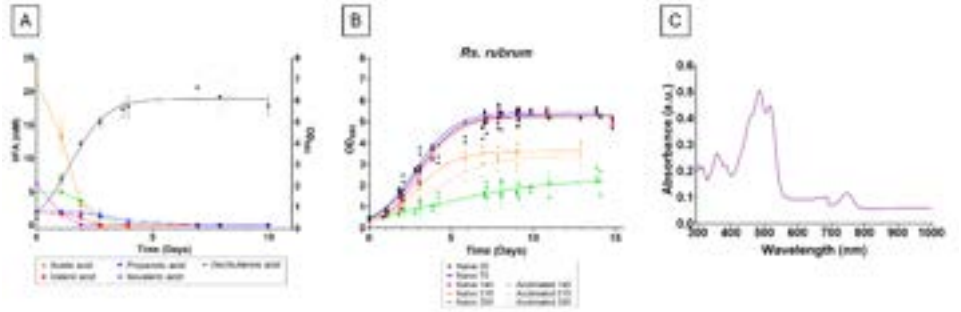


Figure 1: (A) The growth of *Rhodospirillum rubrum* in the synthetic digestate medium (OD_{680}) (right y-axis) and assimilation of the volatile fatty acids (VFA) (left y-axis) over time. Mean ± SD, n = 5. (B) *Rs. rubrum* grows with levels of ammonium of up to 140 mM NH_4Cl without any inhibition. Mean ± SD, $n \geq 3$. (C) Absorption spectrum of spirilloxanthin, the main carotenoid isolated from *Rs. rubrum*.

Abstract ID: 3554 / Poster Number: 343.17P

Valorization of coffee pulp for biobutanol production through anaerobic fermentation by Clostridium acetobutylicum ATCC 824

Presenting author: Prof. Silvia Mau Incháustegui, Universidad Nacional, Heredia, Costa Rica

Co-authors: *Allison Granados Ruiz, Saul Mora Barquero, Jorengeth Rodríguez Rodríguez*

Energy demand is increasing and dependence on fossil fuels is affected by economic, political and environmental factors. Therefore, finding alternative energy sources is becoming a key challenge. Among biofuels, biobutanol represents a potential solution, however, substrates price hinders its production. A prospective fix is to use raw materials such as coffee agroindustrial waste, only 5% of the fresh coffee fruit's weight is utilized for the drink; while the remaining 95% becomes waste.

The aim of this work was to investigate the feasibility of using coffee waste as a substrate to produce biobutanol.

Dry coffee waste was thermochemical pretreated with dilute acid followed by an enzymatic hydrolysis to obtain fermentable sugars. Anaerobic batch fermentation with Clostridium acetobutylicum ATCC 824 was then carried out in a culture medium with 25% and 50% of the hydrolysate to produce biobutanol. Fermentation in a defined medium was used as control. Hexoses, pentoses and biobutanol was determined by HPLC.

Experimental results showed that coffee waste hydrolysate had: 20.42 g/L glucose, 2.62 g/L hemicellulosic sugars and 0.56 g/L arabinose. Specific growth rate (μ) was higher in the fermentations with 25 and 50% of the hydrolysate (0.20 h-1 and 0.15 h-1, respectively) compared with the control (0.11 h-1). Biobutanol final concentration in a defined medium was 5.25 g/L, surprisingly, the fermentations with 25% and 50% of the hydrolysate had biobutanol final concentration of 5.55 g/L and 6.93 g/L, respectively.

This study showed that pretreated coffee waste can be used as an alernative and economic source to produce biobutanol.

Abstract ID: 2272 / Poster Number: 312.16F

Valorization of Hydrothermal Carbonization Liquid (HTC) residues for microbial polyhydroxyalkanoates (PHAs) production using Cupriavidus necator LMG-1209

Presenting author: Dr. Alessandra Filieri, Università degli Studi della Tuscia, Viterbo, Italy

Co-authors: *Federico Di Gregorio, Federico Stotani, Jasmina Jusic, Marco Barbanera, Silvia Crognale*

Waste valorization to obtain value-added products is a promising opportunity that has driven the development of strategies for waste conversion through microbial metabolism. Liquid fraction derived from the Hydrothermal Carbonization (HTC) process of biomasses; it is a byproduct of hydrothermal carbonization. This liquid fraction is a dark byproduct with a pH of 4.5, characterized by high COD, TOC, and nitrogen content, while phosphorus levels depend on process conditions. Its composition includes amides, alcohols, heterocyclic compounds, short-chain organic acids and substances such as phenols and furfurals are toxic to the environment and inhibit microbial growth.

This study utilized *Cupriavidus necator* to valorize HTC liquid waste derived from organic waste and landfill leachate through fermentation for PHA production. *C. necator* is a highly versatile Gram-negative bacterium, capable of growing on a wide range of organic substrates, including toxic compounds, due to its metabolic flexibility, under both aerobic and anaerobic conditions, adopting either heterotrophic or chemolithotrophic metabolism. This microorganism accumulates significant amounts of PHA.

In this study exploratory flask experiments demonstrated that *Cupriavdus necator* was able to grow on HTC liquid fraction, without any addition or dilution. PHA production was observed during the exponential growth phase at 48 hours of fermentation. Given its ability to utilize the liquid fraction *C. necator* was further tested in a fed-batch bioreactor system, achieving good microbial growth and a PHA yield. These findings indicate that *C. necator* has substantial potential to grow on this waste substrate, enabling its sustainable valorization once the fermentation process will be optimized.

Abstract ID: 445 / Poster Number: 262.17P

Valorization of lactic acid for omega-3 fatty acids production by the heterotrophic marine microalga Crypthecodinium cohnii

Presenting author: Ms. Katerina Foka, National Technical University of Athens, Athens, Greece

Co-authors: *Christina Ferousi, Anthi Karnaouri, Evangelos Topakas*

Heterotrophic microalgae have emerged as promising sources of polyunsaturated fatty acids, which are essential nutrients with significant benefits as functional foods for humans. Current research is focused on identifying cost-effective nutrient sources for these microalgae, ideally derived from industrial by-products and waste streams. The objective of this project was to investigate the utilization of lactic acid (LA), derived from biodegradable plastic waste and industrial food by-products, as a feedstock for the sustainable production of omega-3 fatty acids from the marine microalga Crypthecodinium cohnii. Batch cultures demonstrated the ability of C. cohnii to efficiently metabolize pure LA and accumulate significant amounts of docosahexaenoic acid (DHA), a valuable omega-3 fatty acid. The process was scaled up in a 2-L bioreactor under pH-auxostat LA dosing system. Growth was monitored daily via optical density (OD680nm), LA consumption was determined by liquid chromatography and lipid content was measured via gas chromatography. The optimized system successfully supported growth of C. cohnii with concomitant consumption of LA, and resulted in enhanced growth and DHA production compared to batch experiments, achieving DHA levels of over 30% of total lipids. Valorization of LA contained in waste streams as a substrate for fed-batch cultivation of C. cohnii are currently being tested to explore the potential of microbial upcycling into high-value compounds. This approach not only offers an environmentally friendly solution to waste management but also contributes to the development of alternative sources for omega-3 fatty acids.

Abstract ID: 1726

Valorization of pomegranate peel extract and its co-encapsulation with Saccharomyces boulardii CNCM I-745 postbiotic for enhanced antioxidant and antimicrobial activity

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Co-authors: *Ilkin Sengun*

Introduction: In the pursuit of a better quality of life, healthy and bioactive foods, including antioxidants and antimicrobial compounds, have become essential in the diets. The pomegranate peel, as an agro-food waste, has antioxidant and antimicrobial properties. However, *Saccharomyces boulardii* postbiotics possess anti-cancer, antioxidant, and gut barrier-enhancing properties. Encapsulation of these compounds enhances their efficacy, preserves their activity, and ensures targeted release.

Objective: This study aimed to encapsulate the postbiotic with pomegranate peel extract (PPE) in alginate beads using the ionic gelation method to enhance the antioxidant and antimicrobial activity of the postbiotic and to obtain a functional product.

Method: PPE was dissolved in distilled water at concentrations of 10 mg/mL, 50 mg/mL and 100 mg/mL and subsequently sterilised through a 0.45 µm filter. The extracts are designated as follows: CE1 (10 mg/mL crude extract), CE2 (50 mg/mL crude extract), and CE3 (100 mg/mL crude extract). Following the evaluation of the antimicrobial activity (agar diffusion method) of the extracts against *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella* Typhimurium, the PPE was dissolved in 10 mL postbiotic to a concentration of 50 mg/mL for the microencapsulation process. The microcapsules were then examined for encapsulation efficiency, particle size, total phenolic content, antioxidant activity, release profile under gastrointestinal conditions, and antimicrobial activity against *E. coli* O157:H7 and *S. aureus* as Gram (-) and Gram (+) representatives, respectively.

Findings: PPE was a significant source of total phenolic content and antioxidant activity (DPPH) with 397.53 mg GAE/g and 88.35%, respectively, and exhibited strong antimicrobial activity (15.5-22.0 mm for CE1; 17.5-26.0 mm for CE2; 21.0-29.5 mm for CE3) (Figure 1). However, postbiotic had a total phenolic content and antioxidant activity (DPPH) of 417.15 mg GAE/L and 38.23%, respectively while demonstrating no antimicrobial activity. The encapsulation efficiency of phenolic compounds was 31.0%.

The antioxidant activity of microcapsules was 50.98%, demonstrating that the PPE enhanced the antioxidant activity of the postbiotic. The microcapsules showed inhibitory effects on *S. aureus* and *E. coli*. The bio-accessibility of total phenolic content was 30.55% and 17.26% after gastric and intestinal digestion, respectively, indicating the release of a substantial quantity of phenolic compounds in the stomach.

Conclusion: The study results highlight the potential of producing microcapsules with high antioxidant activity and inhibitory effects, which can enhance the functional properties of various food products while promoting the circular economy and the use of wastes.

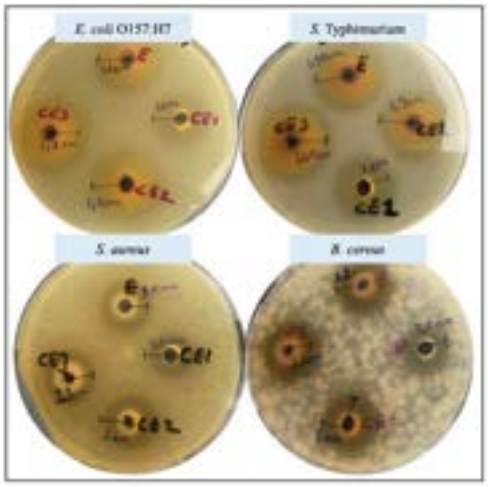


Figure 1: Antimicrobial activity results according to agar diffusion method

Abstract ID: 1845 / Poster Number: 417.16F

Variations and Correlations of Bacterial and Fungal Communities across Three Stages of Traditional Meju Fermentation

Presenting author: Dr. Eunhye Jo, Pusan National University, Busan, South Korea

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Meju, a main ingredient in Korean soybean food Doenjang and Ganjang, is produced through soybean fermentation with spontaneous microorganisms. Metagenomic analyses revealed diverse bacterial and fungal taxa contributing to flavor, aroma, and food safety, with microbial diversity varying based on manufacturing processes. While the crucial role of microorganisms in Meju fermentation is well established, studies on microbial interactions remain limited. This study aimed to predict bacterial-fungal interactions through correlation analysis during Meju fermentation stages. Physicochemical indicators, including pH, water content, and amino nitrogen levels, confirmed fermentation progression. Microbial diversity and abundance, assessed using Shannon and Chao1 indices, exhibited opposite patterns between bacterial and fungal communities. Amplicon-based sequencing identified Bacillaceae as dominant in bacterial communities and Rhizopodaceae and Mucoraceae in fungal communities. The absolute abundance pattern being merged with quantitative data revealed a significant positive correlation between Bacillaceae and Rhizopodaceae. These findings highlight microbial interplay and biotic roles in traditional Korean fermented products, advancing the understanding of fermentation processes and microbial interactions in soybean-based foods.

Meju fermentation, essential for Korean soybean foods like Doenjang, involves diverse microbial interactions. This study used metagenomics to identify dominants and found significant positive correlation based on absolute abundance data. Microbial diversity patterns and physicochemical changes highlight microbial interplay, offering insights into fermentation processes and microbial dynamics in soybean-based foods.

Abstract ID: 398 / Poster Number: 261.17P

Various basidiomycetous yeast strains capable of producing ergothioneine

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Co-authors: *Azusa Saika, Kazunori Ushimaru, Tatsuyuki Koshiyama, Yukihiro Higashiyama, Tokuma Fukuoka, Tomotake Morita*

Backgrounds: Ergothioneine (EGT) is a natural antioxidant of histidine derivative. It was originally found in Claviceps purpurea, and the main source of EGT are now fruiting bodies of some mushrooms. Recent studies revealed versatile functions of EGT for applications EGT in dietary supplements and pharmaceutics, and thus alternative processes for efficient production of EGT are to be developed.

Objective: This study aimed to evaluate capabilities of various basidiomycetous yeasts to produce EGT.

Methods: Sixteen strains of basidiomycetous yeasts grew on liquid cultures and then harvested cells were subjected to extract intracellular constituents. The extracts were analysed by liquid chromatography-mass spectrometry to quantify intracellular EGT.

Results: All the strains tested found positive on EGT production. Among them, Ustilago siamensis CBS9960 showed the greatest productivity at 49.5 ± 7.0 mg/L-culture in 120 h of cultivation. This strain increased EGT production in the presence of external histidine.

Conclusions: Various basidiomycetous yeasts can produce EGT naturally. Optimization of cultivation processes as well as strain engineering with these natural producers could be novel bioprocesses for industrial production of EGT.

Abstract ID: 2438 / Poster Number: 036.17F

Vat (vacuolating autotransporter toxin) plays a role in extraintestinal Escherichia coli survival in the blood of infected mice

Presenting author: Claudia Freire, PhD, Instituto Butantan, São Paulo, Brazil

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Introduction: Proteases produced by extraintestinal *Escherichia coli* (ExPEC) are versatile virulence factors, which can act at different stages of the infection. Among them, the serine protease Vat (vacuolating autotransporter toxin) is one of the most frequently found in isolates from bloodstream infections (BSI), suggesting an important role of this protease in this type of infection. While Vat cytotoxicity on different cell lineages has been demonstrated, immunomodulatory effects of this protease in a BSI context have not been investigated.

Objective: We aimed to investigate the relevance of Vat in the BSI outcome and its role in immune evasion.

Method: Survival rates of strains EC120 and EC120Δ*vat*, a Vat-producing ExPEC isolated from BSI and its respective *vat* mutant, were evaluated in a murine sepsis model and in an in vitro serum resistance assay. Culture supernatants of DH5α(pIJ551) and DH5α(pIJ551*) (hosts of Vat clone and non-functional Vat, respectively) were tested in proteolytic assays against complement proteins using immunoassays.

Findings: Infection with EC120 killed 60% of the mice after 72h, but all animals infected with the *vat* mutant survived during a seven-day observation period. However, no differences were observed between EC120 and EC120Δ*vat* survival rates after one hour of incubation with human serum and C3, C4, C5 and C6 cleavage products were detected more intensely only after 24h of incubation with DH5α(pIJ551) culture supernatant.

Conclusion: Our data suggests that Vat plays an important role in lethality during sepsis which does not appear to be linked to immunomodulation through proteolytic cleavage of complement system proteins.

Abstract ID: 3085

Versatile Blowers: A microbial investigation into the many roles of Blowflies (Diptera: Calliphoridae) in science and medicine

Presenting author: Madison Bone, PhD, Deakin University, Waurn Ponds, Australia

Co-authors: *Michelle Harvey, Andrew Oxley*

Blowflies (Diptera: Calliphoridae) participate in diverse trophic behaviours, including feeding on carrion as the primary source of soft tissue removal during decomposition [1]. Others act parasitically, a specific form of this known as myasis, where larvae feed on the soft tissues of a live host [2]. Advances in medical entomology have highlighted their advantages cleaning and healing properties as "surgical maggots" in debridement therapy [2]. Some of these behaviours can be linked to blowfly specie, though the green bottle blowflies, *Lucilia spp.,* are found to participate in all behaviours [3]. This versatility suggests that there may be another biomechanism that drives these behavioural modes. Recent microbiome studies explore the influence bacterial communities have on their host, and the subsequential behaviours [3,4]. In such, some studies have explored the microbial communities of scientifically relevant blowfly species independently, however, there lacks a global comparison including a collection of locally relevant and abundant blowfly species. Could the difference in constituent bacterial communities be the driving force behind the diverse applications of certain blowfly species?

This research aims to the explore and compare the microbial communities present in the salivary glands and the digestive tract (gut) of a selection of scientifically relevant blowflies, local to Victoria, Australia. This investigation compares *Lucilia sericata*, *Lucilia cuprina*, *Chrysomya rufifacies*, *Calliphora stygia* and *Calliphora augur*, salivary gland and gut bacterial populations and infer substantial diversity differences that may inform the vastness of abilities of these blowfly species. Furthermore, this study intends to identify key bacterial species that may act as biomarkers specific to each species, to be used in future as environmentally contributing specie predictors from foreign samples.

To assess organic microbial communities the selected blowfly species, solar traps were set over two consecutive flystrike seasons, 2023 – 2024 and 2024 – 2025, where wild blowflies trapped from various locations across Victoria and New South Wales, Australia. Collected specimens were identified to a species level, before dissections of the gut and salivary gland were performed. The bacterial community composition of the gut and

salivary glands was assessed by 16S rRNA Next Generation Sequencing and compared across the differing fly species.

Preliminary explorations indicate differences in global bacterial communities dependent on fly species, though there were some similarities between select species, likely from the shared feeding behaviours and cohabitation. Variations were present amongst the key contributing bacterial taxa of the salivary gland in comparison to the gut, independent of fly species, contributing to the characterization of organ specific assemblages. *Pseudomonadacae*, *Enterococcaceae* and *Proteus mirabilis* were identified as potential biomarkers to be further explored to understand their influence on feeding behaviours.

The variations in global bacterial communities between blowfly species may inform the diversified natural behaviours and further indicate the medical/scientific utility of these organisms. Furthermore, the unique microbial profiles of the salivary gland and gut indicate that though their shared involvement in food metabolism, the specifications of these roles are represented by the constituent microbial communities of the respective organs. An advanced exploration into the unique bacterial species specific to fly species and organ would permit a further understanding of the interaction between internal microbiome of blowfly specifies and unlock their potential for medical and scientific applications, whilst also increasing our understanding in blowfly-bacteria biology.

Abstract ID: 2312 / Poster Number: 356.15F

Versatile microbial community responsible for nitrate turnover in a carbonate aquifer in southwest Germany

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Co-authors: *Karsten Osenbrueck, Eva Blendinger, Hermann Rügner, Daniel Straub, Esteban Góngora, Robert Lehmann, Kai Uwe Totsche, Andreas Kappler, Peter Grathwohl, Sara Kleindienst*

Background: Groundwater in agricultural regions is often nitrate-contaminated, posing health risks like methemoglobinemia. Denitrifying microorganisms mitigate this by using nitrate as an electron acceptor, but the influence of hydrogeochemical factors such as oxygen, electron donors, and carbon sources on the activity of denitrifiers remains poorly understood.

Objective: To identify hotspots of nitrate turnover and reveal the electron donors essential for denitrification.

Methods: Limestone cores were aseptically retrieved by drilling from the anoxic saturated zone of the aquifer, reaching depths of 70 meters. Groundwater and rock samples were analyzed using 16S rRNA gene amplicon sequencing, qPCR, geochemical methods, and SEM-EDS to identify key microorganisms and potential electron donors driving denitrification.

Findings: Sequential Fe extraction revealed diverse Fe(II) forms within the rock matrix, with total concentrations reaching up to 19 mg/g. Pyrite (FeS_2) was identified as a main source of Fe(II), with crystals frequently exhibiting oxidized surfaces. Endolithic microbial communities were mainly detected in highly porous rock facies and included taxa capable of nitrate reduction coupled with H_2 oxidation (e.g., *Hydrogenophaga*), reduced sulfur compounds (RSCs) oxidation (e.g., *Sulfuritalea*, *Sulfuricurvum*, *Thiobacillus*), and Fe(II) oxidation (e.g., *Aquabacterium*, *Gallionella*). The RSCs as well as Fe(II) likely originated from pyrite. Groundwater microbial communities were dominated by *Hydrogenophaga* and *Aquabacterium* likely capable of autotrophic denitrification. Additionally, Fe(III)-mineral incrusted cells were observed in groundwater particulate matter.

Conclusion: Nitrate turnover in carbonate aquifers can be mediated by autotrophic microorganisms colonising fractured rock. Endolithic denitrifiers probably preferentially colonize highly porous rock facies enriched with bioavailable Fe(II).

Abstract ID: 360 / Poster Number: 182.15P

Versatile traits of green microalgae in sequestrating an excess of environmental manganese

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Co-authors: *Milan Zizic, Milena Dimitrijevic, Snezana Kovacevic, Isidora Santrac, Valentina Curic, Jelena Danilovic Lukovic, Marina Stanic,*

Microalgae are exposed to manganese excess in metal-infested waters and have to mitigate toxic effects at least in part by accumulating Mn in the form of chelates or clusters. On the other hand, Mn is a co-factor of different catalytic centers and some microalgal species appear to be capable of opportunistic accumulation. Herein, we compared the ways that different green microalgae accumulate Mn, using synchrotron radiation-based techniques – X-ray fluorescence microscopy (XRF) and X-ray absorption fine structure. High sublethal concentrations of Mn(II) have been applied in the early stationary phase of growth of cultures of Chlorella sorokiniana, Haematococcus pluvialis, Chlamydomonas reinhardtii, and Chlamydomonas acidophila. XRF microscopy generated maps of distribution of Mn and other elements within a single cell, which enabled the identification of location of Mn deposits on the subcellular level. In the early phase of the treatment, Mn was accumulated in the cell wall or mucilage. It appears that acidocalcisomes are the final destination for Mn in all analyzed strains, although deposits in other compartments are possible. In C. sorokiniana, Mn was accumulated in the form of a multivalent cubic Mn-O cluster. In H. pluvialis, Mn(II) was coordinated to sulfated polysaccharides. In Chlamydomonas strains, Mn appears to be coordinated to polyphosphates mainly in Mn(II) redox form, with Mn(III) being detected in C. acidophila. Such versatile traits could be exploited in the biosynthesis of Mn compounds.

Abstract ID: 2153 / Poster Number: 186.16P

Viability PCR Reagent: New Tool for Rapid Quantitation of Bacterial Viability and Viral Integrity

Presenting author: Dr. Brigitta Saul, Promega Corp, Madison WI, United States

Co-authors: *Nathan Feirer, Subhanjan Mondal*

Introduction: Culture-based detection methods are widely used in microbial and viral surveillance but are hindered by lengthy time-to-answer and inability to detect viable but non-culturable (VBNC) bacteria. PCR-based approaches address these limitations but lack live-dead discrimination. The Viability PCR Reagent System combines rapid PCR results with the ability to differentiate signals from viable and nonviable cells and viruses, providing an advanced molecular assay for microbial viability.

Objective: This study aims to demonstrate the use of the Viability PCR Reagent System for detecting intact viruses and bacteria in diverse samples, including food, water, and environmental matrices.

Method: The Viability PCR Reagent System uses a membrane/capsid impermeable molecule that binds and modifies nucleic acids from non-viable bacteria and viruses, preventing PCR amplification. We tested the reagent with bacteria (*Legionella*), DNA viruses (AAV), and RNA viruses (Norovirus and SARS-CoV-2). Assay performance was compared to traditional culture-based methods.

Findings: Viability PCR reduced or eliminated PCR signals from defective viruses and non-viable bacteria, while signals from intact microbial species remained unaffected. Heat or pressure-treated AAV, Norovirus, and SARS-CoV-2 were clearly discriminated from control samples. Live *Legionella* bacteria spiked into water samples were differentiated from dead *Legionella* using a filter-based collection and treatment protocol. For both bacteria and viruses, Viability PCR results were similar to corresponding culture-based assays.

Conclusion: The Viability PCR Reagent System offers a rapid, accurate alternative to culture-based assays for microbial monitoring. Its application can facilitate timely risk assessments and outbreak responses, improving the safety of food and water supplies globally.

Abstract ID: 3582 / Poster Number: 030.17S

Vibrio vulnificus and blood cells interaction: a dualRNAseq approach uncovers a fatal retrotransposon storm induced by a toxin of the MARTX family

Presenting author: Carla Hernández-Cabanyero, PhD, Ifremer, Montpellier, France; University of Valencia, Valencia, Spain

Co-authors: *Adrián Santiago, David Pajuelo, Celia Murciano, Eva Sanjuán, Carmen Amaro*

Introduction: *Vibrio vulnificus* is a marine and zoonotic bacterial species linked to climate change. The species is divided into five lineages related to human vibriosis and one pathovar associated with fish vibriosis. This pathovar encompass the *V. vulnificus* strains that belong to a zoonotic clonal-complex, which can cause lethal septicemia through ingestion or contact. This septicaemia, also known as vibriosis, shows very similar clinical signs in humans and fish, with the eel being the most susceptible host. In previous studies we have shown that the RtxA1 toxin, the main virulence factor of the species, is associated with an early cytokine storm in the blood that ends with the death of the infected individual in 24-48 hours, irrespective of the host.

Objective: To perform a comprehensive study of the complex host-pathogen interaction that occurs in the blood during septicemia using eel vibriosis as a model, and to better understand the role of the RtxA1 toxin in that interaction.

Method: We designed an *ex vivo* approach to perform dualRNAseq and analyze in parallel the host and bacterial transcriptomes during early vibriosis. We incubated a representative strain of the *V. vulnificus* zoonotic clonal-complex (parental and mutant strain defective in RtxA1) in eel plasma containing erythrocytes or leukocytes for 3 hours to simulate early vibriosis in blood. After that time, we harvested the RNA and analyzed the bacterial and eel transcriptome. The transcriptomic results that were detected as relevant were confirmed by using bacterial mutants in certain genes and performing a series of phenotypic *in vivo*, *ex vivo* and *in vitro* experiments specifically designed for each gene or cellular process.

Findings: The results obtained in this work show that in contact with blood cells, V. vulnificus expresses a broad arsenal of defense (LPS-modification and nutritional immunity) and attack systems (prominently the RtxA1 toxin, but also its major hemolysin (VvhA) and the VgrG toxin (part of type VI secretion system [T6SS]. In response, blood cells activate their own defense systems (RBCs activate degranulation, while WBCs

activate NO and ROS production). In addition, both cell types show signs of bacterial attack, manifested as the activation of cell death, inflammation markers that correspond to the early stages of a cytokine storm. Finally, we revealed the activation of a potential retrotransposon storm in WBCs, which could underlie the RNA-based immune response previously observed in vivo. This storm is specifically associated with RtxA1 and we propose that it is involved in the dysregulated immune response triggered by this pathogen, the main cause of the death of the host.

Conclusion: This study provides critical insights into the complex host-pathogen interactions during early vibriosis, using eel plasma as a model. Our results deepen our understanding of the molecular interplay underlying septicemia and underscore the importance of RtxA1 in shaping the outcome of the disease. Our results also offer showcase a potential avenue for future therapeutic interventions against animal and human vibriosis.

Abstract ID: 3190 / Poster Number: 389.17P

Violacein-Producing Janthinobacterium and Its Derivatives Activate β-Catenin Signaling to Promote Hair Health

Presenting author: Dr. Hyunggwan Lee, Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea

Co-authors: *Ye Zhuo*

Violacein, a secondary metabolite produced by specific bacteria, is known for its diverse biological activities, including antibacterial, anticancer, anti-nematode, and anti-protozoan properties. This study screened indigenous pigment-producing bacteria for potential applications in the cosmetic industry. From over 300 pigmented bacterial isolates, 14 violacein-producing *Janthinobacterium* strains were identified and subjected to phylogenomic, physiological, and cosmeceutical activity analyses. Notably, certain strains, including J. sp. SUN073, lacked the vio gene cluster and were unable to produce violacein. Physiological characteristics, such as carbon assimilation, enzyme activity, antibiotic resistance, antifungal properties, and violacein productivity, varied among the strains. Interestingly, crude extracts and violacein derived from J sp. SUN098 notably upregulated hair growth-related factors, including ALP, VEGF, and FGF2, and β-catenin signaling pathway, known as a key regulator of hair regeneration. Furthermore, violacein significantly increased the size of human hair follicle dermal papilla cells. This study provides the first evidence of violacein's potential benefits for hair health. Future in vivo studies are necessary to validate these findings and support the development of violacein as a valuable cosmeceutical bioresource.

Abstract ID: 4014 / Poster Number: 074.15P

Viral communities in glacier-fed streams exhibit strong biogeographical signatures as well as high levels of novelty and specificity

Presenting author: Mr. Martin Boutroux, Ecole Polytechnique Federale de Lausanne, Sion, Switzerland

Co-authors: *Hannes Peter*

Viruses are well known to modulate the biomass, diversity and activity of microbial communities in many ecosystems. Yet, little remains known about their role in stream biofilms. Here, we leverage metagenomic data from sediment biofilms in 200 glacier-fed streams (GFS) around the world to unravel the diversity and biogeography of benthic dsDNA viral communities.

These environments are expected to harbour a high degree of viral novelty due to undersampling. This consideration motivated the development of a reference-independent bioinformatics pipeline for viral detection. Our new pipeline roughly doubles the number of putative viruses identified from metagenomic data, shedding new light onto viral dark matter in GFS.

Among the 5000 high-quality viruses identified, almost none of them is found in public viral databases of isolates or metagenomic sequences, confirming the specificity to GFS. As observed for the host communities, most viral populations are sample-specific and only few are observed across all mountain ranges. Nonetheless, clustering by mountain ranges shows strong biogeographical signatures.

The GFS microbiome is characterized by low cell abundances and few but well-adapted bacterial clades, likely reflecting adaptation to the extreme environmental conditions in these habitats. Among phages, it manifests as high host specificity and the encoding of auxiliary metabolic genes that mediate interactions with their hosts, pointing to the prevalence of mutualistic interactions rather than mere lysis of host cells in GFS. Similarly, a comparison of upstream and downstream samples reveals lower viral diversity near the glacier snout but a broader gene repertoire.

Taken together, our work highlights the co-evolutionary history of phages and their hosts in a cryospheric ecosystem, now threatened by climate change.

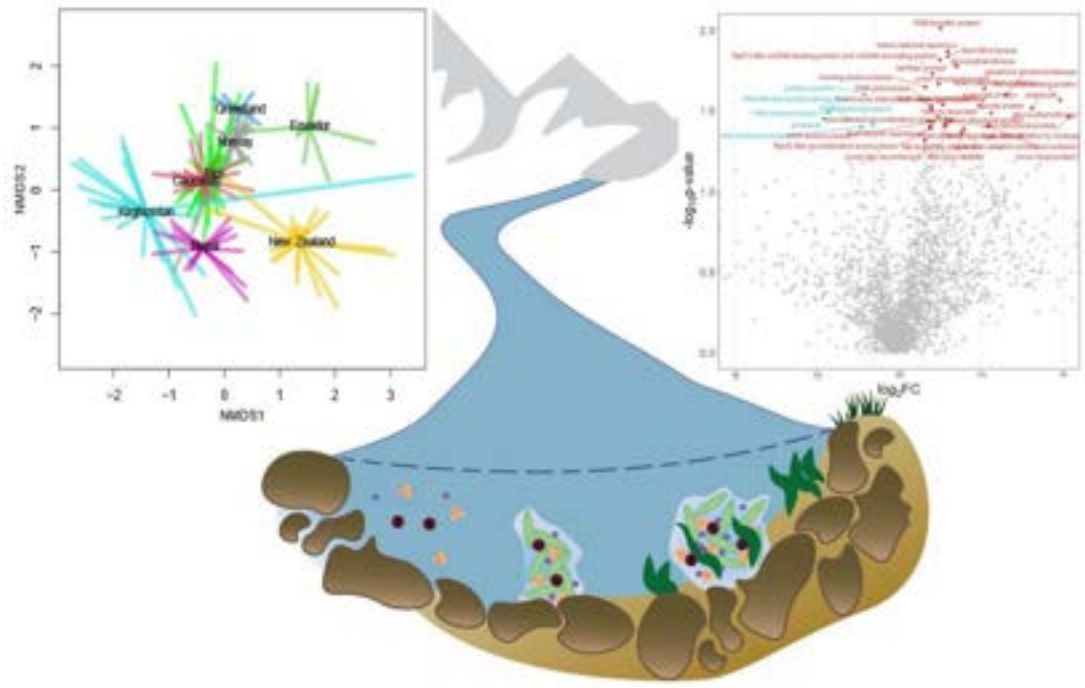


Figure 1: Schematic illustration of a glacier-fed stream, showcasing biofilms at various stages of maturation on the streambed. Top left: NMDS plot showing how viral communities strongly cluster by mountain ranges. Top-right: volcano plot showing viral genes enriched in samples close to the glacier snout (red) or further downstream (blue).

Abstract ID: 1733 / Poster Number: 384.15P

Viral evolution under simulated microgravity

Presenting author: Ms. Alicia Rodríguez-Moreno, Centre of Astrobiology, Torrejón de Ardoz, Spain

Co-authors: *Sergio Martín-Blázquez, Unai López de Heredia, Álvaro Soto, Ester Lázaro*

Introduction and Objective: Experimental evolution is a valuable approach to studying the molecular basis of biological adaptation under controlled laboratory conditions. To this aim, in our lab, we use the RNA bacteriophage Qβ, which infects Escherichia coli and that can be propagated under diverse selective pressures. The comparison of the ancestral and the evolved populations allow us to analyze the phenotypic changes and the evolutionary pathways experienced by the virus. This study investigates the survival and evolution of Qβ under simulated microgravity, a condition relevant to space exploration.

Methods: Qβ was propagated for ten serial transfers in semisolid medium under simulated microgravity, achieved using a 3D-clinostat, or under normal gravity in a standard incubator. We characterized the resulting populations by analyzing their consensus sequences and replicative capacities.

Findings: Our results show that Qβ replicates less efficiently in microgravity than in standard gravity. However, populations propagated under simulated microgravity could adapt to this condition. Genetic analyses identified a specific mutation that enhances the ability of the virus to enter bacteria, which appears to underlie the observed phenotypic changes. These findings suggest that the reduced replication in microgravity is associated with a lower encounter rate between the virus and bacteria.

Conclusion: Simulated microgravity drives the evolution of Qβ populations, favoring mutants with enhanced ability to initiate infections. These results provide insights into viral adaptation to conditions typical of outer space.

Abstract ID: 1570 / Poster Number: 356.16P

Virulence Factors and Cellular Interactions of Staphylococcus aureus in Bovine Mammary Epithelial Cells

Presenting author: Assoc. Prof. Vera Rall, São Paulo State Universit, Botucatu, Brazil

Co-authors: *Erika Bonsaglia, Bruna Rossi, José Pantoja, Ary Fernandes, Juliano Gonçalves, Marcos Santos*

Staphylococcus aureus, a significant bacterial pathogen, plays a pivotal role in the development of clinical and subclinical mastitis. The pathogen's ability to harbor virulence factors contributes to the severity and persistence of infections. This study aimed to investigate the interaction of S. aureus virulence factors in the adhesion and invasion of bovine mammary epithelial cells (MAC-T). PCR was used to detect microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), including fbnA, fbnB, fib, clfA, clfB, can, eno, ebpS, and the agr cluster (I–IV) in 10 clinical mastitis isolates (5 mild, 5 moderate) and 5 subclinical isolates. Confluent layers of MAC-T cells were cultured in 24-well plates and inoculated with S. aureus (1.5 × 10^6 CFU/mL) at 37°C/5% CO_2. After 3 hours, cells were detached, stopped with DMEM, and seeded on TSA plates. For invasion assays, intracellular bacteria were quantified following treatment with Triton X-100. Colony recovery and counting determined positive invasion relative to adhesion test values. Subclinical strains exhibited lower adhesion rates compared to clinical strains. However, all subclinical strains were agr-negative, harboring only one MSCRAMM gene, and demonstrated higher invasion rates than moderate and mild clinical strains, which carried agr types II or III and three or more MSCRAMM genes. These results suggest that the agr system may enhance MSCRAMM expression, increasing adhesion. This study highlights the intricate link between bacterial genetic factors and disease severity, underscoring the importance of understanding bacterial composition for predicting pathogenic potential.

Abstract ID: 4130 / Poster Number: 400.17P

Visualizing antimicrobial resistance burden/trends and engaging scientists, policymakers and the public through the interactive MICROBE tool

Presenting author: Dr. Tomislav Mestrovic, University of Washington, Seattle, United States; Institute for Health Metrics and Evaluation, Seattle, United States; University North, Varazdin, Croatia

Introduction: Effectively communicating antimicrobial resistance (AMR) data is essential for public awareness and policy change efforts, which highlights the need for visualization tools that facilitate data interpretation and stakeholder engagement.

Objective: To demonstrate the importance of science communication techniques in AMR research and highlight how the MICROBE tool can bridge the gap between complex data and stakeholder/public engagement by visualizing AMR burden trends across regions, pathogens and health outcomes.

Methods: The visualization tool includes estimates based on 520 million records across 19,513 study-location-years, which integrate mortality records, data from surveillance systems, hospitals, literature reviews and pharmaceutical sources. Statistical modelling was employed to estimate AMR burden and developed two counterfactual scenarios: (1) replacing all drug-resistant infections with drug-susceptible infections, and (2) replacing them with no infection. The tool was designed to communicate these findings effectively, using interactive maps, age/sex-specific breakdowns, and different comparisons to illustrate AMR trends across countries/years.

Results: The MICROBE tool allows for burden comparisons over time (i.e., from 1990 to 2021) by providing interactive visualizations and age/sex-specific breakdowns of AMR burden. Users can explore fatal and nonfatal health outcomes by selecting specific pathogens, drug-resistance patterns and infectious syndromes. The tool also enables comparisons of AMR-related mortality rates between different countries and regions, highlighting disparities.

Conclusion: Interactive data tools like MICROBE enhance science communication and public engagement by making AMR burden estimates more accessible. By visualizing these differences, MICROBE supports public awareness, evidence-based policymaking, and proactive outreach to ensure that AMR remains a priority on the global health agenda.

Abstract ID: 423 / Poster Number: 432.16P

Viva voce as a tool for achieving mastery learning in introductory microbiology

Presenting author: Dr. Andrew St. James, Wake Forest University, Winston-Salem, United States

Viva voce, or oral examination, is a robust assessment technique that allows instructors to probe knowledge gaps and provide immediate, differentiated feedback to students. In introductory microbiology courses, heavy content loads present a challenge to comprehensive student mastery. While individualized feedback is a necessary component of mastery learning, it may also be time-intensive for the instructor to deliver. The objective of this study was to evaluate the effectiveness of viva voce as a time-sensitive mastery learning tool. Students in an introductory microbiology class were allowed to sit for oral examinations of content that they failed to master on an initial traditional written assessment. Vivas were limited to 15 minutes per student, though most vivas were completed within 10 minutes, and no instructor time-demands were required outside of the 15-minute period. Vivas were administered according to best practices in equity to maximize the validity, reliability, and fairness of the exams. We found that students who completed a viva on previously unmastered material were significantly more likely to achieve and retain content mastery after the intervention as compared to students who did not elect to sit for vivas. We conclude that oral examinations on previously unmastered material may allow students to effectively achieve and retain content mastery. We recommend that viva voces be considered by instructors in small (< 50 students) introductory microbiology courses to improve student learning outcomes.

Abstract ID: 3374 / Poster Number: 116.15F

Warming enhances the active methane sink in alpine soils with strong seasonal dynamics

Presenting author: Mr. Eduard Vico Oton, EPFL, Sion, Switzerland

Co-authors: *Massimo Bourquin, Laureen Ahlers, Ianina Altshuler*

Alpine ecosystems are dominated by barren soils under seasonal snow cover and particularly sensitive to climate change. Despite its relevance, it is largely unknown whether these ecosystems act as greenhouse gas sinks or sources, what microbial communities drive these processes, and how they respond to environmental factors.

We aim to characterise the carbon cycling processes in these high-altitude soils and identify the microbial communities responsible for their activity. We hypothesise that these ecosystems significantly contribute to carbon cycling and that warming will alter both the microbial communities and their functions. Warming experiments were established on high-altitude soils under intermittent snow cover during 2023-2024. We measured *in situ* CH_4 and CO_2 gas fluxes across seasons while collecting soil samples for metabarcoding, metagenomics and metaproteomics to link community composition with functional activity.

Our findings reveal these ecosystems as net CH_4 sinks and CO_2 sources, with fluxes showing strong seasonality and sensitivity to experimental warming. The significant presence of cryosphere-associated bacteria suggests highly specialized communities. Results also indicate distinct environmental drivers for each gas flux, with soil water content affecting CH_4 uptake and temperature controlling CO_2 emissions.

These results provide the first comprehensive characterisation of carbon cycling processes and microbial communities in high-altitude soils through in-depth -omics analyses. The demonstrated sensitivity to warming and the presence of cold-adapted microorganisms suggest these systems may experience significant functional changes under climate warming, potentially affecting their role in global carbon cycling. Future work will focus on identifying specific metabolic pathways and their regulation under warming conditions.

Abstract ID: 451 / Poster Number: 263.17P

Waste reduction through sustainable use of nutrient-rich by-products in lactic acid bacteria (LAB) starter culture and probiotics production

Presenting author: Giulia Arrigoni, PhD, Agroscope, Bern, Switzerland; Proge Farm, Novara, Italy

Co-authors: *Lorenzo Vecchio, Paola Usurini, Cornelia Baer, Fabio Grasso, Ueli Von Ah, Lorenzo Morelli*

Introduction: Using by-products from e.g. production of plant-based protein alternatives as substrates for enhancing LAB starter culture growth is a promising approach to improve sustainability and waste management in food production. Nutrient-rich by-products, such as fruit and vegetable pomace, spent grains, and agricultural residues, can provide suitable environments for microorganisms such as LAB, supporting a circular economy that emphasizes resource efficiency and waste minimization.

Objective: This study aims to explore the potential of okara, a by-product of processing for example soy beans into milk or tofu to enhance microbial growth. By correlating genomic and metabolic peculiarities of bacterial strains with growth in different substrates it is possible to develop sustainable media that can be adapted to meet the specific nutritional needs of various LAB strains, supporting efficient biomass production and circular economy solutions.

Method: Bioinformatic analysis is conducted to identify genes involved in protein metabolism of starter cultures and LAB strains. Tools were used to correlate metabolic pathways with growth in lab-scale bioreactors using different okara substrates, specifically oat, rice, lupin and press cake from sunflower oil.

Findings and conclusions: As a protein source, okara showed potential for waste reduction and enhanced sustainability in LAB cultivation with increased or comparable yields compared to standard procedures. The findings highlight the potential of plant-based protein alternatives, such as okara, as sustainable substrates for microbial growth. By developing strain-specific nutrient profiles, it is possible to optimize fermentation efficiency and contribute to circular economy initiatives that minimize waste.

Abstract ID: 485 / Poster Number: 064.17P

Wastewater monitoring using digital PCR

Presenting author: Mrs. Helena Block, QIAGEN, Hilden, Germany

Co-authors: *Silvia Magyar, Stefanie Schroeer, Dominic O'Neil*

Introduction: The detection and monitoring of microbial pathogens and antibiotic resistance (AMR) in wastewater have rapidly gained importance in both the health and environmental settings for the safety of patients and the general public. However, wastewater, with its high content of inhibitory substances, poses special challenges for both nucleic acid extraction and downstream analysis such as detection by PCR.

Objective: In this study, we examined wastewater samples for the presence of microbial pathogens and AMRs.

Methods: Wastewater samples were collected from different wastewater treatment plants in Germany and DNA and RNA were isolated using extraction kits specifically designed for inhibitory samples. Extraction efficiency and target gene content were assessed by digital PCR and NGS.

Findings: The wastewater samples showed a high content of different AMR markers with a wastewater plant-specific composition. Very high concordance was observed between the NGS read count and dPCR copy number, indicating the potential complementary value of both methods.

This results demonstrates the importance of a reliable nucleic acid extraction kit with a sufficient level of inhibitor depletion and a robust readout method for fast and accurate analysis of wastewater.

Conclusion: Digital PCR provides accurate and rapid insights into microbial pathogen and antibiotic resistance dynamics and could be a powerful method for wastewater surveillance.

These tools can provide insights into the development and dynamic of AMR, and could thus be a powerful method to combat antibiotic resistance and can also be extended to further targets or application fields, improving understandings and outcomes for public health.

Abstract ID: 3429 / Poster Number: 168.15P

Wastewater surveillance of SARS-CoV-2 RNA post vaccination in Baltimore

Presenting author: Dr. Samendra Sherchan, Morgan State University, Baltimore, United States

This study was to determine the presence of SARS-CoV-2 RNA in wastewater samples from two wastewater treatment plants in Baltimore over a period of one year. The samples were concentrated by the Polyethylene Glycol 8000 (PEG) method, and RNA fragments were extracted using the QIAamp Viral RNA Mini Kit. RT-PCR and qPCR assays were performed, and Cq values below 40 were analyzed and presented as gene copies/L. N1 and N2 genes were detected in both WWTP samples, with N1 ranging from 1.38 to 3.34 log10 gc/l and N2 ranging from 1.88 to 3.20 log10 gc/l. COVID-19 hospitalization cases in Baltimore County and City were observed to be 40–42 % positively correlated with the copies of N-genes detected in the WWTP-A. On the contrary, the N-genes (N1 and N2) from both WWTPs exhibited a very weak positive and negative relationship with wastewater physical parameters such as pH, electrical conductivity, total dissolved solutes, salinity, and temperature. The reduction in positive correlation with hospitalization cases could be attributed to an increase in immunity amongst the population surveyed. There is a need to ascertain the effect of physical parameters changes from the sampling point to the processing point on the capture and detection of SARS-CoV-2 RNA in wastewater.

Abstract ID: 827

Wastewater talks: Revealing age-specific antibiotic resistance profiles

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Introduction: Wastewater-based epidemiology (WBE) is a valuable tool for monitoring antimicrobial resistance (AMR) by analyzing antibiotic resistance genes (ARGs) present in wastewater, which contains contributions from human feces produced by communities. Studying the factors that shape the resistome (the whole set of ARGs) and the mobilome (the whole set of mobile genetic elements, MGEs) offers a valuable insight for tailored AMR management strategies. However, the impact of age (a factor influencing the maturity of the gut microbiome, the antibiotic use, and the health status) in the gut resistome remains poorly explored. This study employs WBE at the building-level to analyze the microbiome, resistome and mobilome of age-segregated populations.

Objective: To explore and describe the differences in the microbiome, the resistome and the mobilome among age groups using WBE at the building scale.

Methods: Wastewater from an elementary school (School), university residence (UnivRes), and elderly care facility (ElderlyRes) in Girona (Catalonia, Spain), was analyzed using metagenomics to investigate differences in bacterial communities, ARGs, and MGEs across age groups.

Findings: Results revealed age-linked microbiome variations, with bacterial pathogens more prevalent in the wastewater collected at the School and the ElderlyRes, highlighting public health implications. ARG abundance peaked in school sewage, reflecting frequent antibiotic exposure, while diversity was highest in the elderly residence, likely due to long-term antibiotic use. Moreover, sewer-associated can also influence the microbial composition of the wastewater, making it difficult to discriminate between human commensals and environmental bacteria.

Conclusions: This study demonstrates that building-level WBE provides valuable insights into age, shaping microbiome, resistome, and mobilome profiles. It highlights WBE's potential as a tool for monitoring pathogens and AMR in targeted populations.

Abstract ID: 1909 / Poster Number: 010.17S

What stays in the family? Analysis of gut microbiota dynamics and adaptations within families using strain-resolved metagenomics

Presenting author: Dr. Katarzyna Sidorczuk, Quadram Institute Bioscience, Norwich, United Kingdom; Earlham Institute, Norwich, United Kingdom

Co-authors: *Falk Hildebrand*

Introduction: The gut microbiota is a crucial factor in human health. Our gut microbiota is seeded at birth, starting with mostly facultative aerobes in the first weeks and gradually changing to anaerobes such as Bifidobacterium, Bacteroides, and Clostridiales. Around six months, when solid foods enter the infant's diet, the abundances of Bacteroides and Clostridiales increase, while Bifidobacterium species decrease. Previous studies have shown that most of the early colonizing species are transmitted from mothers and that the diversity of gut microbiota increases as the children get older. Moreover, higher strain transmission rates have been associated with both kinship and cohabitation, which is consistent with observations that families tend to display similar gut microbiota compositions. However, colonization and persistence among multiple family generations remain largely unexplored.

Objectives: This study aims to investigate strain transmission patterns between multiple generations of family members at an unprecedented scale. We want to identify strains that persist for a long time within host and are frequently shared between family members. By investigating the genomes of these strains, we aim to determine factors that contribute to the successful colonization of the human gut.

Method: We established a meta-analysis of metagenomes from families and mother-child pairs from 17 published studies, supplemented by a local cohort. The data came from longitudinal and cross-sectional studies and covered westernized and non-westernized communities. In total, over 5,000 shotgun metagenomes were processed with the MG-TK pipeline to reconstruct metagenome-assembled genomes (MAGs), predict genes, and perform functional annotations.

Findings: We identified over 1,900 metagenomic species (MGS) clustered from >45,000 high-quality MAGs. More than 90% of these MGS were resolved intra-specifically to track strain transmissions within families and investigate their persistence over time based on longitudinal studies. This showed the strong influence family members have on each

other's gut microbiome and identified species with the highest/lowest strain-level family association. The highest transmission rates within families were observed for child-mother and child-child pairs. Moreover, we found that despite lower diversity, strains found in newborns tend to be more persistent than those found in adults. We further analysed de novo reconstructed pangenomes for each MGS to identify genes overrepresented in newborns that could indicate their involvement in the colonization process.

Conclusion: Our strain-resolved analysis of metagenomes provides novel insights into strain dynamics within families. We also identify genes characteristic of persistent and highly transmissible strains, indicating factors that might contribute to successful colonization. Overall, these results contribute to our understanding of microbial colonization and persistence in the human gut.

Abstract ID: 3977 / Poster Number: 400.15F

What the Phacoides: Long-read sequencing reveals genome expansion in ancient intracellular symbionts of marine clams

Presenting author: Dr. Jay Osvatic, Joint Microbiome Facility, Vienna, Austria; Medical University of Vienna, Vienna, Austria

Co-authors: *Joana Séneca, Delano Schleder, Jillian Petersen*

Symbiont populations represent microbial communities of naturally low diversity whose genomes can in principle be easily studied using short-read sequencing. However, issues often remain as genomic diversity within a population can fragment genomic assemblies. This issue is evident in many NCBI-available metagenome assembled genomes (MAGs) of symbionts from the widespread clam family Lucinidae. Symbionts of the lucinid *Phacoides pectinatus* (*Ca*. Sedimenticola endophacoides) are emblematic of these issues as MAGs are smaller and more fragmented when compared to cultured *Sedimenticola* genomes.

To generate the first circularized Lucinidae symbiont MAGs, *Phacoides pectinatus* gill DNA was sequenced using short-read (Illumina) and long-read (ONT) technology. The resulting long-read data was used for symbiont genome assembly while short-read data provided coverage information. A circularized genome of *Ca*. S. endophacoides was assembled and compared to existing MAGs using orthologous gene clustering and annotation.

The circularized symbiont genome is approximately twice as large as available short-read-derived MAGs for the species and encodes over three times as many genes. Orthogroup clustering of these genes revealed that more than 40% of genes were of similar functions, primarily transposases. Coverage information from accompanying Illumina data suggested that some transposases have hundreds of copies in the genome, representing transposon-duplication events. This is a likely mechanism for rapid genome evolution inside of the Lucinidae clam and one of several reasons for fragmented short-read MAGs in low-diversity populations of symbionts. Long-read assemblies will not only enable us to close symbiont genomes but also provide more insight into the genomic evolution of symbiont populations.

Abstract ID: 2751 / Poster Number: 164.15P

Wheat microbiota characterization: the first step to mitigate mycotoxin accumulation in grains

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Co-authors: *Marco Saracchi, Paolo Cortesi, Cristina Pizzatti, Andrea Kunova, Daniela Bulgari, Matias Pasquali*

Deoxynivalenol (DON) and enniatin B (ENB), *Fusarium* mycotoxins, contaminate Italian wheat, resulting in potential synergistic effects on organisms. On the other hand, plant microbiota plays a key role in plant health by protecting against pathogens and promoting growth, offering a sustainable, innovative solution for mycotoxin control. Therefore, the identification of microorganisms with effective activity against ENB-DON accumulation in wheat is the main goal of this work.

Selective growth conditions were used to isolate approximately 50 microorganisms (both bacteria and fungi) from wheat spikes collected from two sources: (*i*) experimental field sites in Northern Italy and (*ii*) plants grown under laboratory conditions and treated with DON and ENB during anthesis.

Subsequently, the DNA was extracted from each sample and the 16S ribosomal RNA (rRNA) and the internal transcribed spacer (ITS) regions Sanger sequencing was performed for the taxonomic profiling of microbiota. According to the results, the identified genera are 11 for both bacteria and fungi, making 22 different genera. Among these, *Kosokonia*, *Bacillus*, *Micrococcus, Linnemannia* and *Trametes* genera are known in literature as potential plant growth promoting microorganisms. The whole genome analysis of the interesting genera is ongoing to exclude possible pathogens. Additionally, the microorganisms will be further screened based on their resistance to the mycotoxins and their ability to degrade them. This work represents the first step to select microorganisms that could mitigate mycotoxin accumulation.

Abstract ID: 1977 / Poster Number: 104.17P

Whole genome analysis of Streptococcus pyogenes clinical strains collected over 15 years in Poland

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Objectives: Genetic characterization of the antibiotic resistance determinants and associated mobile genetic elements among *Streptococcus pyogenes* [Group A streptococci (GAS)] clinical isolates of M77 and M28 serotypes collected in Poland between 2003 and 2017.

Methods: The genomes of 136 M77 and 308 M28 GAS isolates were sequenced using Illumina and selected with a long-read approach (Oxford Nanopore). Whole genome sequences were analyzed to determine the presence of macrolide resistance determinants, and their genetic context.

Results: The strains were collected in the two multicenter surveys from in- and outpatients. Sequencing data analysis revealed four sequence types within the M28 strains: ST52 (N=125), ST456 (N=61), ST458 (N=100), and ST244 (N=22); only the latter carried *tet*(M). The *erm*(TR), *erm*(B), and *erm*T genes were detected in 16.9% of M28 strains (N=52): *erm*(B) within a multiresistant cassette on Tn*6263* in ST52 (N=31), and on Tn*6189* in ST456 (N=4) and ST458 (N=5) strains, 6 *erm*(TR) within the ICE*Sp2905* remnants, and plasmid-located *erm*T in six ST456 strains. All M77 strains were of a single ST63 sequence type and carried *tet*(O). In M77/ST63 isolates *erm*(TR) was detected in 76.5% (N=104), predominantly within the novel hybrid Integrative Conjugative Element (N=74) composed of the ICE*Sp1108*-like sequence and ICE*Sp2906* variant, named ICE*Sp1109*. In strains isolated before 2008, *erm*(TR) was located within ICE*Sp2905* (N=27).

Conclusions: In M28 strains, the *erm* genes were acquired in multiple events over time. In M77 strains, the clonal dissemination of the macrolide-resistant M77/ST63 strain with ICE*Sp1109* was observed between 2008 and 2017.

Abstract ID: 3122 / Poster Number: 336.16P

Whole genome sequence analysis as a driver of innovation in the safety assessment of microorganisms used in the food chain in the European Union

Presenting author: Jaime Aguilera, PhD, European Food Safety Authority, Parma, Italy

Co-authors: *Simone Lunardi, Ana Gomes, Eleonora Marini, Silvia Peluso, Yi Liiu, Estefania Noriega*

Introduction: Scientific and technical advances in microbiology led to the development of improved food and feed products such as enzymes, additives and probiotics. They also enabled innovative production technologies such as precision fermentation. In the European Union, those products must undergo a safety evaluation before being placed into the market, which is conducted by the European Food Safety Authority (EFSA). Applications for food and feed regulated products submitted to EFSA often involve microbial strains used as such (active agents) or as a source of fermentation products (production strains). The amount and the complexity of such applications have increased in recent years, thus requiring a precise and rapid characterisation of microorganisms to assess the risks associated with their use in the food and feed chain.

Objective: To allow a precise and rapid characterization of microbial strains in food and feed products, EFSA has developed cutting-edge tools and guidance, with whole genome sequencing (WGS) analysis as a central methodology.

Method: Several guidance and a collection of bioinformatic tools and pipelines have been developed to support applicants and risk assessors to generate and analyse WGS-derived data. They can be used to identify microorganisms at the species level, to predict the possible production of toxic compounds and virulence factors as well as the presence of acquired antimicrobial resistant (AMR) genes, and to characterise genetic modifications. All these steps are necessary to determine the impact of the strain on the safety of the final product, resulting in accurate and streamlined assessments.

Findings: WGS-based methods allow a faster and more accurate characterisation of microbial strains, revealing safety-linked aspects that previously passed unperceived, such as:

- Identification of acquired AMR marker genes, naturally present or unintendedly remaining after a genetic modification

- Identification of gene clusters involved in the biosynthesis of antibiotics and bioactive compounds
- Correct taxonomic identification of strains

This high throughput characterisation contributes to fine-tune the assessment of the final products.

Conclusion: Scientific advances provide excellent resources for modernising and improving the risk assessment, which is essential to address the growing complexity of food and feed produced using microorganisms. The incorporation of WGS analysis to the safety evaluation of microbial strains intentionally used in food or feed enables a faster and more accurate characterisation of such strains. EFSA is pioneering the application of this method to regulated products, resulting in more comprehensive and robust assessments and ultimately in a higher capacity to protect consumer's health This effort will continue in the future: the advent of synthetic biology is expected to bring new complexity to biotechnological solutions. EFSA has already identified new possible hazards emanating from synthetic biology applied to the food and feed sector, and will continue applying cutting-edge knowledge and technological innovations to improve its risk assessments and face these new challenges.

Abstract ID: 882 / Poster Number: 077.17P

Whole-genome sequencing and analysis of Streptomyces violaceus IMV Ac-5027 in ability to synthesize bioactive metabolites with antitumor and antibacterial properties

Presenting author: Mariia Loboda, PhD, D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, Kyiv, Ukraine

Co-authors: *Andrii Siromolot, Liudmyla Biliavska*

Streptomyces species are valuable sources of clinically and biotechnologically significant natural products. Among these metabolites are classic antibiotics, antivirals, antifungals, antiparasites, and antitumor agents. The *Streptomyces violaceus* IMV Ac-5027 strain was isolated from the coast of the Black Sea in Ukraine and registered in the Microorganisms Depositary of the D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine. It showed high bioactivity in laboratory tests regarding its ability to inhibit phytopathogenic fungi and bacteria, phytonematodes, and stimulated plant growth and development. It is therefore a promising candidate for the search and identification of antimicrobial compounds, including those with antitumor and antibacterial properties.

Whole-genome shotgun sequencing was performed using an Illumina NovaSeq 6000 mode S2 (GATC Biotech, Konstanz, Germany) and assembled with BacFlux v1.1.8, including steps for quality control, assessment of genome completeness, contamination, taxonomic placement, and the quality of assembly. Genome annotation was carried out using DFAST (DDBJ Fast Annotation and Submission Tool) and RAST Server (Rapid Annotation using Subsystem Technology). Biosynthetic gene clusters and secondary metabolites were predicted using antiSMASH tool (Blin et al., 2023). Liquid chromatography-mass spectrometry analysis using the Q Exactive mass spectrometer (Thermo Fisher Scientific, US) was utilized to detect bioactive compounds in the ethanolic extracts of *S. violaceus* IMV Ac-5027 when grown in both organic and synthetic nutrient media. All chromatograms were analyzed using the FreeStyleTM 1.8 software with Excalibur (Thermo Fisher Scientific, US)

A rapid annotation of the genome of *S. violaceus* IMV Ac-5027 revealed to identify different encoded metabolic pathways. This includes tetracycline and fluoroquinolones antibiotic resistance and several beta-lactamases were identified in the genome. The encoded cobalt-zinc-cadmium and copper tolerance potentially allows the strain to survive in polluted environments, and the potent biosynthesis of the ectoine osmoprotectant indicates the high adaptive potential of the streptomycete in conditions of high salinity and extreme temperatures. The capacity of *S. violaceus* IMV Ac-5027 to

synthesize marinocine – a ribosomally synthesized bacteriocin was encoded. This compound is important for combating multi-drug-resistant strains. No virulence genes, toxins, or superantigens have been identified, thereby classifying the strain as risk group 1 (non-pathogenic). The antiSMASH analysis allowed to identify 34 putative biosynthetic gene clusters (BGC). High homology was detected for the BGC of the anthracycline antibiotic cytorhodin which has shown preclinical antitumor activity. Additionally, a high level of homology was detected for the BGC of informatipeptin antibiotic, as well as for the BGC of germicidin.

LC-MS analysis allowed to identify bioactive metabolites in the ethanolic extracts of the streptomycete using positive high-resolution electrospray ionization. Several anthracycline antibiotics, known for their antitumor properties, were found specifically: rhodilunancin A and rhodilunancin B, obelmycin F (788.3476 m/z), and IV B (768.3228 m/z). The molecular formula of rhodilunancin A was calculated as $C_{40}H_{53}NO_{13}$ with an m/z of 756.3590, and the retention time (RT) was observed at 6.12 minutes. The molecular formula of rhodilunancin B was calculated as $C_{40}H_{53}NO_{14}$ with an m/z of 772.3546, and the RT was observed at 5.58 minutes. The analysis identified several peaks corresponding to purpurolide F, water-soluble monascus pigment 7, and purpurquinone D. These compounds are likely responsible for imparting the purple-violet colour to the strain and its extracts.

The detection of the strain's ability to synthesize bioactive metabolites highlights its potential as a valuable source of antimicrobials. Moreover, LC-MS analysis identified several antitumor agents and bioactive pigments, indicating its prospects for therapeutic studies.

Abstract ID: 3146 / Poster Number: 329.17P

Wickerhamomyces anomalus, a new potent Baker's Yeast

Presenting author: Ms. Jeong-Ah Yoon, Kangwon National University, Chuncheon, South Korea

Co-authors: *Chan-Haeng Lee, Sang-Min Shim, Myoung-Dong Kim*

During fermentation, *Wickerhamomyces anomalus* produces aroma compounds such as isoamyl acetate, ethyl acetate, and ethyl hexanoate. This study investigated the potential of *W. anomalus* isolates to enhance bread fermentation's efficiency and aroma profile. Three isolates (NIYL11942, NIYL11971, NIYL25845) with superior specific growth rates and gas production compared to the control s *W. anomalus* KCTC27761 were selected from liquid dough. The isolates and the KCTC27761 were mixed with *Saccharomyces cerevisiae* SAF-instant dry yeast red, a widely used baker's yeast, to prepare the dough. After fermentation, the dough made with the isolates produced over 460 ml of gas, exceeding the production made with the control. In addition, volatile compounds from the dough were analyzed using an electronic nose, and PLS-DA revealed ethyl acetate as the key compound, with a VIP score above 1. The isolate NIYL25845 produced approximately 1.2 times more ethyl acetate than the control, showing the most remarkable performance. These results suggest that *W. anomalus* NIYL25845 has significant potential as an alternative starter for bread fermentation.

Abstract ID: 2630

Widespread horizontal transfer and strong selection for aerotrophy genes underlie microbial adaptation in Antarctic soils

Presenting author: Yongyi Peng, PhD, Department of Microbiology, Biomedicine Discovery Institute, Monash University, Melbourne, Australia; Securing Antarctica's Environmental Future, Monash University, Melbourne, Australia

Co-authors: *Pok Man Leung, Laura Woods, Sophie Holland, Chris Greening*

Introduction: The prevalent taxa in oligotrophic Antarctic soils are metabolically versatile aerobes that use atmospheric trace gases for energy, carbon and hydration needs – a process recently termed aerotrophy. Although the widespread aerotrophy genes play a prominent role in microbial adaptation and survival, their dissemination and selective pressures across Antarctic soils remain largely unexplored.

Objective: In this study, we aimed to demonstrate that key functional aerotrophy genes are widely distributed through frequent horizontal gene transfer in resource-poor Antarctic soils. We also sought to investigate the selection pressures acting on these essential genes by analyzing population microdiversity and their associations with protein structures.

Method: Here, we profiled 676 metagenome-assembled genomes from 16 glacial and mountainous soils, from a combination of short- and long-read datasets. Genes related to energy and carbon acquisition were collected by searching for 52 conserved marker genes in derived genomes. Horizontal gene transfer events were identified through phylogenetic reconciliation and mobile genetic element analysis. We further quantified genetic variations and selection level of aerotrophy genes integrated with protein structure analysis using state-of-the-art methods.

Findings and conclusion: We provide evidences that horizontal gene transfer events occur frequently among these Antarctic bacteria. Surprisingly, some aerotrophy genes are horizontally acquired at multiple taxonomic levels and closely associated with various mobile genetic elements, including 12 CO dehydrogenase and 57 [NiFe]-hydrogenase genes. Hydrogenases responsible for atmospheric hydrogen oxidation are encoded by 12 bacterial phyla, and six Group 1l [NiFe]-hydrogenase genes are horizontally transferred among up to eight phyla. To reveal fine-scale evolutionary dynamics of aerotrophy genes, we further investigate their genetic diversity and selection

signature. Metabolic genes associated with horizontal gene transfer or in proximity to mobile genetic elements exhibit significantly higher nucleotide diversity compared to other genes without such associations. The polymorphism rates indicate most aerotrophy genes are under strong purifying selection that preserves functionality, demonstrated by a predominance of synonymous mutations. Buried and functional constraint sites of aerotrophy enzymes are likely to purify nonsynonymous changes for protein structural stability and function. Moreover, evolutionary metrics in aerotrophy genes are significantly related to physicochemical properties, particularly moisture content (critical for hydration), as well as phosphorus and nitrate levels (essential for microbial growth and metabolism). Overall, these findings uncover unique eco-evolutionary trends of microbial aerotrophy in cold desert ecosystems and provide insights into microbial adaptation in the extreme polar biosphere.

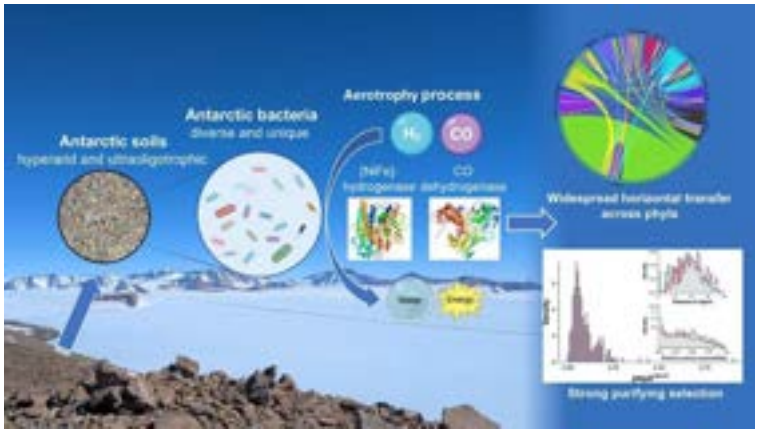


Figure 1: Illustration of eco-evolutionary trends of microbial aerotrophy in Antarctic desert soils.

Abstract ID: 776

Widespread transport of bicarbonate by members of the ancient and ubiquitous SulP family of transporters

Presenting author: Prof. Kathleen Scott, University of South Florida, Tampa, United States

Co-authors: *Jana Wieschollek, Daniel Walsh*

Introduction: SulP transporters are a huge and ancient protein family found in almost every organism; currently, genes that encode SulP transporters have been found in over 95,000 organisms in the IMG genome database, placing the SulP family of transporters in the top 1% of all protein families with respect to its abundance in organisms. Surprisingly, transport has been characterized for only a handful of these transporters. Thus far, they have been shown to be capable of transporting a variety of small, negatively charged molecules. Bicarbonate uptake has been measured for some SulP transporters in *Cyanobacteria*, but the extent of this capability within this massive family of transporters is unclear at this point.

Objective: The objective of this study was to screen SulP transporters sampled from the five clades of this transporter family (A – E) to clarify the extent of bicarbonate uptake within this protein family.

Methods: Gene sequences encoding 218 SulP transporters were gathered from the Integrated Microbial Genomes and Microbiomes database (https://img.jgi.doe.gov/), synthesized, and inserted into pBAD202, where their expression could be regulated by arabinose. These plasmids were transformed into a strain of *E. coli* engineered to require the presence of bicarbonate transporters or carbonic anhydrase to grow under ambient air (*E. coli* Lemo21(DE3) Δ*yadF* Δ*cynT*). Each transporter was screened for its ability to rescue this strain of *E. coli* when its expression was induced by the presence of arabinose.

Findings: The ability to transport bicarbonate was found to be particularly prevalent in members of clade C, which includes many SulP transporters from autotrophic members of phylum *Pseudomonadota*. Previously, bicarbonate transport had been demonstrated in clade D, which includes bicarbonate transporters from *Cyanobacteria*.

Conclusions: Understanding the extent of SulP-mediated bicarbonate uptake is linked to our understanding of the global carbon cycle, since SulP-family transporters facilitate bicarbonate uptake by *Cyanobacteria* responsible for a sizeable portion of ocean primary productivity, as well as by autotrophic bacteria from deep-sea hydrothermal vents and

other habitats. Furthermore, knowing which of the SulP transporters can facilitate bicarbonate uptake will be helpful in efforts to engineer organisms to synthesize compounds of industrial interest from bicarbonate and carbon dioxide. Given the broad range of environmental conditions in which SulP transporters operate, it seems likely that there exists a bicarbonate-transporting SulP transporter suitable for any possible industrial context.

Abstract ID: 156 / Poster Number: 293.16P

Yeast diversity and volatile compounds in the fermentation of Amazonian Robusta coffee clones

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Co-authors: *Sandy Rodrigues Dias, Nádia Nara Batista, Silvia Juliana Martinez, Enrique Anatácio Alves, Disney Ribeiro Dias, Rosane Freitas Schwan*

Amazonian Robusta coffees (Coffea canephora) are known for their high productivity. This study aimed to evaluate the diversity of yeasts and volatile compounds during the spontaneous fermentation of two clones: 08, known for higher productivity, and 025, recognized for its superior quality. The potential impact of this study's findings on the coffee industry must be considered. The coffee fruits underwent fermentation through self-induced anaerobiosis for 21 days. Yeast diversity was assessed using next-generation sequencing (NGS), and volatile compounds were evaluated using gas chromatography-mass spectrometry (GC-MS). A total of 21 yeast species were identified. Among the yeast strains examined, only Torulaspora delbrueckii (clone 08), Candida zeylanoides (clone 025), and Cryptococcus sp. (clones 025 and 08) had an abundance exceeding 1% at the start of fermentation. By the end of the fermentation process, Kazachstania humilis (clones 025 and 08), Kazachstania sp. (clone 025), and Saccharomyces sp. (clones 025 and 08) became the dominant yeast species. A total of 66 volatile compounds were identified and categorized as follows: acids (9), alcohols (12), aldehydes (5), esters (16), fatty acids (8), ketones (2), phenols (8), and others (6). The analysis of the predominant compounds revealed distinct profiles for the clones at the beginning of fermentation. By the end of the fermentation process, the profiles of the clones became more similar. Despite the initial differences among the clones, yeast diversity, and volatile compounds, they exhibited more remarkable similarities by the end of fermentation, along with desirable flavor compounds, underscoring the importance of this research in coffee fermentation.

Abstract ID: 1812 / Poster Number: 299.16S

Yeast diversity associated with Rooibos (Aspalathus linearis) tea production

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Introduction The beverage Rooibos tea is produced from *Aspalathus linearis*, a shrub endemic to the Cape Floristic Region in the Western Cape, South Africa. Its caffeine-free status, antioxidant activity and aroma and flavour contribute to this beverage's popularity world-wide. After harvest the finely cut biomass is heaped in the open air, wetted and bruised before the traditional overnight 'fermentation' process. The next day the heaps are spread out in the sun to dry. This results in a product with a characteristic red-brown colour and desired sensory profile. The 'fermentation' stage is initiated by an enzymatic oxidation process involving oxygen and polyphenol oxidases, released when the biomass is cut and bruised. Historically the process has been considered a chemical process and not microbiological. However, unlike other commodities such as wine, little is known about the yeast diversity naturally present that could impact the quality and sensorial properties of Rooibos tea.

Objective This study investigated the dominant yeast biodiversity during production and the effect of yeast isolates on tea aroma.

Method Rooibos tea samples were collected from two processing facilities in the Piekenierskloof area, South Africa. The most dominant and culturable yeasts were isolated using yeast and mould, and chromogenic agar as isolation mediums. One hundred isolates were selected and subjected to molecular typing techniques (RAPD-PCR, Ribotyping [ITS] and Sequencing).

Findings Sequencing results revealed the presence of several genera including Candida, Cyberlindnera and Pichia. The tea from the two processing facilities showed distinct population profile differences with Site 1 and 2 having nine and ten distinct yeast phenotypes, respectively. However, there was an overlap in species found at both sites. Representative yeast cultures were then used to inoculate green unfermented Rooibos tea in a laboratory-scale protocol to determine the sensory contribution of yeast growth to the tea aroma.

Conclusion Preliminary results showed that the yeast isolates can individually contribute to a desired aroma and could be used in future as inoculants during the production process to enhance Rooibos tea aroma and flavour.

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Yeast ecology and evolution: hints from yeast-insect-environment associations

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Yeasts are widely distributed in diverse environments, where they can form significant relationships with the ecosystems they inhabit. Nevertheless, we are still far from properly knowing yeast ecology. Growing body of studies has revealed intricate interactions between natural yeast populations, their vectors, and the environment. Our previous investigations uncovered a strong *Saccharomyces cerevisiae*-social wasps association that supports the yeasts' persistence, spread, and evolution through sexual reproduction.

Building on these findings, we further explored how yeast ecology and evolution are affected by these factors.

We conducted field surveys and evaluated the composition of yeast populations vectored by arthropods in Piedmont vineyards selected to represent a range of environmental settings and human impacts (e.g. vine management or proximity to buildings and roads). Yeast abundance and diversity were higher in settings closer to woods compared to samplings farther from woods. This effect was particularly relevant for some yeasts, such as *Saccharomyces cerevisiae*, which was vectored only in vineyards close to woods, and only by social wasps (*Polistes* spp. and *Vespa crabro*). Conversely, *Starmerella bacillaris* strains did not show environmental or vector preference, and whole-genome sequencing analysis revealed distinctive genomic features compared to co-specific strains isolated from other natural sources (Fig.1A). The specificity of the *S. cerevisiae*-social wasps association prompted us to investigate physiological and behavioural features promoting this association. Using molecular approaches to assess the pathways necessary for *S. cerevisiae* survival, along with customized chemical probes and physical assays to characterize the wasp intestine environment, we found that factors such as pH, medium viscosity, and presence of amino acids provide a suitable environment for the yeast's persistence. On the other hand, yeast genomics and metabolic analyses highlighted that strains with functional

genes involved in aerobic respiration and producing lower amounts of decanoic acid are more attractive to insects and hence potentially more successful in being vectored to new natural niches (Fig.1B).

Understanding the factors that govern yeast ecology requires a long, multidisciplinary, and collaborative effort. Exploration of yeast-insect associations has proven to provide key insights for supporting this challenging task.

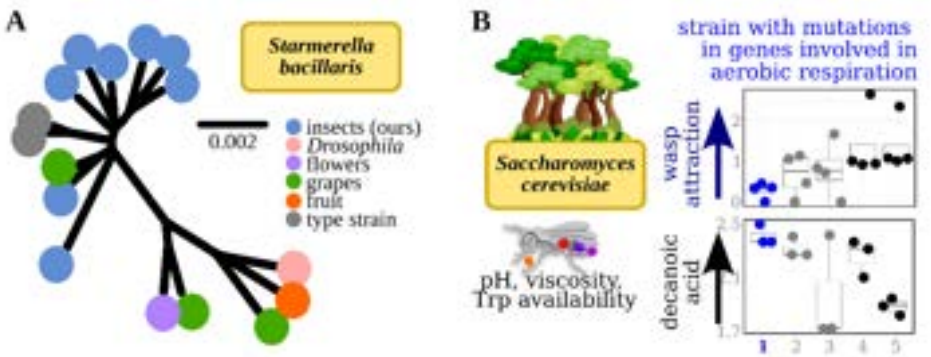


Fig.1: A) *Starmerella bacillaris* strains isolated from Piedmontese insects show distinctive genomic features; B) *Saccharomyces cerevisiae* show genetic and metabolic peculiarities explaining the specificity of the association with social wasps.

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Zinc-peptidase IrrE and repressor DdrO: a key regulatory protein pair required for radiation resistance in Deinococcus bacteria

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Deinococcus bacteria are extremely resistant to radiation (e.g. UV, gamma) and other DNA damage and oxidative stress-generating conditions (e.g. desiccation). They are able to repair a shattered genome after stress exposure, including hundreds of double-strand breaks. This involves specific physiological conditions associated to different molecular mechanisms. An efficient radiation response mechanism to induce the expression of several DNA repair genes is also essential for radiation resistance.

We have deciphered this SOS-independent response mechanism and showed that two key proteins control it: IrrE and DdrO, which are highly conserved in *Deinococcus*. Following radiation or desiccation, the transcriptional repressor DdrO is cleaved and inactivated by the metallopeptidase IrrE. We solved the 3D structure of these two regulators. IrrE is composed of a zinc-peptidase domain and a GAF-like domain. The metallopeptidase may be activated by a zinc signal but also by ssDNA generated by oxidative stress or radiation. DdrO has an N-terminal DNA binding domain, and a C-terminal dimerization domain with IrrE's cleavage site.

To characterize how they interact and which domains or regions are involved, we use integrative structural biology associated to a 'domain dissection approach' (e.g SEC, SEC-MALLS, SAXS, EPR, Far-Western blot). Many pairs of IrrE/DdrO homologs have also been identified in pathogenic, environmental or industrially interesting bacteria but they remain poorly studied.

Better understanding these proteins interactions will expand the information available for this new and large family of bacterial regulators involved in various stress defence mechanisms, for which IrrE/DdrO pair from *Deinococcus* is the reference.

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Zoogloea, the canonical floc-former in WWTPs: Re-evaluation of identity, global distribution and "slime" production

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Zoogloea is regarded as the key floc-former and 4th most abundant genus in wastewater treatment plants (WWTPs) globally. Members of the genus aid floc-formation and nutrient removal, which is vital for treatment efficiency. *"Zoogloea"* is, however, not always what it seems to be. Historically, some taxa were misclassified as *Zoogloea* based on phenotypic characteristics and later reclassified into other families or even orders. Among our new 20,000 high-quality genomes from global WWTPs, we have recovered more than 50 *Zoogloea* genomes including 6 undescribed species. Combining this with abundance data, we have re-evaluated the identity and taxonomy of "*Zoogloea*" as well as the biogeographical distribution, metabolic diversity and genomic machinery for polysaccharide (slime)-formation.

The slimy *Zoogloea* flocs were believed to be caused by the polysaccharide "zooglan", discovered in 1980, produced by *Zoogloea ramigera*-115. However, this species was later reclassified as *Duganella zoogloeoides*, leaving the polysaccharide of true *Zoogloea* uncharacterized. Recently, a novel gene cluster believed to produce the slimy polysaccharide was identified in *Z. resiniphila*. We have screened our global genome catalogue and the GTDB r220 genome database for this gene cluster and found it in several families in the Burkholderiales order including *Zoogloea* and other floc-forming genera such as *Thauera* and *Azoarcus* but also other less closely related taxa highlighting its obvious importance. Although this biopolymer seems central in many natural and engineered aquatic systems, its chemical structure and properties are largely unknown, requiring future research to unlock its huge potential for e.g. resource recovery.

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