SIMGBM
Società Italiana di
Microbiologia Generale
e Biotecnologie Microbiche

Microbiology 2017

XXXII SIMGBM Congress

Palermo, September 17-20, 2017

Programme & Abstracts

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University of Palermo
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PROGRAMME

Sunday, September 17

Palazzo Steri (Piazza Marina 61)

14:30 **Registration**

Plenary Lecture I

(*Chairs:* **M. Sosio, P. Visca**)

16:00 **Marnix Medema** *(Wageningen University, The Netherlands)*
Computational genomic approaches to study microbial biosynthetic diversity

General session - Microbial metabolisms: predictions and modelling

(*Chairs:* **R. Fani, E. Ricca**)

16:45 **Emmanuelle Bouveret** *(CNRS, Marseille, France)*
Regulation of lipid metabolism in bacteria

17:15 **Marco Moracci** *(Federico II University, Naples, Italy)*
A metagenomic approach to the study of microbial metabolic pathways

17:45 **Marco Fondi** *(University of Florence, Italy)*
Genome-scale prediction of metabolic fluxes

18:15 **Donato Giovannelli** *(Earth-Life Science Institute, Tokyo, Japan)*
Extremophiles as a model to understand the emergence and evolution of metabolism

19:00 **Welcome Reception**

Monday, September 18

Polo Didattico, Edificio 19 (Parco D'Orleans, Viale delle Scienze)

General Session - Gene regulation in viruses and pathogenic bacteria

(*Chairs:* **F. Briani, E. Tramontano**)

9:00 **Jonas Blomberg** *(University of Uppsala, Sweden)*
Endogenous retroviruses and regulatory RNAs: sequence traces of past battles between organism and microbe

9:30 **Riccardo Manganelli** *(University of Padova, Italy)*
The sigma factors of Mycobacterium tuberculosis*: physiology and role in virulence*

10:00 **Antonina Dolei** *(University of Sassari, Italy)*
Human endogenous retroviruses: physiological functions, epigenetic dysregulation and transactivation by environmental stimuli

10:30 **Maria Letizia Di Martino** *(University of Uppsala, Sweden)*
One gene and two proteins: a leaderless mRNA supports the translation of a shorter form of the Shigella *VirF regulator*

11:00 **Coffee Break**

General Session - Complex microbial systems

(*Chairs:* **F. Barras, B. Colonna**)

11:30 **Søren Molin** *(Technical University of Denmark, Kgs Lyngby, Denmark)*
Chronic bacterial infections in human airways constitute models for microbial population dynamics and adaptive and evolutionary processes

12:00 **Emilia Mauriello** *(CNRS, Marseilles, France)*
The nucleoid as a scaffold for bacterial chemosensory complexes

12:30 **Sacha Lucchini** *(University of Salerno, Italy)*
Role and diversity of global gene regulators in bacterial adaptation to the host

13:00 **Giordano Rampioni** *(University Roma Tre, Italy)*
Untangling the quorum sensing signaling network of Pseudomonas aeruginosa

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13:30 **Lunch**

14:30 **Posters overview 1 (sessions A and C)**

General Session: Extracellular vesicles from bacteria to animal cells

(*Chairs:* **: E. Affabris, AM. Puglia**)

16:00 **Meta J. Kuehn** *(Duke University, Durham, North Carolina, USA)*
Origins and functionality of outer membrane vesicles

16:30 **Andreas S. Baur** *(University of Erlangen, Germany)*
Plasma extracellular vesicles in health and disease: the biomarker revolution in Medicine?

17:00 **Mariagrazia Pizza** *(GSK, Siena, Italy)*
Outer membrane vesicles in vaccinology

17:30 **Claudia Arenaccio** *(University Roma Tre, Italy)*
Exosomes in HIV-1 pathogenesis

18:15 **Guided tour discovering the Norman-Arab heritage sites of Palermo**

Tuesday, September 19

Polo Didattico, Edificio 19 (Parco D'Orleans, Viale delle Scienze)

General Session - New frontiers in extreme marine microbiology: ecology, biotechnology and evolution

(*Chairs:* **E. Ricca, L. Vezzulli**)

9:00 **Costantino Vetriani** *(Rutgers University, New Jersey, USA)*
Prokaryotic diversity and function at a shallow-water gas vent site in the Tyrrhenian Sea

9:30 **Michail Yakimov** *(Inst. Coastal Marine Environment, CNR Messina, Italy)*
Evolutionary and biotechnological aspects of studying the deep-sea haloarchaea

10:00 **Angelo Fontana** *(Institute of Chemistry of Biomolecules, CNR, Naples, Italy)*
Capnophilic lactic fermentation and hydrogen synthesis by Thermotoga neapolitana

10:30 **Gian Marco Luna** *(Institute of Marine Sciences, Venice, Italy)*
Viruses in the dark, deep ocean

11:00 **Coffee break**

General Session - Plant-microbe interactions reloaded: new frameworks for old partners (*Chairs:* **AM. Puglia, C. Viti**)

11:30 **George C. diCenzo** *(McMaster University, Hamilton, Canada)*
Towards the minimal rhizobium N2-fixing symbiotic genome

12:00 **Davide Bulgarelli** *(University of Dundee, Dundee, Scotland, UK)*
Defining the host control of the rhizosphere bacterial microbiota

12:30 **Alessio Mengoni** *(University of Florence, Italy)*
Genome and metabolic evolution of the symbiotic rhizobia

13:00 **Massimiliano Cardinale** *(IFZ Justus-Liebig-University, Giessen, Germany)*
Diversity and specificity of the seed microbiota

13:30 **Lunch**

14:30 **Posters overview 2 (session B)**

Plenary Lecture

(*Chairs:* **F. Briani, P. Visca**)

16:00 **E. Gerhart H. Wagner** *(University of Uppsala, Sweden)*
It's ok to be different – featuring toxin-antitoxin-driven persisters

16:45 **"Franco Tatò" Prize 2017**

Sabrina Duranti *(University of Parma)*
Genetic adaptation of bifidobacteria to the human gut: insights from genomics and transcriptomics analyses

17:10 **"Mario Campa" Prize 2017**

Anella Saggese *(University of Naples)*
Analysis of the spore structure in the model species Bacillus subtilis *and* Bacillus megaterium

17:35 **Naicons Prize 2017**

Angela Casillo *(University of Naples)*

Anti-biofilm activity of a long-chain fatty aldehyde from Antarctic Pseudoalteromonas haloplanktis *TAC125 against* Staphylococcus epidermidis *Biofilm*

18:00 **Annual Society Members' Meeting**

20:30 **Social Dinner - *Palazzo Fatta, Piazza Marina 19***

Wednesday, September 20

Polo Didattico, Edificio 19 (Parco D'Orleans, Viale delle Scienze)

Plenary Lecture

(*Chairs*: **P. Branduardi, M. Ruzzi**)

9:30 **Michael Sauer** *(Boku University of Wien, Austria)*
Synthetic and diverse - microbiology on duty in industry

10:15 **Coffee break**

Parallel sessions: Oral presentation of selected abstracts

Session A: Microbial genetics and genomics

(*Chairs:* **L. Baccigalupi, F. Iannelli**)

10:45 **F. Ardizzone** *(University of Palermo, Italy)*
A "fat" Streptomycete strain overproduces antibiotics

Federica Briani *(University of Milan, Italy)*
Temperature-responsive regulation of the Escherichia coli *lpxT gene*

Marilena Falcone *(University of Milan, Italy)*
Dissecting the role of the small RNA ErsA in Pseudomonas aeruginosa *motility and biofilm regulation*

Alessandro Cascioferro *(Institut Pasteur, Paris, France)*
PknG senses amino acid availability to control metabolism and virulence of Mycobacterium tuberculosis

Martina Pasqua *("Sapienza" University, Rome, Italy)*
Understanding the expression profile of efflux pumps during the intracellular life of Shigella

Daniela Visaggio *(Roma Tre University, Rome, Italy)*
Bioluminescence-based biosensor for the detection of the Pseudomonas aeruginosa *siderophore pyochelin*

Eleonora Gaspari *(Naicons Srl, Milan, Italy)*
Insights into the biosynthesis of pseudourydimycin, a new RNA polymerase inhibitor

Marco Di Salvo *(University of Salento, Lecce, Italy)*
G4PromFinder: an algorithm for predicting transcription promoters in GC-rich bacterial genomes based on AT-rich elements and G-quadruplex motifs

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Session B: Environmental and industrial microbiology

(*Chairs:* **L. Leoni, M. Petruccioli**)

10:45 **Cristiana Sigona** *(University of Pisa, Italy)*
Effects of ciliates and rotifers on fungal degradation of natural tannins in bench tests

Marta Mangifesta *(University of Parma, Italy)*
Unveiling gut microbiota biogeography across the mammalian branch of the tree of life

Matteo Daghio *(University of Milano-Bicocca, Italy)*
BTEX removal with bioelectrochemical systems

Elisa Binda *(University of Insubria, Varese Italy)*
VanYn, a novel enzyme involved in glycopeptide resistance: a target for screening in the search of novel inhibitors of antibiotic resistance

Emanuela Claudia La Marca *(University of Palermo, Italy)*
Biological diversity of the microbial film associated with the central-Mediterranean Dendropoma cristatum (Biondi, 1859) reefs

Mariamichela Lanzilli *(University of Naples Federico II, Naples, Italy)*
Adsorption of a thermoacidophilic peroxiredoxin of Sulfolobus solfataricus *on* Bacillus megaterium *spores*

Eleonora Carota *(University of Tuscia, Viterbo, Italy)*
Cryptococcus curvatus*: a versatile yeast for the bioconversion of agro-industrial wastes into microbial oils of industrial interest*

Alberto Sutera *(University of Palermo, Italy)*
Enhancement of antibiotic productions by plasma modified PLA electrospun membranes in Streptomyces coelicolor *immobilized-cell cultivations*

Concetta Lauro *(University of Naples "Federico II", Naples, Italy)*
Polar marine bacteria as novel source of anti-biofilm agents

Daniele Ghezzi *(University of Bologna, Italy)*
Exploring the microbial diversity featuring the unique biospeleothems from the quartz-sandstone cave Imawarì Yeuta in Auyan Tepui (Venezuela)

Session C: Interactions between microbes/viruses and their hosts

(*Chairs:* **P. Alifano, O. Jousson**)

10:45 **Alessandra Aiello** *(University Roma Tre, Rome, Italy)*
Characterization of the effects induced by the HIV-1 recNEF$_{sf2}$ protein on plasmacytoid dendritic cells

Angela Corona *(University of Cagliari, Italy)*
A structure-based virtual screening and site directed mutagenesis approach identify new promising HIV-1 RNase H inhibitors

Francesca Esposito *(University of Cagliari, Italy)*
Discovery of dual allosteric Integrase and Reverse Transcriptase associated Ribonuclease H activities inhibitors: investigation of dihydroxyindole-2-carboxylic acids in the HIV-1 integrase sucrose binding pocket

Elisa Fanunza *(University of Cagliari, Cagliari)*
Single compound from Onopordum illyricum *is able to counteract Ebola virus VP35 protein inhibition of the interferon cascade*

Marco Maria D'Andrea *(University of Siena, Siena, Italy)*
Isolation and characterization of lytic bacteriophages targeting major multi-drug resistant high-risk clones of carbapenemase-producing Klebsiella pneumoniae

Daria Bottai *(University of Pisa, Pisa, Italy)*
Sensitivity to oxidative stress and hypoxia in ancestral and modern Mycobacterium tuberculosis *strains*

Giulia Giallonardi *(University Roma Tre, Rome, Italy)*
Alkyl-quinolone-dependent quorum sensing controls prophage activation, autolysis and antibiotic resistance in Pseudomonas aeruginosa *biofilm*

Makrina Totsika *(Queensland University of Technology, Queensland, Australia)*
E. coli ST131: a versatile multidrug resistant pathogen in and outside the gut

Francesca Turroni *(University of Parma, Parma, Italy)*
The fimbriome of the genus Bifidobacterium *represents extracellular structures that modulate interactions with the mammalian gut*

Annamaria Bevivino *(ENEA Casaccia Research Center, Rome, Italy)*
Time-resolved metagenomic identifies key features in the co-evolution of bacterial communities and cystic fibrosis

ABSTRACTS

Plenary Lectures

Computational genomic approaches to study microbial biosynthetic diversity

M. H. Medema
Bioinformatics Group, Wageningen University, Wageningen, The Netherlands

Microorganisms produce a wealth of specialized metabolites, which are of great importance from both ecological and clinical perspectives. Due to the accelerated accumulation of omics data, computational methods have become more and more important to identify these molecules and to assess their biological activities. In this lecture, I will highlight the work performed in my research group on using these approaches to accelerate natural product discovery, as well as to study microbe-microbe and host-microbe interactions in human, plant and animal microbiomes.

It's ok to be different: featuring toxin-antitoxin-driven persisters

E. Gerhart H. Wagner
Department of Cell and Molecular Biology, Uppsala University, 75124 Uppsala, Sweden

In my talk, I will address the fascinating topic of phenotypic heterogeneity in isogenic bacteria that grow in a homogeneous environment, here exemplified by persisters. Considered as a manifestation of bet-hedging, persisters are phenotypic variants that emerge as a small subpopulation of cells that have entered a dormant state in which they are tolerant to various antibiotics. Since persisters are not mutants, resuscitation after hours, days, or weeks can regenerate the entire population. It appears that many pathways can generate persisters, but toxin-antitoxin (TA) systems probably are dominant factors. The major classes of TA systems are type 1 where an antisense RNA (antitoxin) prevents translation of a toxin mRNA, and type 2, where an antitoxin protein blocks the activity of the toxin. Toxins can cause dormancy/ slow growth by impeding many vital growth-related functions through effects on translation, replication, mRNA stability etc. The *istR-tisB* TA system (of type 1) has been implicated in setting persister frequencies, and may therefore be considered a persister gene locus. I will describe the intricate regulation of TisB toxin translation and indicate how two RNA elements - a structure within the mRNA and the antitoxin RNA - set persister frequencies and the duration of dormancy. TisB's effect is exerted through membrane insertion, followed by membrane depolarization, and consequently severely decreased ATP levels. I will also touch upon questions that are yet unanswered. For instance, are persisters generated stochastically or deterministically? Is there a threshold which is only transgressed by few cells, and if so, what is it?

Synthetic and diverse - microbiology on duty in industry

M. Sauer
Department of Biotechnology, BOKU University of Natural Resources and Life Sciences, Vienna, Muthgasse 18, 1190 Vienna; CD Laboratory for Biotechnology of Glycerol, Muthgasse 18, 1190 Vienna; Austrian Centre for Industrial Biotechnology, Muthgasse 11, 1190 Vienna

In our societies quest to mitigate greenhouse gas emissions and petroleum use, industrial microbiology plays a key-role for the provision of processes for fuel and chemical production from renewable resources.

Clearly, the microorganism is in the center of the process and care should be taken for

its choice. Industrial production conditions are generally very harsh for the microorganism. Nevertheless, the host cells should be very efficient, which opens a vast area of conflict for the industrial microbiologist. Synthetic biology and metabolic engineering provide optimal tools for the rational design of biocatalysts. However, biodiversity is a major resource which should be tapped first. Nature solved many problems, which we face in industrial context – be it natural stress resistance or efficiency of metabolic pathways. However, all too often the rich source of natural diversity is neglected in favor of "pet" or model organisms. I propose that the fastest and most reliable path to efficient and economically viable microbial production processes uses both – natural diversity and synthetic biology. This concept shall be exemplified with bacterial and yeast host systems for the production of 1,3-propanediol, or sugar alcohols, respectively.

General session - Microbial metabolisms: predictions and modelling

Regulation of lipid metabolism in bacteria

E. Bouveret
LISM, CNRS, Aix-Marseille University, Marseille, France

Tight coordination of fatty acid (FA) and phospholipid (PL) biogenesis with growth and in response to stress is a crucial feature for all organisms. In particular, bacteria must adapt to important fluctuations of their environments, which can directly affect their membrane properties. In *Escherichia coli*, the biochemistry of FA and PL synthesis pathways is very well described. However, the study of their genetic regulation has been neglected until now.

The signaling nucleotide ppGpp, which is involved in the stringent response, shuts down FA and PL synthesis in response to nutritional downshift, presumably by direct inhibition of the first step of PL synthesis. However, recent genomic studies and our results show that ppGpp also regulates the expression of most FA and PL synthesis genes. This regulation by ppGpp results in tuning the production of lipid synthesis machineries to the growth rate. Furthermore, several envelope stress response pathways affect PL synthesis. We are currently studying how the alternative SigmaE factor and the CpxRA two-component pathways control key steps of PL to adapt and reprogram PL synthesis in response to envelope perturbations. Overall, our results highlight the importance and diversity of the genetic regulation of the FA and PL synthesis pathways.

A metagenomic approach to the study of microbial metabolic pathways

M. Moracci[1,2], A. Strazzulli[1,2], R. Iacono[2], L. Maurelli[2], R. Giglio[2], B. Cobucci-Ponzano[2]
[1]Department of Biology, University of Naples "Federico II", Via Cupa Nuova Cinthia 21, 80126 Naples, Italy, [2]Institute of Biosciences and BioResources, CNR, Via P. Castellino 111, 80131, Naples, Italy.

Communities of hyperthermophiles, composed of Bacteria, Archaea, and viruses, populating marine and terrestrial hydrothermal sites is of interest to understand their evolution in extreme conditions and as a source of biocatalysts for innovative bioprocesses as the intrinsic stability of thermozymes to common protein denaturants made them interesting tools for industrial applications.

In the framework of the discovery of new hyperthermophilic microorganisms, we decided to explore the diversity of the solfataric field of Pisciarelli, Agnano (Naples, Italy), by performing a metagenomic analysis of the microbial community living in two mud/water pools that, although very close (4 meters), greatly differ in both temperature and pH (T=85°C and pH 5.5; T=94°C and pH 1.5, for Pool1 and Pool2, respectively). DNA from Pool1 was extracted from the mud/water suspension while Pool2 DNA was obtained from the sediment. The deep sequencing by Illumina technology and the following *in-silico* analysis showed that the *phylum* Crenarcheaota was prevalent, but with great *genera* variance in the two pools according to the different environmental T and pH. The analysis of the functional categories and of the inferred prevalent metabolism found will be discussed together with the possible mechanisms of selection occurred in the two extremophilic environments.

Acknowledgement
This work was supported by the projects PON01_01966 "ENERBIOCHEM" and PON03PE_00107 "BioPolis" of the Programma Operativo Nazionale Ricerca e Competitività 2007-2013 -MIUR-Italy and by the Short-term mobility Programme "STM 2013" of the National Research Council of Italy

Genome-scale prediction of metabolic fluxes

M. Fondi, E. Bosi, L. Presta, A. Mengoni, R. Fani
Dep. of Biology, University of Florence

Understanding how the genetic potential of a microbe translates into its phenotype is a fundamental challenge in biology. Genome sequence information alone falls short in providing a dynamic and functional perspective on the real working scheme of a cell. Computational methods, such as constraint-based metabolic modelling (CBMM), are often used to bridge this knowledge gap. CBMM consists in the use of a mathematical representation of metabolism to perform genome-scale simulations and predict metabolic features at the cell level. This approach is rapidly expanding, as it combines reliable predictive abilities with conceptually simple frameworks. Among the possible outcomes of CBMM, the capability to i) guide a focused planning of metabolic engineering experiments and ii) provide a systems level understanding of (single or community-level) microbial metabolic circuits also represent primary aims in present-day microbiology. We will briefly introduce the theoretical formulation behind CBMM and then review the most recent and effective study-cases in microbes and microbial communities. These will include the use of CBMM for simulating growth in a nutritionally complex environment and the integration of gene expression data for generating of context-specific metabolic reconstruction. Also, emerging challenges and possibilities in the use of such methodologies in microbiology/biotechnology will be discussed.

Extremophiles as a model to understand the emergence and evolution of metabolism

D. Giovannelli
Earth-Life Science Institute, Tokyo, Japan; Rutgers University, New Brunswick, NJ, USA

Life may have arisen on our planet as far back as four billion years ago. Unlike today, the Earth's atmosphere at the time had no oxygen and an abundance of volcanic emissions including hydrogen, carbon dioxide and sulfur gases. How did the ancient microorganisms that inhabited our early planet make a living? And how has microbial life co-evolved with the Earth? Anaerobic thermophiles inhabit relic environments that resemble the early Earth. However, the lineage of these modern organisms co-evolved with our planet. Hence, these extremophiles carry both ancestral and acquired genes and serve as models to reconstruct early metabolism, and tease apart ancestral *vs* acquired metabolic traits. By using comparative genomic, physiologic and proteomic analyses, we identified two distinct groups of genes in a phylum of early branching bacteria, the *Aquificae*, that live in geothermal environments: the first codes for enzymes that do not require oxygen and use substrates of geothermal origin; the second appears to be a more recent acquisition, and may reflect adaptations to cope with the rise of oxygen on Earth. Our results provide insight into how the metabolism of microbes has co-evolved with the Earth's changing conditions and increased its flexibility while retaining ancestral metabolic traits.

General session - Gene regulation in viruses and pathogenic bacteria

Endogenous retroviruses and regulatory RNAs: sequence traces of past battles between organism and microbe

J. Blomberg[1], L. Vargiu[2,3], P. Rodriguez-Tomé[3], E. Tramontano[2], G. O. Sperber[4]
[1]Section of Virology, Department of Medical Sciences, Uppsala University, [2]Dept. of Life and Environmental Sciences, Cagliari University, Italy, [3]Nurideas SRK, Cagliary, Italy, [4]Physiology unit, Department of Neuroscience, Uppsala University

Present-day vertebrate genomes have been shaped by radical rearrangements and continuous (re)introduction of transposon sequences over evolutionary time. This dynamic situation allows flexibility in spite of DNA stability, but it is also a threat for organismic function. An even more chaotic situation exists at the RNA level, where diverse epigenomic RNAs, many of transposon origin, compete and collaborate in a Darwinistic fashion. This occurs in both somatic and germ line cells, in all of us. An elaborate control system is necessary to keep these "selfish" autoregulatory circuits at bay. One of several inhibitory RNA (RNAi) systems is the piwi anti-transposon system, which stores short (24-32 nt) concatenated transposon copies in genomic DNA clusters. It is especially active during early embryogenesis. It is not known if the piwi system is active against retroviruses and related retrotransposons in humans. We therefore searched for sense and antisense piwi sequences in our collection of human endogenous retroviral (HERV) sequences. It turned out that proviruses containing piwi sequences occurred on all chromosomes. As will be detailed in the presentation, the piwi system probably participates in RNAi retroviral restriction, an example of dynamic host-microbe interaction and hierarchic organismic control.

The sigma factors of *Mycobacterium tuberculosis*: physiology and role in virulence

R. Manganelli
Department of Molecular Medicine, University of Padova, Via Gabelli 63, 35121, Padova

Rapid adaptation to changing environments is one of the keys of the success of microorganisms. Since infection is a dynamic process, it is possible to predict that *Mycobacterium tuberculosis* adaptation involves continuous modulation of its global transcriptional profile in response to the changing environment found in the human body. Sigma factors are small interchangeable subunits of the RNA polymerase holoenzyme that are required for transcriptional initiation and that determine promoter specificity. The *M. tuberculosis* genome encodes 13 of these proteins, one of which: the principal sigma factor SigA is essential. Of the other 12 sigma factors, at least 6 were proved to be essential for virulence. One of the best characterized *M. tuberculosis* sigma factors is SigE. Beyond being involved in surface stress, it is essential for the arrest of phagosome maturation and consequently, virulence. In the last few years we showed that a *sigE* mutant of *M. tuberculosis* is more attenuated and more protective than BCG when used as a vaccine in animal models of infection and that SigE is involved in the determination of the basal level of resistance to several antitubercular drugs and in the development of persisters cells resistant to treatment.

Human endogenous retroviruses: physiological functions, epigenetic dysregulation and transactivation by environmental stimuli

A. Dolei
Department of Biomedical Sciences, University of Sassari, Italy

To replicate, retroviruses integrate within host DNA. If infection occurs in germ-line cells, it can be transmitted through generations. During speciation, this occurred repeatedly in our ancestors, with loss, damage, reinsertions and rearrangements, proportionate to the time from initial integration. The human genome contains the remnants of these ancient retroviruses, named human endogenous retroviruses (HERV), constituting, on average, ~8% of our DNA. Generally, HERVs are highly defective, but few complete proviruses persisted.

The expression of HERVs depends on multiple factors. Pre-requisites are preservation of functional long terminal repeats, at least one intact open reading frame, and the chromatin state.

Several triggers may modify the epigenetic control of HERVs, including environmental stimuli. Cells of different tissues vary for nuclear microenvironment, chromatin state of tissue-specific coding regions, and pool of transcription factors. Hence, baseline predisposition of a HERV to be activated may be tissue-, cell- or differentiation-specific. Many HERVs are expressed in highly proliferating tissues (placenta, embryo tissues, cancer cells). Inflammatory stimuli may activate HERVs, when epigenetically dysregulated.

Activation of specific HERVs was proposed to have role in pregnancy, sexual dimorphism, cancer progression/metastasis, and some neurological diseases. Transgenic mice show neurological diseases resembling human multiple sclerosis (HERV-W) and lateral amyotrophic sclerosis (HERV-K).

One gene and two proteins: a leaderless mRNA supports the translation of a shorter form of the *Shigella* VirF regulator

M. L. Di Martino[a,b], C. Romilly[c], E. Gerhart H. Wagner[c], B. Colonna[b], M. E. Sellin[a], G. Prosseda[b]
[a]Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Box 582, S-751 23 Uppsala, Sweden; [b]Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Biology and Biotechnologies "C. Darwin", Sapienza Università di Roma, Via dei Sardi 70, 00185 Roma, Italy; [c]Department of Cell and Molecular Biology, Uppsala University, Box 596, S-75124 Uppsala, Sweden

VirF is an AraC-like regulator controlling virulence gene expression in *Shigella* spp., one of the main cause of bacillary dysentery in humans. VirF expression is activated upon entry into the host and depends on many environmental signals. VirF protein has been identified in different forms, but only the longer one, $VirF_{30}$, has been considered as an active form. Here we show that in *Shigella*, the VirF protein is present in two different forms, independently translated. $VirF_{30}$ is responsible for activation of the virulence system, whereas $VirF_{21}$ negatively autoregulates *virF* expression itself. In addition we provide evidence supporting that $VirF_{30}$ and $VirF_{21}$ are translated by a single full length mRNA. $VirF_{21}$ can be also translated exclusively by a leaderless mRNA form, transcribed from a newly identified gene internal promoter. These new insights into the fine-tuned regulation of *Shigella* virulence open unexpected possibilities in the study of *Shigella* virulence and host cell invasion mechanisms.

General session - Complex microbial systems

Chronic bacterial infections in human airways constitute models for microbial population dynamics and adaptive and evolutionary processes

S. Molin
Novo Nordisk Center for Biosustainabilty, Technical University of Denmark, Lyngby, Denmark

The airways of cystic fibrosis (CF) patients are usually colonized and infected by a plethora of microorganisms of which the bacterium *Pseudomonas aeruginosa* is particularly frequent and persistent. We have for several years investigated such infections by *P. aeruginosa* – both from a medical point of view in order to improve treatment, and from a biological point of view in order to learn about adaptive evolution in complex and dynamic environments. Based on intensive characterization of bacterial isolates from a large number of CF patients covering decades of infection and more than 200,000 bacterial generations, we have been able to describe the major steps of colonization and infection. The early stages of colonization are typically characterized by accumulation of mutations in global regulatory genes and increases in fitness and evolvability. The later stages of infection show reduction of fitness increase, stable polymorphisms and sudden bursts of innovative changes.

Despite intensive treatment of the CF patients with antibiotics resistance development is slow and scattered. Central metabolism is changed significantly reflecting the nutrient composition of the human airway environment. Most importantly, growth rate is gradually decreased to eventally reach generation times that are 2-5 fold higher than the original ancestors isolates.

The presentation will provide an overview of the early and late adative stages of these airway infections, and discuss the evolutionary processes behind the successful persistence of the bacteria in the CF airways over periods of several decades.

The nucleoid as a scaffold for bacterial chemosensory complexes

I. Vergara Alvarez[1], A. Guiseppi[1], S. T. Islam[2], C. Morrone[1], T. Mignot[1], **E. M. F. Mauriello[1]**
[1]*Laboratoire de Chimie Bactérienne, CNRS Aix-Marseille University, Marseille, France;* [2]*INRS-Istitut Armand-Frappier, Laval, Quebec, Canada*

In the δ-proteobacterium *Myxococcus xanthus*, the formation of specialized multicellular biofilms termed fruiting bodies is mediated by the production and excretion of a matrix of proteins and exopolysaccharides (EPS). Our bioinformatics and phenotypic analyses suggest that the synthesis/export of the EPS is mediated by three genetically distinct Wzx/Wzy transporter-dependent pathways. To understand to biological function of the three EPS, we developed an *in vivo* transcriptional assay where fluorescent reporters expressed under the control of different Wzx/Wzy promoters could be observed, at the microscope, during fruiting body and biofilm maturation. Results indicate that the production of the three EPS is spatio-temporally regulated within fruiting bodies. More in particular, EPS1 is produced inside mature fruiting bodies serving as a cohesive agent; EPS2 functions as a surfactant favoring the movement and spreading of cells at the edge of biofilms; finally EPS3 constitutes the sugar part of the myxospore coat. With this work, we show that the formation of complex bacterial communities requires a spatio-temporally regulated production of matrix components.

Research in our lab is supported by an ANR Jeune Chercheur-Jeune Chercheuse to EM

Role and diversity of global gene regulators in bacterial adaptation to the host

S. Lucchini[1], I. Porcelli[2], L. Searle[2], G. Meric[2]
[1]Universita' di Salerno, Fisciano, Italy; [2]Institute of Food Research, Norwich, United Kingdom

The indigenous microorganisms of the gastrointestinal tract are not mere bystanders, but play a critical role in health maintenance. The last decades have seen changes in the population structure of commensal *Escherichia coli* in humans, with an increase in the proportion of isolates belonging to the phylogenetic group B2, which have been linked to inflammatory bowel diseases. It is therefore important to understand the factors determining which bacteria can stably colonise the gut. To address this question, we used a range of genomic and functional approaches to identify traits associated with gut colonisation by *E. coli*.

Comparative analysis of *E. coli* strains isolated from faeces or the external environment identified differences in stress resistance and nutrient acquisition abilities that correlated with strain origin and competitiveness in the GI-tract. Gene content analysis could only partially explain the observed phenotypic differences. However, gene expression analyses revealed that variations in the 'fine tuning' of global regulatory pathways are major contributors to niche adaptation. These regulatory differences were found to be phylogenetically distributed, with *E. coli* belonging to phylogenetic group B2 displaying distinct characteristics, possibly explaining their relative abundance in humans. Our findings also highlight the need to complement genomics approaches with functional analyses.

Untangling the quorum sensing signaling network of *Pseudomonas aeruginosa*

G. Rampioni[1], M. Cámara[2], P. Williams[2], P. Visca[1], L. Leoni[1]
[1]Department of Science, University Roma Tre, Viale Marconi 446, 00146, Rome, Italy; [2]Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD, UK.

Quorum sensing (QS) is a widespread communication system that allows single bacterial cells to coordinate gene expression in response to cell-density and environmental cues. Most QS systems rely on a simple molecular mechanism that involves the synthesis of a single signal molecule released in the environment and perceived (directly or indirectly) by a global transcriptional regulator, hence driving gene reprogramming in the bacterial population. However, some bacteria evolved complex QS systems based on multiple signal molecules and cognate receptors, effectors and modulators, thus complicating the understanding of their mechanism of action and physiological role. This is the case of the opportunistic pathogen *Pseudomonas aeruginosa*, in which a complex network of four interwoven QS systems based on signal molecules belonging to three chemical classes controls the expression of key virulence traits.

In this talk recent data contributing to untangle the complexity of the *P. aeruginosa* QS circuitry will be presented, with a special focus on *i)* the *pqs* system, in which multiple QS signal molecules control the activation state or expression level of different effector proteins, and *ii)* the *las* system, in which the opposite effect of two QS transcriptional factors leads to emerging regulatory properties increasing phenotypic plasticity.

General session - Extracellular vesicles from bacteria to animal cells

Origins and functionality of outer membrane vesicles

M. J. Kuehn
Department of Biochemistry, Duke University Medical Center, Durham NC 27710 USA

Membrane vesicles are released into the environment by cells in all domains of life. Specifically, Gram-negative bacteria produce vesicles from their envelope termed "outer membrane vesicles" (OMVs). OMVs are produced in a variety of physiological and geological environments and are variable for diverse species and strains. Environmental cues cause differences in OMV abundance, size, content, and function. Many of our studies of OMVs have generally focused on the function of OMVs, particularly as it relates to bacterial pathogenesis. OMVs promote the transport and pathogenic properties of virulence-related factors. OMVs can be directly targeted to specific host cell types via specific receptor-adhesin interactions. We also found that OMV can absorb and inactivate antimicrobial compounds. Utilizing genetic and biochemical analyses, we are now elucidating mechanistic aspects of OMV production and aspects of OMV function in non-pathogens. Our studies demonstrate that OMVs can enable surface remodeling and contain non-stochastic amounts of specific membrane components. Evaluation of envelope architectures of wildtype and mutants linked multiple and specific aspects of envelope structure to the budding of OMVs. The characterization of vesiculation properties in model organisms provides the foundation for future understanding of OMV production in diverse strains and species.

Plasma extracellular vesicles in health and disease: the biomarker revolution in Medicine?

A. Baur
Department of Dermatology, University Hospital Erlangen

As a clinical institution, we focus on the assessment of plasma extracellular vesicle (pEV)-derived biomarkers for the prediction and monitoring of human diseases. It is currently assumed that plasma contains at least two types of pEV, namely exosomes and microvesicles. We described an additional type of pEV, budding directly from endosomal compartments and containing numerous biomarkers including cytokines, chemokines and soluble factors (CCF), matrix metalloproteinases and miRNAs, all of which are not found in pEV of healthy individuals. These pEV have a surface marker pattern and a structural composition that is very different from exosomes. Since they derive directly from endosomal compartments by a budding process, we termed them endosomal pEV. Upon disease development, the concentration of endosomal pEV can increase dramatically. In cancer we find up to 10fold and in HIV infection up to 20fold higher concentrations of pEV. These newly appearing pEV contain a large number of the above described biomarkers in various combinations. The combination of factors is highly specific for different diseases and disease conditions. Hence these endosomal pEV-derived biomarkers will be of great importance for translational medicine in the foreseeing future and data to support this assumption will be presented from different diseases.

Outer membrane vesicles in vaccinology

M. Pizza
Research and Development GSK Vaccines, Siena, Italy

Outer Membrane vesicles are non-replicative vesicles with a spheroid structure of 50–200 nm in diameter, naturally produced by Gram-negative bacteria, and composed mainly by outer membrane proteins and periplasmic proteins, phospholipids and LPS. Because of their

composition OMVs represent a unique reservoir of surface-exposed proteins, the bacterial accessible target for antibody recognition and binding. OMVs are therefore ideal vaccine antigens as demonstrated by the Meningococcal OMV vaccines used to fight meningococcal B outbreaks in more than 30 million individuals worldwide. Although safe and effective, meningococcal OMVs have the limitation to induce protection mainly against strains expressing the homologous PorA antigen, the most abundant and immunodominant antigen, that is highly variable among strains. This aspect has restricted the utility of OMVs vaccines only to large epidemics and has emphasized the importance of a vaccine against meningococcal B disease with a broader protection spectrum able to address the global epidemiological need. To address this need, a multicomponent vaccine, 4CMenB, that includes the OMV form the New Zealand outbreak strain has been designed. This vaccine has been licensed in 38 countries worldwide and shown to be 84% effective in a mass infant immunization campaign in UK.

The OMVs used for large scale immunization campaign ad as component of the 4CMenB vaccine are produced by detergent extraction. Proteomic analysis of detergent extracted OMVs (dOMVs) or spontaneously released native vesicles from overblebbing bacterial mutants GMMA (Generalized Membrane Modules Antigen) show that they composition is very different, with GMMAs significantly enriched in outer membrane proteins. The antigen composition of GMMAs and the high immunogenicity makes them ideal vaccine antigens.

Identification of mutations able to confer the OMV-overblebbing phenotype represents a valuable approach to study bacterial membrane composition and architecture and to design new effective vaccine formulations

Exosomes in the pathogenesis of HIV-1

C. Arenaccio[a], E. Affabris[b], M. Federico[a]

[a]National Center for Global Health, Istituto Superiore di Sanità, Viale Regina Elena, Rome, Italy; [b]University Roma Tre, Department of Science, Viale G. Marconi, Rome, Italy

Cells secrete various membrane-enclosed microvesicles from their cell surface (shedding microvesicles) and from internal, endosome-derived membranes (exosomes). Intriguingly, these vesicles have many characteristics in common with viruses, including biophysical properties, biogenesis, and uptake by cells. Accumulative findings suggest that exosomes resemble HIV-1 particles in many aspects, from their physical properties to composition. Interestingly the Nef protein of HIV-1, an important virulence factor, induce the production of Nef-containing exosome that mediate its transfer to uninfected cells. In addition Nef is transferred cell to cells also through nanotubes or cell to cell contact depending on the Nef producing and Nef receiving cells. This transfer might have pathogenetic consequences.

$CD4^+$ T lymphocytes are one of the major target of HIV-1 infection and replicate HIV-1 when activated, but resist HIV-1 replication when they are in a quiescent/resting state. We reported that treatment of resting $CD4^+$ T lymphocytes with exosomes produced by HIV-1 infected cells induces cell activation and susceptibility to HIV replication through a Nef- and ADAM17-dependent mechanism[1,2]. Next, we present data regarding the effects of these exosomes on cells latently infected with HIV-1. HIV-1 latency is the major hurdle toward HIV-1 eradication. Infact, HIV-1 infection is efficiently counteracted by combination anti-retroviral therapy (cART) which, despite preventing disease progression, does not eradicate virus infection which persists in a latent form. Our results strongly suggest that latent HIV-1 can be activated by TNFα released by cells upon ingestion of exosomes released by infected cells. This effect depends on the activity of exosome-associated ADAM17, that cleaves the membrane-bound precursor of TNF-alpha to its mature soluble form[3].

Morever, recent findings show that HIV-1 and exosomes can be internalized by dendritic cells (DCs) through a common pathway leading to their transmission to $CD4^+$ T lymphocytes by means of mechanisms defined as trans-infection and trans-dissemination, respectively. We

observed that mDC-mediated trans-dissemination of exosomes from HIV-1-infected cells to resting $CD4^+$ T lymphocytes induces efficient trans-infection and HIV-1 expression in target cells. Most relevant, when both mDCs and $CD4^+$ T lymphocytes were isolated from cART-treated HIV-1-infected patients, trans-dissemination of exosomes from HIV-1-infected cells led to HIV-1 reactivation from the viral reservoir. These evidences shed new light on the mechanism of HIV reactivation in latent reservoirs suggesting the important role of exosome trans-dissemination in this process[4].

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General session - New frontiers in extreme marine microbiology: ecology, biotechnology and evolution

Prokaryotic diversity and function at a Shallow-Water Gas Vent Site in the Tyrrhenian sea

C. Vetriani[1, 2]

[1]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ 08901, USA; [2]Institute of Earth, Ocean and Atmospheric Sciences, Rutgers University, New Brunswick, NJ, USA

The Tyrrhenian margin of Southern and Central Italy is a volcanic geothermal region with a thinned continental crust. It is characterized by heat flows and degassing of CO_2 of deep magmatic/mantle origin due to the volcanoes present in the region. The Tor Caldara site is characterized by gas emissions associated with the volcanic system of Colli Albani. The study site is a shallow submarine vent degassing mainly CO_2 and H_2S, with no evidence of thermal anomalies. In this study, we surveyed the prokaryotic diversity of the established filamentous biofilms growing in the vicinity of the gas vents, encrustation seen around the orifice of the gas vents as well as the sediment in the venting area. Our findings indicate that the sediment community is most diverse and includes members of *Proteobacteria, Acinetobacteria Bacteroidetes, Planctomycetes, Acidobacteria*, *Firmicutes* and *Cyanobacteria,* among other groups. The crust community is slightly less diverse comprising predominantly members of *Proteobacteria*, *Cyanobacteria* and *Bacteroidetes*. The established filamentous biofilm community is the least diverse and most specialized and is dominated primarily by *Epsilonproteobacteria, Gammaproteobacteria* and *Bacteroidetes.* Integrated metagenomic and metaproteomic analyses were further carried out on representative samples to assess the metabolic potential and the expressed functions of the Tor Caldara microbial communities. In conjunction with the omics-based assessment of diversity, metabolic potential and function, we enriched and isolated bacteria using different culture conditions. Two novel bacterial strains, $TC3^T$ and $TC8^T$, 92.76% and 91.25 % similar to *Sulfurimonas gotlandica* and *Magnetovibrio blackemorei* respectively, were isolated. $TC8^T$ was further characterized. I will discuss how the integration of culture-based and molecular analyses is providing qualitative and quantitative insight into prokaryotic diversity and function at the shallow-water gas vent of Tor Caldara.

Evolutionary and biotechnological aspects of studying the deep-sea haloarchaea

M. Yakimov

Institute for Coastal Marine Environment, CNR Messina, Italy

Extremely halophilic archaea represent a unique class *Halobacteria* within the phylum *Euryarachaeota*. Their main property is a massive adaptation of cell proteins to extreme salinity. Although apparently originated from anaerobes, these archaea had acquired approximately 1,000 eubacterial genes (30% of their genomes) by a massive lateral LTG. As a consequence of this acquisition, most of the known haloarchaeal species are aerobic heterotrophs inhabiting oxygenated layers of brines with very few of them possessing the ability for fermentative growth with sugars or arginine and for anaerobic respiration using nitrate, fumarate, DMSO or TMAO as terminal electron acceptors. Despite the molecular evidences on the presence of diverse uncultured haloarchaeal lineages in anoxic sediments of hypersaline habitats, no attempts until recently, been undertaken to obtain this physiological group in the culture.

Our recent research aimed at microbial sulfur respiration at extreme salinity in anaerobic sediments of hypersaline chloride-sulfate lakes with neutral pH uncovered two novel functional

groups of obligately anaerobic haloarchaea using sulfur compounds as electron acceptors. One group, described as *Halanaeroarchaeum sulfurireducens*, is using acetate as electron donor for elemental sulfur-dependent respiration - a catabolic route not known previously in the whole Archaeal Kingdom. The second group, related to *Haa. sulfurireducens* and described as *Halodesulfuriarchaeum formicicum*, represents a first example of lithoheterotrophy in haloarchaea. These organisms are using formate or H_2 as electron donor and elemental sulphur, thiosulfate or DMSO as acceptor. The discovery of these groups of obligately anaerobic sulphur-respiring haloarchaea, widely present in anoxic hypersaline environments, including deep-sea brine lakes, showed that the dominant paradigm on the haloarchaeal physiology is far from completeness.

Their placement on the phylogenetic tree constructed with concatenated ribosomal proteins within order *Halobacteriales* is strongly supported. Following approach of Wolf et al. (2012) to reconstruct common ancestors, we tried to infer gene gains and losses on the branches leading to these physiologically novel haloarchaeal groups. Two scenarios were scrutinized: (i) the history of this "obligate anaerobic" clade during diversification appears to have involved gradual extensive gene loss (cytochromes oxidase genes, rhodopsin, gas vesicle system) compared to gene gain; (ii) this "obligate anaerobic" clade was never facing the massive acquisition of eubacterial genes and remained anaerobic, colorless and non-buoyancing organism as common *Halobacteriales*/*Methanomicrobiales* ancestor should be. Besides the outcomes of this evolutionary study, several attempts in biotechnological application of these novel haloarchaeal organisms will be demonstrated during presentation.

Capnophilic lactic fermentation and hydrogen synthesis by *Thermotoga neapolitana*

A. Fontana, L. Dipasquale, G. d'Ippolito
CNR - Istituto di Chimica Biomolecolare, Bio-Organic Chemistry Unit, Via campi Flegrei 34, 80078 Pozzuoli, Napoli, Italy

The heterotrophic bacterium *Thermotoga neapolitana* produces hydrogen (H_2) by fermentation of organic substrates. The process is referred to as dark fermentation (DF) and is completed by synthesis of organic acids whose composition varies in agreement with the culture conditions. Here we show that DF is shunted by an unprecedented mechanism of capture and sequestration of carbon dioxide into lactic acid (**1-3**). This novel pathway is based on direct coupling of CO_2 with C_2 substrates, such as exogenous acetate or acetyl-CoA derived from fermentation of carbon-rich materials. When the bacterial cultures are supplemented with carbohydrates, synthesis of lactic acid occurs to the detriment of acetic acid and, in contrast with the currently accepted DF model, does not affect H_2 productivity (**4**). The CO_2-dependent fermentation process, which we named capnophilic lactic fermentation (CLF), yields at the same time production of H_2 and conversion of CO_2 into added value chemicals without biomass deconstruction (**5-6**).

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Viruses in the dark, deep ocean

G. M. Luna

Istituto di Scienze Marine, Consiglio Nazionale delle Ricerche (CNR - ISMAR), Largo Fiera della Pesca, 60125 Ancona (Italy)

Life in the largest ecosystem on the planet is dominated by microbes. In addition to cellular microbes, viruses constitute fundamental components of the deep sea biota, but the lack of viral isolates, coupled with the paucity of data on viral diversity, limits our knowledge about which viruses reside in the dark ocean, the phage/host interactions and the ecological role that viruses play. In this talk, I will summarize results of recent collaborative researches that investigated, by integrating culture-dependent and -independent approaches, the diversity, phage-host interaction and ecological role of deep sea viruses. We assessed patterns in abundance and processes across the bathypelagic ocean, to demonstrate how important viruses are in the major pathways of carbon cycling. Also, we isolated and characterized the first phages from the deep sea, infecting one of the most abundant deep sea bacterium (Alteromonas), and mapped their genomes over a collection of oceanic viromes, to investigate their abundance across the ocean. Lastly, we produced a sequence dataset of 18 dsDNA viral-fraction bathypelagic metagenomes, to assess the diversity of populations, identify abundant and rare bathypelagic viruses, and make predictions of their hosts. Taken together, our results shed light on one of the most common phage/host interaction in the deep sea, provide the first laboratory models to better understand mechanisms of viral infection, and serve as foundational knowledge to comprehend more fully the ecological role by viruses in the deep ocean.

General session - Plant-microbe interactions reloaded: new frameworks for old partners

Towards the minimal rhizobium N_2-fixing symbiotic genome

G. C. diCenzo, M. Zamani, B. Milunovic, T. M. Finan
Department of Biology, McMaster University, Hamilton, Ontario, Canada

The rhizobium – legume symbiosis is well-studied, and many key bacterial genes involved in this process have been identified through decades of genetic studies. However, the lack of an appropriate system has hindered our ability to decisively elucidate the necessary and sufficient set of genes required for symbiotic nitrogen fixation. To this end, we have constructed a *S. meliloti* strain in which 45% of the genome, representing nearly 3000 genes, was deleted. This strain was unable to form nodules on its legume host, while re-introduction of all 3000 genes fully restored symbiotic capabilities. To begin identifying the minimal set of these 3000 genes necessary for symbiosis, we constructed a series of smaller, defined deletions cumulatively removing the majority of these genes, and examined their symbiotic consequences. Only four regions were found, which accounted for less than 12% of the 3000 genes, that were absolutely essential for symbiosis. These regions will now serve as our initial target of the necessary and sufficient symbiotic gene set. Additionally, the path towards the minimal symbiotic genome has led to new genetic knowledge of the symbiosis, including the identification of a novel genes involved in symbiosis, and the divergent roles of the BacA protein.

Defining the host control of the rhizosphere bacterial microbiota

R. Alegria Terrazas[1], S. Robertson-Albertyn[1], M. Blank[1], L. Pietrangelo[1,2], F. Concas[1], **D. Bulgarelli**[1]
1-Plant Sciences, School of Life Sciences, University of Dundee at the James Hutton Institute. Invergowrie DD2 5DA, Scotland, United Kingdom; 2- Department of Biosciences and Territory, University of Molise, Campobasso I-86100, Italy

The rhizosphere, the thin layer of soil surrounding plant roots, represents a distinct microbial habitat whose composition is influenced by plant growth and development. In turn, the bacterial communities inhabiting the rhizosphere, the rhizosphere bacterial microbiota, establish relationships with their host which include parasitism, commensalism and mutualism. My group uses barley (*Hordeum vulgare*) as a model to gain novel insights into the genetic basis of host-microbiota interactions.

We recently characterised the microbiota populating the rhizosphere of 30 wild and cultivated barley genotypes grown in distinct agricultural soils. High-resolution 16S rRNA gene profiles revealed that community composition can recapitulate the major genetic groups emerged during barley domestication, providing further support to the notion that microbiota recruitment can be genetically inherited. In particular, we identified members of the barley microbiota, overrepresented by Actinobacteria, whose recruitment pattern mirrors the domestication trajectory of modern cultivated barleys. We are now using this information to link microbial diversity with host genetic diversity, defined at a depth of thousands SNPs in the barley genome.

In a parallel line of investigation, we demonstrated that mono-Mendelian mutations in a single root trait can displace the microbiota associated with barley plants in different soils. Interestingly, this host genotype-dependent effect on the rhizosphere microbiota impacted on a taxonomically narrow group of bacteria, predominantly Actinomycetales, Burkholderiales, Rhizobiales, Sphingomonadales and Xanthomonadales. To ultimately determine whether members of the microbiota are causally linked to given plant phenotypes, we have developed recolonization assays to quantify the contribution of synthetic bacterial communities to rhizosphere formation and functioning.

Genome and metabolic evolution of the symbiotic rhizobia

A. Mengoni
Dept. of Biology, University of Firenze, Florence, Italy

Many bacterial species contain a multipartite genome, that is, a genome divided into two or more large DNA fragments (replicons). This genome architecture is particularly interesting as it is common in societally relevant organisms, including rhizobia, but also *Vibrio* and *Burkholderia* genera. Many aspects of the evolution and biological role of this genome organization remain unclear, such as the contribution of each replicon in environmental adaptation. The N_2-fixing endosymbiont of legumes *Sinorhizobium meliloti* contains a multipartite genome consisting of three main replicons, one chromosome, one chromid and one megaplasmid. We performed comparative genomic analyses and reconstruction of the regulatory and metabolic network to investigate the differential functional and evolutionary patterns of the replicons. Results presented strong evidences consistent with the conclusion that replicons have nearly independent evolutionary routes and, in particular, that secondary replicons evolved to fulfill specialized functions, particularly host-associated niche adaptation. Transplantation experiments of secondary replicon have been also performed to test their metabolic independence.

Diversity and specificity of the seed microbiota

M. Cardinale[1], MD Mahafizur Rahman[1], E. Flory[1], A. Weimer[1], C. Suarez[1], H.–Werner Koyro[2], Z. Abideen[2,3], S. Schnell[1]
[1]*Institute of Applied Microbiology, Justus-Liebig-University Giessen, Giessen, Germany;* [2]*Institute of Plant Ecology, Justus-Liebig-University Giessen, Giessen, Germany;* [3]*Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi, Pakistan*

The seed microbiome is getting interest due to its importance for the "plant holobiont". In this work, the endophytic microbiota of six different seed batches of barley (*Hordeum vulgare* L.), representing four cultivars, four geographical sites and four harvest years, was investigated by cultivation–dependent and –independent analysis, microscopy and functional assays. Eighty isolates were obtained, grouped at intraspecific level and identified by phylogenetic analysis. Some isolated bacteria were shared between seed samples: the dominant was *Pantoea agglomerans*, confirmed by metagenomics analysis. *Paenibacillus kyungheensis* was another shared isolate but was not detected by metagenomics, which in turn showed *Pseudomonas* as a further shared *taxon*. Fluorescence *in situ* hybridization and confocal laser scanning microscopy showed low–abundant bacteria residing in the seed endosphere able to colonize massively the root system after seed germination. Enrichment assays with the dominant *Pantoea agglomerans* strain promoted barley growth in greenhouse, and improved plant mineral nutrition. Possible mechanisms of action included auxin and siderophores production, phosphate solubilisation and nitrogen fixation. This work showed a new type of highly specific and stable plant–microbe association, which is maintained across boundaries of plant genetics, geography and time by using seeds as both storage and vectors.

PARALLEL SESSIONS: Oral presentation of selected abstracts

Session A - Microbial genetics and genomics

A1. A "fat" Streptomycete strain overproduces antibiotics

F. Ardizzone[1], E. Rimini[1], G. Renzone[2], A. Palumbo Piccionello[1], M. Galardini[3], M. Morgante[1], A. Maggio[1], G. Gallo[1], A. Mengoni[3], A. Scaloni[2], A. M. Puglia[1]
[1]STEBICEF Department, University of Palermo, Viale delle Scienze Ed. 16, 90128 Palermo (PA), Italy; [2]Proteomic and Mass Spectrometry Laboratory, ISPAAM, National Research Council, 80147 Naples, Italy; [3]Laboratory of Microbial and Molecular Evolution, Dept. of Biology, University of Florence, 50019 Sesto Fiorentino (FI), Italy

The filamentous actinomycete *Streptomyces coelicolor* is a model organism for microbial morphological differentiation and production of secondary metabolites like antibiotics. The *bldF* (166) strain is unable to differentiate, because it forms red–orange pigmented colonies lacking both aerial hyphae and spores.

Proteomic analysis showed that glycerol-3-phosphate dehydrogenase (SCO1661) is strongly up-regulated in the 166 strain in the respect of wild type. Based on KEGG metabolic database, this enzyme is involved in the biosynthesis of secondary metabolites and glycerophospholipid metabolism, catalyzing the conversion of glycerol-3-phosphate into glyceron phosphate.

Accordingly, the 166 strain overproduces two mycelium-associated compounds belonging to prodigiosin group, namely undecylprodigiosin and streptorubin B, as revealed by HPLC-ESI-MS analysis. These red-pigmented antibiotics possess also antifungal, antitumor, and antimalarial activities. Interestingly, 166 strain overproduces oleic and stearic acids as highlighted by GC/MS analysis on the fatty acid content. Fatty acids are components of cell membranes, source of metabolic energy and effector molecules regulating metabolism; thus the different fatty acid content may be associated with the different physical-chemical property of hyphae (i.e. observed hydrophobicity) and/or different regulation of metabolism (i.e. morpho-physiological differentiation).

This study expands our knowledge on the relationship between primary and secondary metabolism in *Streptomyces*.

A2. Temperature-responsive regulation of the *Escherichia coli lpxT* gene

F. Briani, B. Sciandrone, S. Perego, C. Portugalli
Dip. di Bioscienze, Università degli Studi di Milano

RNA thermometers (RNATs) are thermo-labile mRNA secondary structures modulating translation initiation in response to temperature changes. We found that the expression of *Pseudomonas aeruginosa* LpxT, a membrane protein phosphorylating the lipid A moiety of the lipopolysaccharide, was induced in response to a temperature upshift and we proposed that an RNAT was responsible for such regulation.

Here we show that the expression of the *Escherichia coli LpxT* orthologous protein (EcLpxT) is also temperature-responsive. Increased Ec*LpxT* expression at high temperature (i.e. 37-42 vs. 28°C) depends on post-transcriptional mechanisms. *In vitro* assembly of the ribosome on the Ec*lpxT* transcript is less efficient at 28° than at 37-42°C. The short *lpxT* 5'-UTR is predicted to form an unstable stem-loop involving the Shine-Dalgarno region (SD). Point mutations changing the stability of the stem-loop or ameliorating the complementarity of the SD with the 16S rRNA affect thermoregulation, showing that both elements cooperate in *lpxT* gene regulation. Our results suggest that Ec *lpxT* is regulated by an unusual RNAT that exploits the combination of sub-optimal elements to confer temperature-responsive translation to the mRNA.

A3. Dissecting the role of the small RNA ErsA in *Pseudomonas aeruginosa* motility and biofilm regulation

M. Falcone[1], S. Ferrara[1], E. Rossi[2], H. Krogh Johansen[2], S. Molin[3], G. Bertoni[1]
[1]University of Milan, Department of Biosciences, Milan, Italy; [2]Department of Clinical Microbiology, Rigshospitalet, Copenhagen Ø, Denmark; [3]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby, Denmark

ErsA is a novel *Pseudomonas aeruginosa* small RNA responsive to infection-relevant host *stimuli*. Its transcription is under the control of the alternative sigma factor σ^{22} (AlgT/U), implicated in bacterial virulence.

In strain PAO1, ErsA exerts a direct negative post-transcriptional regulation on the bi-functional enzyme AlgC, which is involved in alginate and esopolysaccharide products.

We aim at the characterization of novel target genes regulated by ErsA to expand the knowledge about the ErsA-based regulatory network by multiple approaches.

Our results comprise:

i) *In silico* analyses identified new putative target genes mainly involved in biofilm formation and exopolysaccharides production.

ii) We specifically validated the interaction between ErsA and *amrZ* mRNA, both *in vitro* and *in vivo*

iii) The RNA-seq analysis showed that some of the genes whose expression is positively controlled by ErsA are important for biofilm development (i.e. genes belonging to the *pel* operon, *algD*).

iv) In line with the other results, the ErsA deletion mutant shows a hyper-motile phenotype compared to the wildtype and develops a thin and flat biofilm.

Overall, our results suggest that the small RNA ErsA might represent a relevant post-transcriptional regulator in biofilm development at different levels, likely interacting with different mRNA targets.

A4. PknG senses amino acid availability to control metabolism and virulence of *Mycobacterium tuberculosis*

B. Rieck¶,[1], G. Degiacomi¶,[2], M. Zimmermann¶,[3], A. Cascioferro¶,[4], F. Boldrin[2], N. R. Lazar-Adler[6], A. R. Bottrill[6], F. le Chevalier[4], W. Frigui[4], M. Bellinzoni[5], M.-N. Lisa[5], P. M. Alzari[5], L. Nguyen[7], R. Brosch[4], U. Sauer[3], R. Manganelli[2], H. M. O'Hare[1,8]
[1]Department of Infection, Immunity and Inflammation, University of Leicester, Leicester LE1; 7RH, UK; [2]Department of Molecular Medicine, University of Padua, Via Gabelli 63, Padova, 35121 Italy; [3]Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland; [4]Institut Pasteur, Integrated Mycobacterial Pathogenomics Unit, Paris 75724, France; [5]Institut Pasteur, Unité de Microbiologie Structurale and CNRS-UMR3528, 75015 Paris,; France; [6]Core Biotechnology Services, University of Leicester, Leicester LE1 7RH, UK.; [7]Department of Molecular Biology and Microbiology, Case Western Reserve University School; of Medicine, Cleveland, OH 44106, USA; [8]Department of Molecular and Cellular Bioscience, University of Leicester, Leicester LE1 7RH, UK
¶These authors contributed equally

A key feature of the pathogen *Mycobacterium tuberculosis* is its ability to survive and replicate within human macrophages. Protein kinase G (PknG) is known to be required for virulence of *M. tuberculosis* and is the only bacterial serine/threonine protein kinase to be known as a virulence factor. However, the molecular mechanisms underlying its function in virulence are unknown and the role(s) of PknG are controversial. Here, we disrupted the genes encoding PknG and its putative substrate GarA in *M. tuberculosis* and related non-pathogenic *Mycobacterium smegmatis*. We observed changes in protein phosphorylation that suggest GarA is the substrate

of PknG, and changes in growth and metabolome that establish this pair of proteins as a bone fide system for metabolic regulation. We also observed a dramatic impact on the ability of GarA-deficient *M. tuberculosis* to grow and survive in macrophages and mice. This highlights the link between metabolism and virulence and suggests that *M. tuberculosis* inside macrophages may have restricted access to amino acids. Our study also provides a first indication of the nutrients that may be sensed by *M. tuberculosis* inside macrophages and provides new insights into the rate and reversibility of serine/threonine phosphorylation in bacteria.

A5. Understanding the expression profile of efflux pumps during the intracellular life of *Shigella*

M. Pasqua[a], M. Grossi[a], S. Scinicariello[a], L. Aussel[b], F. Barras[b], M. L. Di Martino[c], G. Prosseda[a], B. Colonna[a]

[a]Department of Biology and Biotechnology "C. Darwin", Sapienza Università di Roma, Via dei Sardi 70, 00185 Roma, Italy; [b]Laboratoire de Chimie Bactérienne, Aix-Marseille Université - CNRS, Institut de Microbiologie de la Méditerranée, 31 Chemin Joseph Aiguier 13009 Marseille, France; [c]Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Biomedical Center, Uppsala University, Box 582, S-751 23 Uppsala, Sweden

In several bacterial pathogens, efflux pumps, besides exporting antimicrobial agents, play a role in bacterial pathogenicity.

Shigella, the causative agent of bacillary dysentery, during its evolution towards pathogenicity, has conserved 14, out of 20 present in the genome of its commensal ancestor *Escherichia coli*, operons encoding efflux pumps systems.

The aim of this work is to analyze the induction of efflux pumps conserved in *Shigella* during the infection of macrophages and epithelial cells and to identify those that are potentially involved in the pathogenesis of *Shigella*.

Our results allowed us to identify efflux pump encoding genes up (*mdtJ*)- or downregulated (*acrA*) in macrophages and epithelial cells. Among them, *emrK*, encoding the membrane subunit of the EmrKY multidrug efflux pump, is specifically up-regulated within macrophages. Moreover, an *emrK* mutation negatively affects *Shigella*'s fitness.

A deeper analysis of the efflux system EmrKY obtained through GFP assays and microscopy reveals that, while its role is still unknown, EmrKY expression during infection is specific and pH and ion concentration dependent and is mediated by the two-component system EvgA/EvgS.

A6. Bioluminescence-based biosensor for the detection of the *Pseudomonas aeruginosa* siderophore pyochelin

D. Visaggio, M. Pirolo, C. Bonchi, E. Frangipani, P. Visca

Department of Sciences, Roma Tre University, Rome, Italy

Iron is an essential nutrient for bacteria. To face the severe iron limitation encountered *in vivo* during infection, the opportunistic pathogen *Pseudomonas aeruginosa* has evolved several strategies to acquire iron, including the production of two siderophores, pyoverdine (PVD) and pyochelin (PCH), which chelate ferric iron and deliver it to the cell.

We have recently shown that the iron-mimetic metal Gallium [Ga(III)], the active component of the FDA-approved drug Ganite®, can successfully be repurposed for antibacterial chemotherapy, and that the entrance of Ga(III) in *P. aeruginosa* cells can be potentiated by complex formation with PCH.

Here, we report the generation of a bioluminescence-based biosensor to investigate the response of *P. aeruginosa* to the presence of exogenously provided PCH, either in its apo-form or complexed with Fe(III) and Ga(III). To this aim, the promoter region of the *pchE* gene for

pyochelin biosynthesis was fused to the *luxCDABE* operon, and inserted into the chromosome of the *P. aeruginosa* siderophore null mutant Δ*pvdA*Δ*pchD*. The resulting biosensor was capable of responding to PCH, either in its apo-form or complexed with Fe(III), displaying excellent sensitivity range (5 nM - 5 μM). Interestingly, the biosensor also responded to activation by PCH-Ga(III), indicating that PCH-Ga(III) is actively transported in *P. aeruginosa* and activates *pchE* expression via the PchR regulator, hence increasing PCH synthesis. The positive regulatory loop triggered by PCH-Ga(III) results in auto-induction of both synthesis and transport of PCH, further potentiating the entrance of Ga(III).

A7. Insights into the biosynthesis of pseudourydimycin, a new RNA polymerase inhibitor

E. Gaspari[1], M. Sosio[1], S. Pessina[1], S. Maffioli[1], A. Bernasconi[1], S. Serina[1], M. Iorio[1], Y. Zhang[2], D. Degen[2], R. Ebright[2], S. Donadio[1]

[1]Naicons Srl, 20139 Milan, Italy; [2]Rutgers University, Piscataway, NJ 08854, USA;

Pseudourydimycin, PUM, is a potent and selective inhibitor of bacterial RNA polymerase (RNAP) that shows no cross-resistance with the clinically used RNAP inhibitors rifampicin and fidaxomicin. PUM inhibits RNAP from both Gram-positive and Gram-negative bacteria, has broad-spectrum antibacterial activity and shows efficacy in animal infection models. PUM is a natural product produced by *Streptomyces* strains.

Structurally PUM comprises a guanidinylated, N-hydroxylated Gly-L-Gln dipeptide conjugated to 5'-amino-pseudoridine,attesting to be the first nucleoside-analog inhibitor (NAI) that selectively inhibits bacterial RNAP. Crystal structure of RNAP in complex with PUM suggests the compound binds key residues in the enzyme, in DNA and in nascent RNA.

Running the *Streptomyces* ID38640 genome sequence with the antiSmash platform led to the identification of 29 distinct clusters directing the synthesis of different specialized metabolite. Among the clusters we have identified a genomic locus involved in the synthesis of this inhibitor. PUM formation is directed by a small biosynthetic gene cluster -- designated *pum* cluster -- comprising 12 predicted genes. The correctness of the identified cluster was confirmed using a method for the genetic manipulation of *Streptomyces* sp. ID38640, based on intergeneric conjugation from *Escherichia coli* that allows to knock out selected *pum* genes.

Analysis by LC-MS of the intermediates accumulated by the KO mutants, feeding experiments and bioinformatic predictions of the roles of the *pum* genes led to a hypothesis for the PUM biosynthetic pathway.

Keywords: Pseudourydimycin, RNAP inhibitor, *pum* cluster

A8. G4PromFinder: an algorithm for predicting transcription promoters in GC-rich bacterial genomes based on AT-rich elements and G-quadruplex motifs

M. Di Salvo[a], E. Pinatel[b], A. Talà[a], M. Fondi[c], C. Peano[b], P. Alifano[a]
[a]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; [b]Institute of Biomedical Technologies National Research Council, Segrate, Milan, Italy; [c]Department of Biology, University of Florence, Florence, Italy

Over the last few decades, computational genomics has tremendously contributed to decipher biology from genome sequences and related data. Considerable effort has been devoted to prediction of transcription promoter and terminator sites that represent the essential "punctuation marks" for DNA transcription. In this study we investigated the possibility to identify putative promoters in prokaryotes based on evolutionarily conserved motifs, and focused our attention on GC-rich bacteria in which promoter prediction with conventional, consensus-based algorithms is often not-exhaustive. We developed an algorithm to identify putative promoters based on AT-rich elements and G-quadruplex DNA motifs, and tested its performances by using available genomic and transcriptomic data of the model microorganisms *Streptomyces coelicolor* A3(2) and *Pseudomonas aeruginosa* PA14. Results demonstrated that the algorithm was more accurate than currently available consensus-based bioinformatic tools. In fact G4PromFinder was able to predict up to 40% and 33% of promoters, respectively, in *S. coelicolor* and *P. aeruginosa*, compared to up to 22% and 29% of promoters that could be predicted by using the most recent consensus-based tool PePPER. Moreover, our search method could implement existing consensus-based tools since we observed a low overlap-value between the promoters herein identified and those pinpointed by available methods.

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Session B - Environmental and industrial microbiology

B1. Effects of ciliates and rotifers on fungal degradation of natural tannins in bench tests

C. Sigona[a], A. Bardi[a], F. Spennati[b], G. Mori[c], S. Di Gregorio[a], G. Munz[b], G. Petroni[a]
[a]Biology Department, University of Pisa, Via L. Ghini 13, 56126 Pisa, Italy; [b]Department of Civil and Environmental Engineering, University of Florence, Via S. Marta n.3, 50139 Florence, Italy; [c]CER2CO (Centro Ricerca Reflui Conciari), Via Arginale Ovest 8, 56020 San Romano-San Miniato, Pisa, Italy

Tannery wastewater presents high concentrations of pollutant recalcitrant molecules, such as tannins, characterized by high refractory organic load, which reduce the efficiency of biological treatment processes. Recent studies showed that several fungal strains, including *Aspergillus tubingensis* MUT 990, thanks to their ability to produce the enzyme tannase, were effective in the degradation of such recalcitrant molecules. However, the growth of *A. tubingensis* and, consequently, its degradation performance decrease when the bacterial load is high.

The aim of the study is to evaluate whether the introduction of bacterivorous organisms, such as protist ciliates and/or rotifers, in bench scale experiments using fungi to remove natural tannins could favor fungal growth and recalcitrant compounds removal.

To achieve this goal, rotifers and different functional categories of ciliates were isolated and cultures established. *Paramecium calkinsi*, *Tetrahymena* sp., *Vorticella* sp. and a culture of rotifers were later selected due to their capability to survive and actively grow in Tara medium (tannin extract of *Caesalpina spinosa* 0.9 g/l). A preliminary batch experiment has been performed to investigate whether the selected organisms could enhance recalcitrant compounds removal of *A. tubingensis*, immobilized on polyurethane foam cubes, toward Tara tannin medium. The following parameters have been monitored: bacterial concentration, fungal dry weight, soluble chemical oxygen demand, total organic carbon (TOC), and A280 (indicative of phenol concentration). Preliminary results suggest an effect of some treatment (e.g. in the presence of *Vorticella* sp. or rotifers) on bacterial load reduction, increase in fungal dry weight and a reduction on final TOC. A second wider batch of experiments is presently ongoing and results will be also presented.

B2. Unveiling gut microbiota biogeography across the mammalian branch of the tree of life

M. Mangifesta[1,2], C. Milani[1], L. Mancabelli[1], G. A. Lugli[1], K. James[3], S. Duranti[1], F. Turroni[1], C. Ferrario[1], M. C. Ossiprandi[4], D. van Sinderen[3], M. Ventura[1]
[1]Laboratory of Probiogenomics, Department of Life Sciences, University of Parma, Italy; [2]GenProbio srl, Parma, Italy; APC Microbiome Institute and School of Microbiology, Bioscience; [3]Institute, National University of Ireland, Cork, Ireland; [4]Department of Medical-Veterinary Science, University of Parma, Parma, Italy

Internally Transcribed Spacer (ITS) rRNA profiling is a novel tool for detailed analysis of microbial populations at low taxonomic ranks. Here, we exploited this approach to explore species-level biogeography of the *Bifidobacterium* genus, across 291 adult animals that fall within the mammalian branch of the tree of life. These include humans and 13 other primates, domesticated animals, such as dogs, cats, cows, sheeps, goats, horses and pigs, and 46 additional mammalian species. The collected profiles revealed the presence of 89 putative novel bifidobacterial taxa in addition to 45 previously described species. Remarkable, in contrast to what currently known for many gut commensals, we did not observe host-specialization among bifidobacterial species, but rather their widespread distribution across mammals. Moreover, ITS rRNA profiling of wild

relatives of domesticated dogs, rabbits and pigs clearly indicates that domestication and close contact with humans have impacted on the composition of the fecal bifidobacterial population. These data were complemented by analysis of bifidobacterial communities in milk of eight mammalian families, showing that bifidobacteria represent prototypical early gut microbiota members which are inherited by new-borns from their lactating mother. Thus, this study highlights the role of bifidobacteria as pioneering gut colonizers of a wide range of mammals.

B3. BTEX removal with bioelectrochemical systems

M. Daghio[1], A. Espinoza Tofalos[1, 2], B. Leoni[1], P. Cristiani[3], M. Papacchini[4], E. Jalilnejad[1, 5], G. Bestetti[1], A. Franzetti[1]

[1]Department of Earth and Environmental Sciences – University of Milano-Bicocca, Piazza della Scienza 1, 20126, Milano, Italy; [2]Department of Chemistry, Universidad Técnica Federico Santa María, Av. España 1680, Valparaíso, Chile; [3]Ricerca sul Sistema Energetico - RSE Spa, Department of Sustainable Development and Energy Sources, Via Rubattino, 54, 20134 Milan, Italy; [4]INAIL Settore Ricerca, Certificazione e Verifica, Dipartimento di Innovazione Tecnologica (DIT) Laboratorio di Biotecnologie, Rome, Italy; [5]Faculty of Chemical Engineering, Urmia University of Technology, Urmia, Iran

Benzene, toluene, ethylbenzene and xylenes (BTEX) are hazardous contaminants that can accidentally impact groundwater. Biological strategies can be used to remove BTEX compounds usually by adding oxygen to sustain the aerobic degradation. However, this approach can be expensive and technically difficult. The use of bioelectrochemical systems (BES) has been suggested as an alternative strategy.

Anaerobic single chamber BES reactors (120 mL) have been set up using volcanic pumice as support material for the microbial growth and refinery wastewater as microbial inoculum. Graphite electrodes were connected to a power supply (external voltages of 0.8 V, 1.0 V and 1.2 V were applied over 160 days). A BTEX mixture was supplied as carbon source. Abiotic and open circuit controls were set up.

Current production and sulphate reduction were associated to hydrocarbons degradation at all the potentials. The highest current output were observed at 0.8 V. The first order kinetic constants calculated for toluene, *m*-xylene and *p*-xylene were respectively 0.4 ± 0.1 $days^{-1}$, 0.34 ± 0.09 $days^{-1}$, 0.16 ± 0.02 $days^{-1}$ at 0.8 V. Next generation sequencing of the 16S rRNA gene showed that the family *Desulfobulbaceae* was the most enriched in the anodic biofilms highlighting the importance of the sulphur cycle.

B4. VanYn, a novel enzyme involved in glycopeptide resistance: a target for screening in the search of novel inhibitors of antibiotic resistance

E. Binda[1,2], S. Gomez Quevedo[3], M. José Hernaiz Gomez-Degano[3], F. Marinelli[1,2]

[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese Italy; [2]The Protein Factory research center, Politecnico di Milano and University of Insubria, Milan Italy; [3]Organic & Pharmaceutical Chemistry Department, Complutense University, Madrid Spain

VanYn is a D,D-carboxypeptidase[1] involved in self-resistance mechanism of *Nonomuraea gerenzanensis*[2,3,4], which produces the glycopeptide A40926, precursor of the recently FDA-approved dalbavancin[5]. VanYn heterologous expression allowed its characterization as bi-functional zinc-dependent enzyme, which produces a modified cell wall resistant to glycopeptides[1]. Moreover, the over-expression of VanYn in *Streptomyces* spp. and *N. gerenzanensis*, increased their glycopeptide resistance, confirming its role *in vivo*[4,6]. Surprisingly, VanYn activity is inhibited by β-lactams although it lacks the Ser-X-X-Lys motif found in the penicillin-binding-proteins[1,4,6]. We recently produced a mutant form of the enzyme for better understanding its

interaction with β-lactams. Additionally, we have used the surface plasmon resonance (SPR)[7] to describe in details the specific modification produced by VanYn on peptidoglycan analogues immobilized on microchips. Our goal is using VanYn, as a novel target to screen for specific inhibitors, which could be used in combination with antibiotics to face emerging resistant infections.

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B5. Biological diversity of the microbial film associated with the central-Mediterranean *Dendropoma cristatum* (Biondi, 1859) reefs

E.C. La Marca[a], V. Catania[b], M. Tagliavia[b, d], M.T. Sardina[c], R. Di Gerlando[c], M. Milazzo[a], R. Chemello,[a] P. Quatrini[b]
[a]Dipartimento di Scienze della Terra e del Mare, DISTEM, Via Archirafi 22, 90123, Palermo; [b]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, STEBICEF, Viale delle Scienze Ed. 16, 90128, Palermo; [c]Dipartimento di Scienze Agrarie, Alimentari e Forestali SAAF Viale delle Scienze Ed. 4 90128 Palermo; [d]Institute for Coastal Marine Environment, National Research Council (IAMC-CNR), Torretta Granitola-Campobello di Mazara, Trapani

Microbial films may provide physical and bio-chemical cues which positively affect the settlement dynamic of a variety of benthic marine organisms, driving community succession. Biofilm maturity has been found to enhance the settlement pattern of the gastropod *Dendropoma cristatum* (Biondi, 1859), which builds up the Mediterranean intertidal vermetid reefs. However, the microbial diversity associated with these bioconstructions has never been described. This study investigates the *D. cristatum* reef bacterial assemblage composition and temporal evolution in two localities in NW Sicily. Biological diversity of the reef-associated biofilm and of 3 progressively older biofims obtained on artificial surfaces exposed to field conditions was described by Automated Ribosomal Intergenic Spacer Analysis (ARISA). Out of 55 detected OTUs, only 6 were shared between the 2 localities. Hierarchical grouping of taxa abundance showed two major groups that separated the 2 localities. Within each group, reef-associated and experimentally obtained biofilms formed individual clades. SSU-rRNA NGS sequencing of the reef-associated biofilm is underway.

These data highlight a high variability of biofilm composition at local scale and between maturity stages. Structural differences among biofilm successional stages might be responsible for the increase of *Dendropoma cristatum* settlement, indicating a positive inter-kingdom relationship with likely implications on the reef development.

B6. Adsorption of a thermoacidophilic peroxiredoxin of *Sulfolobus solfataricus* on *Bacillus megaterium* spores

M. Lanzilli, G. A. Tipaldi , G. Donadio, F. Fusco, D. Limauro, E. Ricca, R. Isticato
Department of Biology, University of Naples Federico II, Naples, Italy

Bacterial spores displaying heterologous proteins have been proposed as a safe and efficient system to deliver antigens and enzymes to animal mucosal surfaces. Initial studies have been performed using Bacillus subtilis spores but then other spore formers have been considered.

B. megaterium spores have been shown able to display large amounts of a model heterologous

protein that localized on the spore surface and in part infiltrated in the below layers.

We used B. megaterium spores to adsorb Bcp1, a thermoacidophilic peroxiredoxin of *Sulfolobus solfataricus*, known to have a strong antioxidant activity.

We report that purified Bcp1 was efficiently adsorbed on spores of B. megaterium QM B1551 localizing on the surface and underneath the exosporium. Adsorbed Bcp1 retained its enzymatic activity and was somehow protected against acidic pH values and simulated gastric or intestinal conditions. The spore outermost layer, the exosporium, was essential in allowing an efficient adsorption of Bcp1 and in protecting the adsorbed enzyme.

The efficiency of the adsorption, the protection of Bcp1 activity in simulated intestinal conditions and the well-documented safety and robustness of B. megaterium spores propose the spore of this species as a valid system for the oral delivery of molecules with health beneficial properties.

B7. *Cryptococcus curvatus*: a versatile yeast for the bioconversion of agro-industrial wastes into microbial oils of industrial interest

E. Carota[1], J. Springer[2], S. Crognale[1], A. D'Annibale[1], R. A. Weusthuis[2], G. Eggink[2], M. Petruccioli[1]

[a]Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF),University of Tuscia, Via S. Camillo De Lellis, 01100 Viterbo, Italy; [b]Bioprocess Engineering, Wageningen University, Wageningen UR PO Box 16, 6700AA, Wageningen, The Netherlands

Microbial oils are on the spotlight as alternative feedstock to crude and vegetable oils in the oleo chemical and biodiesel industry. For this purpose, the use of low cost and widespread substrates combined with the identification of a versatile and efficient oleaginous strain is required. Within the frame of a screening program, *Cryptococcus curvatus* emerged as a potential candidate, able to grow on different substrates such as ricotta cheese whey (RCW) and orange peel aqueous extract. Biomass production reached the theoretical yield on both substrates, while lipid accumulation was favoured on RCW arriving up to 63% on dry weight basis, with a predominance of oleic acid (50%), followed by palmitic acid (20%). Thus, the possibility of modulating the lipid profile through metabolic engineering techniques was explored. In particular, in view of a potential application in biodiesel industry, where a high percentage of monounsaturated fatty acids is preferred, the main target was decreasing the content of C16:0 in favour of C16:1, through the insertion of a specific Δ9-desaturase. Mutants showed an increase in C16:1 during exponential phase, while they were not able to accumulate it in later stages. Thus, further studies are still in progress to better understand regulation mechanisms.

B8. Enhancement of antibiotic productions by plasma modified PLA electrospun membranes in *Streptomyces coelicolor* immobilized-cell cultivations

A. Sutera[1,2], F. Lopresti[3], R. Dolcemascolo[1], R. M. Fontana[1], L. Botta[3], F. Amato[4], C. Miccichè[4], S. Agnello[2,4], A. M. Puglia[1], R. Scaffaro[3], G. Gallo[1,2]

[1]Laboratory of Molecular Microbiology and Biotechnology, STEBICEF Dept., University of Palermo, Italy; [2]ATeN Center, University of Palermo, Italy; [3]DICAM, University of Palermo, Italy; [4]Department of Physics and Chemistry, University of Palermo, Italy

Actinomycetes, Gram-positive filamentous bacteria, are industrially-relevant producers of bioactive metabolites, including antibiotics. Antibiotic production is usually performed by liquid-medium cultivations, where biomolecule yield is negatively affected by mycelial-cell pellet formation. This aspect could be due to planktonic growth that does not reproduce the usual lifestyle of terrestrial actinomycetes which, instead, grow adhering on organic surfaces.

Therefore, immobilized-cell cultivations of *Streptomyces coelicolor,* a model actinomycete

producing undecylprodigiosin (RED) and actinorhodin (ACT) antibiotics, were performed using polylactic acid (PLA) membranes modified or not by an O_2-plasma treatment. The immobilized-cells formed a dense "biofilm-like" mycelial network on the PLA membranes, as observed by scanning electron microscope. In addition, ACT, produced by immobilized cells, was adsorbed on the PLA fibers of membranes as observed by Raman spectroscopy. Interestingly, *S. coelicolor* immobilized-cells showed more than 4-fold RED and ACT yields in comparison to planktonic-mycelial cells, with O_2-plasma treated PLA membranes the most effective ones.

Moreover, a differential proteome analysis, based on 2D-DIGE and MS analysis, is in progress to highlight metabolic and molecular processes differentially regulated in immobilized- and planktonic-cell cultivations.

Thus, this study, encouraging the use of PLA membranes for actinomycetes cultivations, could unveil functional insights associated with antibiotic production and *S. coelicolor* cell-immobilization.

B9. Polar Marine Bacteria As Novel Source Of Anti-Biofilm Agents

A. Ricciardelli[1], C. Lauro[1], A. Casillo[1], M. Ziaco[1], R. Papa[2], M. Tilotta[2], L. Selan[2], M. M. Corsaro[1], M. L. Tutino[1], M. Artini[2], E. Parrilli[1]

[1]Chemical Sciences, University of Naples "Federico II", Naples, [2]Department Of Public Health And Infectious Diseases, Sapienza University, Rome.

Staphylococcus aureus and the coagulase-negative *Staphylococcus epidermidis* are among the most leading causes of biofilm-related nosocomial infections. Cold-adapted marine bacteria synthesize a wide range of bioactive compounds often involved in quorum sensing modulation and biofilm inhibition.

The present research project aims to exploit cold-adapted microorganisms as a sustainable source of novel anti-biofilm compounds active against staphylococci.

In this work, a selection of Polar marine bacteria were grown in planktonic conditions in GG medium, a synthetic medium based on gluconate and glutamate. Whole bacterial cultures were subjected to liquid-liquid extraction. Obtained total organic extracts were analysed for their anti-biofilm activity against different strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* species. The screening results highlight that several extracts show anti-biofilm activity against staphylococci biofilm. Further analysis are still ongoing aimed to purify and identify the compounds responsible for the anti-biofilm activity.

B10. Exploring the microbial diversity featuring the unique biospeleothems from the quartz-sandstone cave Imawarì Yeuta in Auyan Tepui (Venezuela)

D. Ghezzi[1], F. Sauro[2,3], A. Columbu[2], J. De Waele[2], P.-Y. Hong [4], D. Zannoni[1], M. Cappelletti[1]

[1]Department of Pharmacy and BioTechnology, University of Bologna, Italy; [2]Department of Biological, Geological and Environmental Sciences, Italian Institute of Speleology, University of Bologna, Italy; [3]La Venta Geographic Explorations Association, Treviso, Italy; [4]Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology (KAUST), Saudi Arabia

The Imawarì Yeuta cave in the table-top mountain Auyan Tepui (Venezuela) is the longest quartz-sandstone cave ever explored. The uniqueness of this cave resides in its great age (over 30 Ma) and its complete isolation from anthropogenic activities. This subterranean "uncontaminated" environment is featured by low nutrient level and the absence of light and represents a putative reservoir of new microbial species as well as an extreme oligotrophic location comparable to extra-terrestrial environments. During two speleological expeditions in 2014 and 2016, several samples were collected from different silica-based biospeleothems

detected in the Imawarì cave[1,2].

Geochemical and microscopic analyses defined the elemental compositions and morphological structures of the biospeleothems, while a Next-Generation Sequencing approach was used to define the composition of the microbial populations based on 16S rRNA. *Proteobacteria*, *Acidobacteria*, *Actinobacteria* resulted to be the most representative phyla inside the cave although their proportion within each community varied significantly. The sequence analysis of the most abundant operational taxonomic units (OTUs) indicated the phylogenetic correlation with reference sequences previously detected in extreme environments such as Antarctic soils and lava caves. Further, a large ratio (around 30%) of the microbial communities detected in Imawarì Yeuta was not taxonomically defined, indicating the presence of novel bacterial genera/species putatively associated with still unexplored metabolic networks.

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[2]Mecchia et al. (2014) J. Hydrol.

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Session C - Interactions between microbes/viruses and their hosts

C1. Characterization of the effects induced by the HIV-1 recNEF$_{sf2}$ protein on plasmacytoid dendritic cells

A. Aiello[1], Z. A. Percario[1], M. Cruciani[2], C. Arenaccio[3], M. Severa[2], E. Coccia[2], E. Affabris[1]
[1]Dept.of Science, University Roma Tre, Rome, Italy; [2]Dept. of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanita', Rome, Italy; [3]National Center for Global Health, Istituto Superiore di Sanità, Rome, Italy

It is emerging fact that the viral protein Nef of Human Immunodeficiency Virus type 1 (HIV-1) has an important impact on the chemo-cytokine network, possibly contributing to the chronic inflammation observed during HIV disease progression (Percario et al., 2015). In this context plasmacytoid dendritic cells (pDCs), specialized to produce type I IFN, play a pivotal role (O'Brien et al., 2013). Since we observed that peripheral blood lymphocytes (PBL) depleted of pDCs fail to respond to Nef, we decide to investigate on Nef-pDC interactions analysing the effects induced by the viral protein with respect the production of pro-inflammatory cytokines including type I IFN.

We performed preliminary experiments on primary human pDCs treated with a recombinant myristoylated HIV-1 Nef$_{SF2}$. We observed that recNef is internalized by the cells and the analysis of the IFN-inducible gene, *mxA*, suggested that pDCs treated with Nef could produce type I or type III IFN. However, as confirmed by the ELISA assay, the viral protein seems not to be able to induce the IFN-α production.

Further studies are in progress to analyse the production of other pro-inflammatory cytokines in primary pDCs and in a more stable and reproducible system that involves a pDC cell line.

C2. A structure-based virtual screening and site directed mutagenesis approach identify new promising HIV-1 RNase H inhibitors

A. Corona[1], V. Poongavanam[2], N. Grandi[1], J. Kongsted[3], F. Esposito[1], E. Tramontano[1-4]
[1]Department of Life and Environmental Sciences, University of Cagliari, Italy; [2] BMC, Department of Chemistry, Uppsala University, Sweden; [3] Department of Physics, Chemistry, Pharmacy, University of Southern Denmark, Denmark; [4]Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche (CNR), Monserrato(CA)Italy

HIV persistent infection requires a life-long treatment, often hampered by the selection of viral strains resistant to the approved drugs, hence, a constant and timely effort is required to identify and develop new inhibitors endowed with innovative mechanisms.

The HIV-1 Reverse Transcriptase (RT) associated Ribonuclease H (RNase H) is the only viral encoded enzymatic activity that still lacks an efficient inhibitor, although is a well validated target whose functional abrogation compromises the viral infectivity. The identification of new drugs is a long and expensive process that can be speeded by new in-silico methods. In the present study, a quantum mechanics structure-based virtual screening is performed to identify a new class of compounds that show inhibition against HIV-1 RNase H. Out of 45 compounds selected for testing, 15 of these inhibited the RNase H function below 100 μM and the four most potent inhibitors possess IC_{50} value < 10μM. The structure activity relationship (SAR), the binding mode investigations of these compounds and site directed mutagenesis studies performed on recombinant HIV-1 RTs showed significant interaction with highly conserved residues in the RNase H domain, leading the bases for further lead optimization.

C3. Discovery of dual allosteric Integrase and Reverse Transcriptase associated Ribonuclease H activities inhibitors: investigation of dihydroxyindole-2-carboxylic acids in the HIV-1 integrase sucrose binding pocket

F. Esposito[1], N. Pala[2], M. Sechi[2], P. C. Koneru[3], M. Kvaratskelia[3], L. Naesens[4], A. Caredda[1], A. Corona[1], S. Cosconati[5], F. Di Leva[6], E. Tramontano[1]

[1]Department of Life and Environmental Sciences, University of Cagliari, Italy; [2]Department of Chemistry and Pharmacy, University of Sassari, Italy; [3]Center for Retrovirus Research and Comprehensive Cancer Center, College of Pharmacy, The Ohio State University, Columbus ,Ohio; [4]Rega Institute for Medical Research, KU Leuven, Belgium; [5]DiSTABiF, University of Campania "Luigi Vanvitelli", Caserta, Italy; [6]Department of Pharmacy, University of Naples "Federico II", Naples, Italy

The prolonged use of drugs to treat Human Immunodeficiency Virus type 1 (HIV-1) infection causes chronic toxicity and may lead to selection of drugs resistant strains. Hence the need of discovery antiviral agents with new mode of action are still needed. An innovative approach is aimed to identify new dual HIV-1 allosteric inhibitors, small molecules that can act one or two viral functions.

In this work, we investigate the effects of a series of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) derivatives on both HIV-1 Integrase (IN) and Reverse Transcriptase (RT)-associated Ribonuclease H (RNase H) activities in biochemical assays.

Among tested DHICA derivatives, compound **5** was able to inhibit the IN-LEDGF/p75 binding, the IN-IN dimerization and, as a consequence, the IN catalytic activity in the low micromolar range (1-18 μM). Moreover, compound **5** inhibited the RT-associated RNase H function, showing to be a dual HIV-1 IN and RNase H inhibitor, and the HIV-1 viral replication. Docking and mode of action studies on HIV-1 IN mutated enzymes showed that compound **5** binds to the previously described IN sucrose binding pocket, but demonstrates a different behavior with respect to previously published IN allosteric inhibitors.

Overall we identified a new scaffold for dual HIV inhibitors for further developments.

C4. Single compound from *Onopordum illyricum* is able to counteract Ebola virus VP35 protein inhibition of the interferon cascade

A. Frau[a], G. Daino[a], E. Fanunza[a], F. Esposito[a], C. Sanna[a], D. Rigano[b], O. Taglialatela Scafati[b], A. Corona[a], E. Tramontano[a]

[a]Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy. [b]Department of Pharmacy, University of Naples Federico II, Naples, Italy

Ebola virus (EBOV) is the causative agent of the Ebola virus disease (EVD) determining unpredictable epidemics for which no drug is currently available. Among EBOV encoded proteins, the multifunctional VP35 protein effectively suppresses the innate immune response and contributes to EVD severity. EBOV VP35 inhibits the RIG-I-like receptor (RLR) signaling cascade activation down-regulating the interferon-α/β production by several mechanisms, among which is the competition with RIG-I for viral dsRNA binding and the interaction with a number of cellular components of the RIG-I cellular cascade. Thus, VP35 is a validated drug target for anti-EBOV drug development. Natural extracts have traditionally been used for medicinal treatments and as a source of natural products with biological activities. Hence, we selected a number of herbal extracts and tested them for their ability to interfere with EBOV VP35 immune suppression by both VP35-dsRNA binding and luciferase reporter gene assays. Results showed that some extracts were able to counteract EBOV VP35 immune suppression. Among them, the extract from *Onopordum illyricum* was effective in both biochemical and cellular assays. Extract fractionation led to the identification of single compounds that are able to inhibit EBOV VP35 anti-IFN functions and that are interesting hits for further drug development.

C5. Isolation and characterization of lytic bacteriophages targeting major multi-drug resistant high-risk clones of carbapenemase-producing *Klebsiella pneumoniae*

M. M. D'Andrea[1], P. Marmo[2], N. Ciacci[1,2], L. Henrici De Angelis[1], E. Demattè[1], P. Lupetti[3], GM. Rossolini[4,5], M. C. Thaller[2]

[1]Department of Medical Biotechnologies, University of Sienna, 53100 Sienna, Italy; [2]Department of Biology, University of Rome "Tor Vergata", 00133 Rome, Italy; [3]Department of Life Sciences, University of Sienna, 53100 Sienna, Italy; [4]Department of Experimental and Clinical Medicine, University of Florence; [5]Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, 50134 Florence, Italy

Klebsiella pneumoniae (Kpn) is a major cause of serious healthcare-associated infections often associated to high mortality rates. The species has a remarkable propensity to become resistant to a wide number of antibiotics, both by acquisition of exogenous resistance genes and by accumulation of chromosomal mutations. These features, together with the ability to rapidly disseminate in hospital settings causing even large outbreaks, make Kpn one of the most worrisome Gram-negative opportunistic pathogen. Here we describe three novel lytic bacteriophages targeting major clonal lineages of Kpn, namely those belonging to Clonal Group (CG)258 clade I, CG258 clade II, CG101 and CG307. Hospital wastewaters were screened for the presence of bacteriophages. The obtained bacteriophages were characterized by determination of host-range, structure (by transmission electron microscopy), whole DNA sequencing, stability to physicochemical conditions, and their efficacy in protecting larvae of *Galleria mellonella* from death caused by *K. pneumoniae* infection. All bacteriophages were composed by a dsDNA genome. One bacteriophage belonged to the *Podoviridae* family, while the others to the *Myoviridae* family. Each bacteriophage selectively targeted specific Kpn clones and was able to protect larvae of *G.mellonella* from death in an experimental infection model, even when challenged with colistin-resistant or hypermucoviscous strains.

C6. Sensitivity to oxidative stress and hypoxia in ancestral and modern *Mycobacterium tuberculosis* strains

D. Spadoni, W. Frigui[2], A. Pawlik[2], A. Tavanti[1], R. Brosch[2], D. Bottai[1]

[1]Department of Biology, University of Pisa, Pisa, Italy; [2]Institut Pasteur, Unit for Integrated Mycobacterial Pathogenomics, Paris, France

Mycobacterium tuberculosis strains have been classified in seven phylogenetic lineages (L1-L7). While lineages L2-L4 ("modern" strains) are spread all over the word, lineages L1, L5-L7 ("ancestral strains") are restricted to specific geographic areas (South Asian countries and Horn of Africa). We have compared the survival of selected ancestral and modern *M. tuberculosis* strains following *in vitro* exposure to stresses mimicking active or latent phases of infection, including exposure to reactive oxygen and nitrogen intermediates (ROI and RNI), low pH, hypoxia. Ancestral strains resulted more sensitive than modern strains to ROI, while no differences were observed in response to low pH and RNI. Furthermore, ancestral strains showed reduced survival in hypoxia, as evaluated in the Wayne model of progressive oxygen depletion. These results indicate an enhanced adaptation of modern *M. tuberculosis* strains to the human host potentially accounting for their successful widespread. Identification of genetic determinants underlying increased ROI-hypoxia resistance will help understanding evolutionary adaptation of modern strains.

C7. Alkyl-quinolone-dependent quorum sensing controls prophage activation, autolysis and antibiotic resistance in *Pseudomonas aeruginosa* biofilm

G. Giallonardi[1], M. Messina[1], E. Frangipani[1], S. Heeb[2], M. Cámara[2], F. Polticelli[1], P. Visca[1], L. Leoni[1], G. Rampioni[1], P. Williams[2]

[1]Department of Science, University Roma Tre, Rome, Italy; [2]Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK.

The *pqs* quorum sensing (QS) system of *Pseudomonas aeruginosa* directs the production of 2-heptyl-4-hydroxyquinoline (HHQ), 2-heptyl-3-hydroxy-4-quinolone (PQS) and 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO). While HHQ and PQS act as QS signals, the physiological role of HQNO is unclear.

Here we show that a *P. aeruginosa* PAO1 *pqsL* mutant, impaired in HQNO synthesis, shows autolysis plaques when grown as colony biofilm. This phenotype is associated to the production of biofilm with enhanced antibiotic resistance, as a consequence of extracellular DNA release. The formation of autolysis plaques is caused by HHQ accumulation and consequent induction of Pf4 prophage lytic cycle.

Colony biofilms produced by 51 out of 126 *P. aeruginosa* clinical isolates tested showed an autolytic phenotype. In the majority of the autolytic strains, the *pqsL* gene was mutated, and wild type phenotype could be restored by introducing *in trans* the *pqsL* gene.

Overall, these data indicate that PqsL-mediated synthesis of HQNO might serve to avoid HHQ accumulation and autolysis in wild type *P. aeruginosa*. However, the autolytic phenotype is associated with increased antibiotic resistance. Hence, loss of *pqsL* functionality might represent a pathoadaptative mutation in *P. aeruginosa* strains isolated from chronic infections.

C8. *E. coli* ST131: a versatile multidrug resistant pathogen in and outside the gut

M. Totsika[1], S. Sarkar[1]

[1]Institute of Health and Biomedical Innovation and School of Biomedical Sciences, Queensland University of Technology, Queensland, Australia

Background. Epidemiological studies point to the gut as a key reservoir of multidrug resistant *E. coli* ST131, a globally dominant pathogenic clone causing urinary tract and bloodstream infections. Here we report a detailed investigation of its intestinal lifestyle.

Methods. Clinical ST131 isolates and type 1 fimbriae null mutants were assessed for colonisation of intestinal epithelia and in mouse intestinal colonisation models. Mouse gut tissue was examined by histology for pathology and ST131 localisation. Key findings were corroborated in mucus-producing human cell lines and intestinal biopsies.

Results. ST131 strains adhered to and invaded into human intestinal epithelial cells more than probiotic and commensal strains. The reference ST131 strain EC958 established persistent intestinal colonisation in mice and expression of type 1 fimbriae mediated higher colonisation levels. Bacterial loads were highest in the distal parts of the mouse intestine and did not cause any obvious pathology. Further analysis revealed that EC958 could bind to both mucus and underlying human intestinal epithelia.

Conclusions. ST131 strains can efficiently colonise the mammalian gut and allow long-term persistence. Type 1 fimbriae enhance ST131 intestinal colonisation suggesting that mannosides, currently developed as therapeutics for bladder infections and Crohn's disease, could also be used to limit intestinal ST131 reservoirs.

C9. The fimbriome of the genus *Bifidobacterium* represents extracellular structures that modulate interactions with the mammalian gut

C. Milani[1], M. Mangifesta[2], L. Mancabelli[1], G. A. Lugli[1], W. Mancino[1], A. Viappiani[2], A. Faccini[3], D. van Sinderen[4], M. Ventura[1], F. Turroni[1]

Laboratory of Probiogenomics, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy[1]; GenProbio srl, Parma, Italy[2]; Interdepartmental Measure Centre Giuseppe Casnati, University of Parma, Parma, Italy[3]; APC Microbiome Institute and School of Microbiology, Bioscience Institute, National University of Ireland, Cork, Ireland[4]

Bifidobacteria represent key gut commensals of mammals irrespective of their age. However, the molecular mechanisms by which these microorganisms establish themselves in the mammalian gut and persist in this environment are largely unknown. Here, we analyzed the genetic diversity of the predicted arsenal of sortase-dependent pili of all members of the *Bifidobacterium* genus and constructed a bifidobacterial fimbriome database. Our analyses revealed considerable genetic variability of the fimbriome among bifidobacterial (sub)species, which appears to have been acquired through numerous horizontal gene transfer events, for which we could reconstruct an evolutionary mapping. Functional analyses involving both transcriptomics as well as binding assays involving different substrates, demonstrated how the bifidobacterial fimbriome is pivotal in promoting various adhesion abilities to glycans and extracellular matrix proteins, thereby sustaining the genetic/evolutionary success of bifidobacteria in adapting to the mammalian gut.

C10. Time-resolved metagenomic identifies key features in the co-evolution of bacterial communities and cystic fibrosis

G. Bacci[1], F. Armanini[2], G. Taccetti[3], V. Lucidi[4], D. Dolce[3], P. Morelli[5], E.V. Fiscarelli[4], N. Segata[2], A. Mengoni[1], A. Bevivino[6]

[1]University of Florence, Department of Biology, Florence, Italy; [2]University of Trento, Centre for Integrative Biology, Trento, Italy; [3]Cystic Fibrosis Center, Anna Meyer Children's University Hospital, Department of Pediatrics Medicine, Florence, Italy; [4]Children's Hospital and Research Institute Bambino Gesù, CF Microbiology and CF Center, Rome, Italy; [5]Cystic Fibrosis Center, G. Gaslini Institute, Department of Pediatrics, Genoa, Italy; [6]ENEA Casaccia Research Center, Sustainable Territorial and Production Systems Department, Rome, Italy

Cystic fibrosis (CF) is characterized by chronic airway infections composed of polymicrobial communities. To date, little is known about the genetic composition and function of CF airways microbial communities, and their relationship to disease benchmarks. Here, we carried out longitudinal metagenomic analysis of sputum samples in order to understand how CF microbiome evolves over time. The sputum samples of twenty-one subjects were collected during clinic visits over a 15-months period. Library construction and metagenomic sequencing were performed following standard pipelines in Illumina Hiseq 2000 platform. An extraordinary resilience of the main and emerging CF pathogens was detected. Hierarchical clustering based on microbial strain-level profiling of marker genes detected from metagenomics samples produced one cluster for each patient, suggesting within-subject strain retention. Taxonomy distribution was quite heterogeneous both across patients and within longitudinally collected samples from patients, with some species that were not detectable during recovery, probably due to the antimicrobial treatment that might have drastically reduced the abundance of pathogen species below the revelation threshold. Our results revealed that the airway colonization is highly selective and that a single strain, which started it, continues to survive and thrive in the lung of the patient. Funded by Italian CF Foundation (FFC#14/2015).

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POSTER SESSIONS

Session A - Microbial genetics and genomics

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A9. Epigenetic influence on *Streptomyces coelicolor* morphological and physiological differentiation

A. Pisciotta[1], F. Attardo[1], A. Manteca[2], R. Alduina[1]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy; [2]Área de Microbiología, Departamento de Biología Funcional, and IUOPA, Facultad de Medicina, Universidad de Oviedo, Oviedo, Spain

DNA methylation is an epigenetic modification regulating many aspects of biological processes; for instance, in bacteria adenine methylation is well known to be associated with DNA repair and coordination of replication, while the role of cytosine methylation has been elucidated only in a few examples.

Streptomyces coelicolor is a mycelial soil microorganism, producer of several antibiotics, with a complex life cycle that includes three different cell types: unigenomic spores, a compartmentalized mycelium (MI) and a multinucleated mycelium (MII).

The main objective of this study was to investigate the role of DNA cytosine methylation along the morphological and physiological differentiation of *S. coelicolor*.

Liquid and solid cultures of *S. coelicolor* were treated with 5-aza-2'-deoxycytidine (aza-dC, a cytidine analogous that inhibits DNA-methyltransferase activity) demonstrating that methylation influences spore germination, aerial mycelium formation and antibiotic production. Sporulation on solid culture was also affected.

To further demonstrate the role of cytosine methylation, knock out mutants in putative methyltransferase genes were generated. Interestingly, disruption of a methyltransferase gene caused a delay in physiological and morphological differentiation similarly to what was obtained treating the cultures with aza-dC.

Altogether these results demonstrate the involvement of DNA cytosine methylation on both morphological and physiological differentiation of *S. coelicolor*.

A10. NGS approach to analyse candidate genes involved in phenotypic resistance to bedaquiline and delamanid in *M. tuberculosis* clinical strains

S. Battaglia[1], M. Chiacchiaretta[1], M. Rossi[1], M. R. De Filippo[1,2], A. M. Cabibbe[1], A. Trovato[1,2], E. Borroni[1], F. Masood[3], A. Hassad[3], S. Tahseen[3], D. M. Cirillo[1]

[1]San Raffaele Scientific Institute, Division of Immunology, Transplantation, and Infectious Diseases, Emerging Bacterial Pathogens Unit, Milan, Italy; [2]San Raffaele Scientific Institute, Centre for Translational Genomics and Bioinformatics, Milan, Italy; [3]National TB Reference Laboratory National TB Control Program, Islamabad, Pakistan

The increasing incidence of multi- and extensively- drug resistant tuberculosis (M/XDR-TB) represents an emergency world-wide. Early diagnosis and treatment is mandatory to achieve good cure rate and stop transmission. Bedaquiline (BDQ) and delamanid (DLM) were recently approved for MDR/XDR TB treatment. We aimed at investigating the mutations conferring resistance to those drugs and their association with phenotypic resistance. Mutations conferring resistance to BDQ are found in *atpE*, encoding for the ATP synthase c-F0 subunit and in *Rv0678*, a regulator which control expression of MmpS5-MmpL5 efflux pump. Mutations leading resistance to DLM are found in *Rv3547* and in genes involved in F420 biosyntesis

pathway. Previous studies demostrated that DLM resistance may also occurred in MDR-TB isolates without drugs exposure. We used Whole Genome Sequencing to analyse a panel of 470 MTB clinical isolates from Pakistan to identify SNPs associated with BDQ- and DLM-DR. Both drugs were tested by microdilution method and MGIT system to determine MICs. The obtained results allowed to identify and discriminate SNPs which correlate with DR phenotype from SNPs without any DR correlation.

A11. A high-throughput screen on *Staphylococcus aureus* clinical isolates identifies increased capsule expression in a subset of strains lacking Protein A

T. Brignoli[1,2], A. Manetti[1], R. Rosini[1], A. F. Haag[1,3], V. Scarlato[2], I. Delany[1]
[1]GSK Vaccines, Siena; [2]University of Bologna; [3]University of Glasgow

Staphylococcus aureus is a major human pathogen, responsible for a wide range of diseases. One of the causes of its success as a pathogen is the peculiar array of immune evasion factors that enable the bacterium to avoid host defenses. Among them, the staphylococcal protein A (SpA) is able to bind the Fc region of IgGs, hence preventing recognition of the Fc by the host immune system and allowing escape from antibody-mediated phagocytosis. We analyzed a panel of circulating strains and, despite the presence of the gene, about 10% of the isolates did not express SpA. Furthermore, the analysis of a subset of isolates belonging to USA100 lineage showed that SpA-negative strains express significantly lower amounts of *spA* transcript than SpA-positive isolates. A high-throughput qRT-PCR analysis on a set of 90 virulence factors showed a negative correlation between *spA* transcripts and capsule biosynthesis-related genes. The capsule is another important virulence factor that inhibits opsonophagocytosis, hence our data suggest that this immune evasion mechanism is enhanced in strains that express low amount of SpA.

There is a tight regulation between spA and capsule, and we hypothesize a different relevance of these two immune evasion mechanisms in different *S. aureus* strains.

This study was sponsored by Novartis Vaccines, now acquired by the GSK group of companies. All authors have declared the following interests : TB, AM, RR, AH and ID were employees of Novartis Vaccines at the time of the study. VS is an employee of [University of Bologna], a contract research organization contracted by GSK Vaccines srl in the context of this study.

A12. Deciphering the cellular mechanism of action of the antitubercular prodrug TP053

L. R. Chiarelli[1], B. S. Orena[1], G. Mori[1], V. Makarov[2], F. Gosetti[3], M. Manfredi[3], J. Kordulakova[4], M. Guerin[5], K. Mikušová[4], E. Marengo[3], G. Riccardi[1], M. R. Pasca[1]
[1]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Italy; [2]A. N. Bakh Institute of Biochemistry, Russian Academy of Science, Moscow, Russia; [3]Department of Science and Technological Innovation, University of Piemonte Orientale, Alessandria, Italy;[4]Department of Biochemistry, Comenius University Bratislava, Slovak Republic; [5]Unidad de Biofísica, Universidad del País Vasco, Leioa, Bizkaia, Spain.

New antitubercular compounds with novel mechanisms of action are needed in order to fight the worrying spread of *Mycobacterium tuberculosis* drug-resistant strains. The thienopyrimidine compound TP053 is a new promising antitubercular active against both replicating and non-replicating *M. tuberculosis* bacilli, with an MIC *in vitro* of 0.125 μg/ml. TP053 has been well characterized as a prodrug activated by the thioredoxin-like oxidoreductase Rv2466c, which reduces the nitro group of the compound. However, its intracellular mechanism of action is still unknown. To identify the cellular target, several *M. tuberculosis* TP053 resistant mutants have

been isolated. Many of them harbored L240V mutation in Rv0579 protein, and recombineering method confirmed its role in TP053 resistance. However, the function ofRv0579 is still unknown, thus to understand its role in TP053 metabolism, the biochemical characterization of the recombinant protein have been performed. Moreover, to demonstrate a direct interaction between the TP053 active metabolite and Rv0579, click chemistry was exploited. In parallel, to get insight into the mechanism of action of the prodrug, and to identify its active metabolite(s), the possibility that the compound also acts through a release of toxic radicals, such as nitric oxide, has been investigated, together with proteomic and metabolomic approaches.

A13. Identification and characterization of the acid-nitrosative inducible mycobacterial TetR-like transcriptional regulators Rv1685c/MSMEG_3765

F. Perrone[1], B. De Siena[1], L. Muscariello[1], S. Kendall[2], S. Waddell[3], M. Sacco[1]
[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DiSTABiF), Università degli Studi della Campania Luigi Vanvitelli, Via Vivaldi 43, Caserta; [2]Department of Pathology and infectious Diseases, The Royal Veterinary College, Centre for Emerging, Endemic and Exotic Disease, Hawkshead Lane, Hertfordshire, AL97TA, UK; [3]Wellcome Trust Brighton and Sussex Centre for Global Health Research, Brighton and Sussex Medical School, University of Sussex, Brighton, BN1 9PX.

Mycobacterium tuberculosis is a pathogen able to survive under acid-nitrosative stress inside the macrophages. Previous studies indicated that the expression profiles of *M. tuberculosis* and *Mycobacterium smegmatis,* exposed to acid-nitrosative stress, show up-regulation of *M. tuberculosis Rv1685c* and of its orthologue in *M. smegmatis, MSMEG_3765*. Both genes are annotated as TetR transcriptional regulators.

Microarray and RT-qPCR analysis, conducted on *M. smegmatis* wild type and ΔMSMEG_3765 strains, show that *MSMEG_3765* is a repressor of the *MSMEG_3762/63/65* operon. This was confirmed by GFP analysis performed on both *MSMEG_3762* and *Rv1687c* upstream regions. By electrophoretic mobility shift assay (EMSA) with the purified recombinant MSMEG_3765 protein we were able to confirm its binding motif, which had been previously identified by bioinformatics analysis. This 36 bp motif is located in the upstream regions of *MSMEG_3762/63/65* and *Rv1687c/86c/85c* operons, spanning into the coding sequences. *MSMEG_3762* and *MSMEG_3763* are annotated as ABC transporter ATP-binding protein and ABC transporter, respectively, as well as their orthologues in *M. tuberculosis.* TetR like proteins regulate a wide range of cellular activities, including efflux pumps. In this contest, strains carrying deletions in *MSMEG_3763* and *Rv1686c* are under investigation.

A14. Genome analysis of *Rhodococcus opacus* R7 strain unravel genetic determinants involved in its environmental persistence

A. Di Canito[1], J. Zampolli[1], P. D'Ursi[2], A. Orro[2], L. Milanesi[2], P. Di Gennaro[1]
[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy; [2]ITB-CNR, Segrate, Milano, Italy

Rhodococcus spp. are able to degrade a wide range of organic compounds and to survive in presence of high doses of toxic compounds, under desiccation conditions, carbon starvation, wide range of temperatures, UV irradiation and osmotic stress. Consistently, they usually possess large genomes and high gene redundancy. *Rhodococcus opacus* R7 can be defined as a powerhouse of degradative abilities, indeed it was isolated from a polycyclic aromatic hydrocarbon-contaminated site and it is characterized for its ability to grow on naphthalene and *o*-xylene as sole carbon and energy source. In a previous work a Phenotype Microarray approach was used to assess metabolic potential of this strain.

R7 whole-genome sequencing and its genome analysis revealed different enzymatic classes putatively involved in xenobiotic degradation, such as mono-, dioxygenases and hydroxylases. Considering these annotated enzymes and reference sequences, a phylogenetic cluster analysis was performed. All these analyses allowed to identify several gene clusters involved both in hydrocarbon degradation and in stress response such as naphthenic acid, betaine and arsenic gene clusters. In conclusion, this work highlighted thepeculiar capacity of R7 strain to persist in the environment, useful for environmental remediation technologies and toxic compound biotransformations.

A15. DksA-dependent regulation of quorum sensing in *Pseudomonas aeruginosa*

G. Rampioni, A. Fortuna, G. Giallonardi, F. D'Angelo, P. Visca, L. Leoni
Department of Science, University Roma Tre, Rome, Italy

Pseudomonas aeruginosa pathogenic potential is controlled *via* multiple quorum sensing (QS) systems, which in turn are modulated by environmental and metabolic cues. This complex regulatory network allows adaptation to challenging environmental niches such as infection sites. However, little is known about the molecular mechanisms connecting QS and other signalling pathways.

DksA is a global regulator, which alters the expression of many genes, often in combination with the stringent response alarmone. The *P. aeruginosa* genome encodes two DksA paralogs: DksA1 is constitutively expressed and contains a canonical Zn-finger motif present in all bacterial DksA orthologs; DksA2 is expressed only under Zn starvation conditions and does not contain a Zn-finger motif. The current model predicts that DksA2 might complement DksA1 function under Zn-starvation, a condition frequently found at the infection site.

Here, single and double deletion mutants in *dksA1* and *dksA2* genes have been originated in *P. aeruginosa* and the effect of these mutations on QS signalling has been studied, in the presence and in the absence of available Zn. The first results of a research aimed at investigating the connections among QS, DksA- and Zn-dependent regulation at both physiological and molecular levels in *P. aeruginosa* will be presented.

A16. A comparative study on the antimicrobial activity of gallium compounds against ESKAPE pathogens

S. Hijazi[1], M. Pirolo[1], D. Visaggio[1], E. Frangipani[1], L. Bernstein[2], P. Visca[1]
[1]Department of Sciences, Roma Tre University, Rome, Italy; [2]Terrametrix, Menlo Park, CA 94025, USA

ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) are the major cause of multidrug-resistant infections. Given the importance of iron in bacterial physiology and pathogenicity, iron-uptake and metabolism have become attractive targets for the development of new drugs. The iron mimetic metal gallium [Ga(III)] has been shown to successfully inhibit bacterial growth by interfering with iron-dependent metabolic pathways.

Here, we have performed a comparative study on the antibacterial activity of Ga(III)-maltolate [GaM], Ga(III)-protoporphyrin IX [GaPPIX] and Ga(III)-nitrate [$Ga(NO_3)_3$] on 24 ESKAPE strains, grown in media with different iron availability [*i.e*. Mueller-Hinton (MH), iron-depleted MH (DMH) and RPMI-1640 supplemented with 10 % of human serum (RPMI-HS)].

In MH and DMH all bacterial pathogens tested were resistant to the three Ga(III) compounds, except *S. aureus* and *A. baumannii* strains, which were susceptible only to GaPPIX, displaying a minimum inhibitory concentration (MIC) $\leq$0.12 μM and 16 – 32 μM, respectively. Conversely, in RPMI-HS, GaM and $Ga(NO_3)_3$ inhibited the growth of 74% and 52% of all strains tested,

respectively, while GaPPIX was only effective against 35% of strains. The susceptibility range of GaPPIX among *A. baumannii* strains grown in RPMI-HS was very broad (0.25 – 128 μM), suggestive of a highly variable response to Ga(III) compounds within the same species.

A17. Immunoassays targeting the major capsid protein for detection of phage φ1207.3 in *Streptococcus pneumoniae*

F. Santoro, G. Pastore, G. Pozzi, F. Iannelli
LAMMB, Dipartimento di Biotecnologie Mediche, Università di Siena

Φ1207.3 is a prophage of *Streptococcus pyogenes* conferring macrolide resistance. We transferred Φ1207.3 in *Streptococcus pneumoniae* where it produces phage particles with a *Siphoviridae* morphology. The presence of phage particles associated to the host in a bacterial culture of *S. pneumoniae* was demonstrated by immunoassay methods. Orf42 protein was predicted to be Φ1207.3 major capsid protein and was used to produce a polyclonal antibodies. *S. pneumoniae* carrying Φ1207.3 was grown to early exponential phase and induced with mitomycin C. Whole bacterial lysate was then assayed by Western Blot with the Orf42-antibody which detected a specific 24 KDa peptide. The lower molecular weight of this band compared to the predicted capsid protein (44 KDa) could be due to cleavage by the Clp protease Orf41. Bacterial cultures were analyzed by flow cytometric and immune transmission electron microscopy assays. Both methods showed that phage particles, in early exponential phase of the bacterial growth, were associated to bacterial surface. In conclusion Φ1207.3 was detected both inside and on the surface of the pneumococcus.

A18. Insights into the role of LptE in LPS transport and cell viability in *Pseudomonas aeruginosa*

A. Lo Sciuto[1], A. Martorana[2], F.-P. Regina[1], A. Polissi[2], F. Imperi[1]
[1]Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome; [2]Department of Pharmacological and Biomolecular Sciences, University of Milan.

Lipopolysaccharide (LPS) is an essential structural component of the outer membrane (OM) of most Gram-negative bacteria. In the model organism *Escherichia coli*, LPS transport requires a set of seven essential (Lpt) proteins, which have been proposed as promising targets for the design of novel antimicrobials. In *Pseudomonas aeruginosa* we have recently demonstrated the essentiality of LptH, the periplasmic component of the *P. aeruginosa* Lpt system. Surprisingly, two transposon mutagenesis studies identified viable insertion mutants in the *P. aeruginosa lptE* gene, suggesting that it might be dispensable in this bacterium. To verify this hypothesis, we generated an *lptE* conditional mutant in *P. aeruginosa* PAO1. LptE appears to be not essential for *P. aeruginosa* viability as its depletion only slightly impaired *in vitro* growth. Conversely, LptE was found to be important for cell-envelope stability, antibiotic resistance and infectivity in an insect model. Interestingly, in LptE-depleted cells the levels of the OM component LptD are significantly reduced, indicating that, as in *E. coli*, LptE is required for the correct insertion and/or stability of LptD. Experiments are in progress to verify the amount and localization of LPS in LptE-depleted cells. Overall, these data imply that *P. aeruginosa* can tolerate an LptE-independent LPS transport system.

A19. *Bacillus cereus* FlhF forms homodimers and is involved in protein targeting to the membrane of the L_2 component of hemolysin BL

D. Mazzantini[1], R. Fonnesu[1], A. Cara[1], F. Celandroni[1], S. Senesi[2], E. Ghelardi[1]
[1]Department of Translational Research and New Technologies in Medicine and Surgery, and [2]Department of Biology, University of Pisa.

FlhF is the third bacterial signal recognition particle (SRP) GTPase. In *Bacillus subtilis*, FlhF forms homodimers that are structurally similar to the heterodimeric (Ffh/FtsY) SRP-targeting complex. This suggests that FlhF can act in the cotranslational transport of secretory and membrane proteins to the membrane. We recently showed that FlhF depletion in *Bacillus cereus* causes a significant reduction in the secretion of the L_2 component of hemolysin BL (Mazzantini *et al*., *Front Microbiol*. 2016; 7:1644,).

In this study, by quantitative Real Time-PCR analysis, we show that the reduced L_2 secretion by the *DflhF* mutant of *B. cereus* is not due to altered expression of the L_2 encoding gene. In bacterial adenylate cyclase two-hybrid screens, *B. cereus* FlhF directly interacts with L_2 and with itself. These results suggest that the protein dimerizes *in vivo* and is involved in the delivery of L_2 to the membrane.

Further experiments are still ongoing to confirm FlhF activity and to identify the protein domains involved in protein/protein interaction.

A20. Large-scale genome manipulation in *Sinorhizobium meliloti* strains: experimental evaluation of genomic interactions in bacterial multipartite genomes

A. Checcucci[1], M. Bazzicalupo[1], A. Becker[2], G. diCenzo[3], F. Decorosi[4], D. Johannes[2], T. Finan[3], M. Fondi[1], V. Ghini[5], P. Turano[6], T. Vignolini[7], C. Viti[4], A. Mengoni[1]
[1]Department of Biology, University of Florence, Sesto Fiorentino, Italy; [2]LOEWE-Center for Synthetic Microbiology, Marburg, Germany; [1,3]Department of Biology, McMaster University, Hamilton, Ontario, Canada; [4]Department of Agri-food Production and Environmental Science, University of Florence, Florence, Italy; [5]CERM & CIRMMP, University of Florence, Sesto Fiorentino, Italy; [6] CERM and Department of Chemistry, University of Florence, Sesto Fiorentino, Italy; [7] European Laboratory for Non-Linear Spectroscopy, LENS, Sesto Fiorentino, Italy

The facultative N_2-fixing symbiont *Sinorhizobium meliloti* has become a model of bacteria with multipartite genome architecture (i.e. formed by more than one replicon). Its multipartite genome is composed by a chromosome of 3.6 Mbp, and two accessory replicons of 1.6 Mbp and 1.3 Mbp (a chromid and a megaplasmid). Comparative genomic analyses and genome-scale metabolic model reconstruction suggested independent evolutionary patterns and the specialization to different ecological niches, as well as a nearly independent transcriptional networks for each replicon. To experimentally verify such predictions we set up experiments of replicons transplantation, starting from a secondary replicons-cured strain, representing the putative paleogenome of *S. meliloti*. We then created novel strains having the same chromosome and containing chromid and/or megaplasmid coming from different donor *S. meliloti* strains. On these genomically hybrid strains, we performed metabolic model reconstruction and lab testing for assessing the robustness and additivity of phenotypes conferred by the whole new replicons (namely growth in different media, symbiosis, intracellular and extracellular metabolome, transcriptome and substrate utilization phenotypes on Phenotype Microarray). Preliminary results indicate that most of the phentoypes are additive, paving the way for the development of genome-wide replicon-based remodeling of bacterial strains to potentially improve plant-bacteria symbiosis.

A21. Structural characterization of the LCP domain from the *Lactobacillus plantarum* FlmC protein

G. D'Abrosca, G. Malgieri, A. Di Pasquale, V. Vastano, M. Sacco, C. Isernia, R. Marasco, L. Muscariello

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Via Vivaldi 43, 81100 Caserta (Italy)

Lactobacillus plantarum has been widely used in food fermentation and, more recently, as probiotics in health-promoting food product. Moreover it is one of the most predominant species in the human gut microbiota of healthy individuals. In a previous study we reported that *L. plantarum* strains carrying null mutations in the *flmA*, *flmB*, and *flmC* genes, initially identified as orthologues of *Streptococcus mutans brpA* (biofilm regulator protein A), were partially impaired in biofilm development. Among these, the *L. plantarum* LM3-6 strain, carrying a deletion in *flmC*, showed a high rate of autolysis, supporting the hypothesis that FlmC might be involved in cell wall integrity. FlmA, FlmB, and FlmC, as well as BrpA, contain the highly conserved domain LytR-CpsA-psr (LCP). Some members of the LCP family are involved in the biogenesis of wall teichoic acids and anionic polysaccharides, and in antibiotic resistance of important human pathogens. In order to gain insight into the structure and function of the Flm proteins we performed an *in silico* analysis of their LCP domains, showing the presence of a N-terminal trans membrane (TM) domain. We expressed the delta-TM version of the FlmC protein and here we report a preliminary structural characterization of the recombinant protein.

A22. Characterization of the microbiome of a thermal spring with therapeutic properties

R. Pedron, A. Esposito, O. Jousson

Centre for Integrative Biology, University of Trento, Italy

The thermal spring of Terme di Comano (Trentino, Italy) is known for its therapeutic properties against inflammatory skin diseases. Here we present the first study of the microbiome of a thermal spring. The investigation consisted in a culture-independent (metagenomic) analysis and an high-throughput cultivation approach with the aim to create a strain collection for further functional characterization. About 230 strains were isolated in pure culture, and part of them were genome-sequenced using Illumina MiSeq platform. A whole genome shotgun sequencing using Illumina HiSeq 2500 was performed on four sampling points at Terme di Comano spa. We evaluated sequence composition and coverage and obtained a number of metagenome-assembled-genomes (MAGs). The comparison of the genomic features of MAGs and single genomes of the isolates from different sites can be used to get a deeper understanding of this ecosystem and to get clues on the possible role of this microbiome in the therapeutic properties of Comano spring water.

A23. Subfunctionalization influences the expansion of bacterial multidrug antibiotic resistance

E. Perrin[1], M. Fondi[1], E. Bosi[1], A. Mengoni[1], S. Buroni[2], V. C. Scoffone[2], M. Valvano[3], R. Fani[1]

[1]Department of Biology, University of Florence, Via Madonna del Piano 6, 50019, Sesto Fiorentino (Fi), Italy; [2]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Via Ferrata 9, Pavia, Italy; [3]The Wellcome-Wolfson Institute for Experimental Medicine, Queen's University of Belfast; 97 Lisburn Rd. Belfast, UK BT9 7BL

Multidrug resistance (MDR) efflux pumps of the RND family are responsible of antibiotic resistance in Gram-negative bacteria. Their genomes often contain several copies of different

classes of MDRs, and gene duplication and the consequent assumption of new functions by the duplicate copies is key for the expansion of drug-resistance.

To understand how these events affect antibiotic resistance, we provide here computational and experimental evidence on the evolution and functional diversification of two members of the RND superfamily in *Burkholderia*.

We assessed the conservation and distribution of these two systems together with their regulation mechanisms. This information was then used to design and perform genetic manipulation of these strains aimed at identifying both the substrate range of these transporters and their eventual interchangeability. The possible role of antibiotics in the activation of expression of these systems was also evaluated, through a direct evolution experiment combined with NGS.

Our results indicate that the first step to diversify the functions of these pumps arises from changes in their regulation (subfunctionalization) instead of functional mutations. Further, these pumps could rewire their regulation to respond to antibiotics, thus maintaining a high genomic plasticity.

A24. Microevolution of *Pseudomonas aeruginosa* chronic phenotypes in a Cystic Fibrosis longitudinal collection

E. Piffer, I. Bianconi, O. Jousson
Centre for Integrative Biology, University of Trento

Pulmonary infections caused by *Pseudomonas aeruginosa* are the main cause of morbidity and mortality in Cystic Fibrosis (CF) patients. *P. aeruginosa* adapts itself to the CF lung environment by loss-of-function mutations that enhance fitness and sustain its clonal expansion during chronic infection.

We aimed to associate the phenotypic microevolution of a *P. aeruginosa* persistent clonal population with mutations within the genome.

Forty *P. aeruginosa* isolated from the sputum of a single CF patient over an eight-year period were characterized with 14 phenotypic assays and genome-sequenced.

Acute virulence traits were lost by the population over time, while traits typical of the chronic phase of the infection were acquired. A strong phylogenetic signal linked the phenotypes to evolutionary relationships within the population and most phenotypes were consistently distributed in relation to population structure. Comparative sequence analysis of the virulence genes in the longitudinal isolates identified a number of putative pathoadaptive mutations.

Further studies are needed to determine causality between the distribution of mutations in the genome and the phenotypic traits of the population.

A25. Role of the glucose uptake pathway in *Pseudomonas aeruginosa* virulence

M. Raneri, C. Dalmasio, P. Ferrante, D. De Vita, F. Briani
Dip. di Bioscienze, Università degli Studi di Milano

Hyperglycaemia has been associated with augmented risk of poor outcome of bacterial lung infections. This has been ascribed to growth stimulation of pathogens such as *P. aeruginosa* due to increased glucose concentration in the airway surface liquid. However, different data suggest that *P. aeruginosa* may exploit glucose not only as a nutrient, but also as a signal molecule that induces virulence functions.

To address this hypothesis, we engineered *P. aeruginosa* mutants in genes encoding transporters of both the phosporylative and oxidative routes of glucose uptake. Interestingly, the deletion of both pathways in the glucose-uptake null mutant (GUN) prevents growth on glucose as sole carbon source. We characterized the mutants for different virulence-related

phenotypes. Protease and pyocyanin secretion were increased in the GUN and in the oxidative route-defective mutants. Transcriptomic analysis of the GUN strain showed a strong divergence in the transcription profile relative to the wt. Interestingly, in preliminary experiments, the GUN mutant exhibited attenuated virulence in the *Galleria mellonella* larvae infection model.

A26. Molecular investigations on the quorum sensing synthase CepI of *Burkholderia cenocepacia*

V. C. Scoffone, M. Fumagalli, L. Spiga, G. Trespidi, L. R. Chiarelli, G. Riccardi, S. Buroni
Dipartimento di Biologia e Biotecnologie, Università degli Studi di Pavia, Pavia, Italy

Burkholderia cenocepacia is a particularly dangerous pathogen for cystic fibrosis patients, difficult to eradicate due to its high level of resistance to most antimicrobial agents. Consequently, new antimicrobials, as well as compounds able to inhibit bacterial virulence, are needed. In this context, quorum sensing (QS) represents a good target for anti-virulence therapies, being involved in biofilm formation and in the production of several virulence factors, such as proteases and siderophores. Recently, we reported new diketopiperazine inhibitors of *B. cenocepacia* acyl homoserine lactone synthase CepI able to reduce virulence factors and biofilm formation, and showing good *in vivo* activity in a *Caenorhabditis elegans* infection model. Understanding their precise mechanism of action, as well as their effects on the whole cellular metabolism, is of great importance to further optimize them. To this aim, to get insight into the binding of the compounds with CepI, we performed an *in silico* analysis of the enzyme structure, followed by site directed mutagenesis and biochemical analyses. Moreover, being QS involved in the regulation of several pathways, a proteomic analysis of *B. cenocepacia* cells treated with the compounds was performed to investigate the whole effects of CepI inhibition.

A27. Whole-Genome enrichment provides deep insights into *Vibrio cholerae* Metagenome from an African river

G. Tassistro[1], C. Grande[1], I. Brettar[2], M. Hofle[2], A. Pallavicini[3], C. Pruzzo[1], L. Vezzulli[1]
[1]University of Genoa; [2]Helmholtz Centre for Infection Research; [3]University of Trieste

This study applied, for the first time to our knowledge, a whole-genome enrichment (WGE) and next-generation sequencing (NGS) approach for direct genotyping and metagenomic analysis of low abundant *V. cholerae* DNA (<50 genome unit/L) from natural water collected in the Morogoro river (Tanzania). The protocol is based on the use of biotinylated RNA baits for target enrichment of *V. cholerae* metagenomic DNA via hybridization. An enriched V. cholerae metagenome library was generated and sequenced on an Illumina MiSeq platform. Up to 1.8 $\times 10^7$ bp (4.5× mean read depth) were found to map against *V. cholerae* reference genome sequences representing an increase of about 2500 times in target DNA coverage compared to theoretical calculations of performance for shotgun metagenomics.

Analysis of metagenomic data revealed the presence of several *V. cholerae* virulence and virulence associated genes in river water including major virulence regions (e.g. CTX prophage and Vibrio pathogenicity island-1) and genetic markers of epidemic strains (e.g. O1-antigen biosynthesis gene cluster) that were not detectable by standard culture and molecular techniques. This study provides a 'proof of concept' on the methodological gap that might currently preclude a more comprehensive understanding of toxigenic *V. cholerae* emergence from natural aquatic environments.

A28. *CoERG11* A395T mutation confers azole resistance in *Candida orthopsilosis*

N. Poma[1], C. Rizzato[2], A. Lupetti[2], M. Zoppo[1], D. Bottai[1], A. Tavanti[1]
[1]Department of Biology, University of Pisa, Pisa, Italy; [2]Department of Traslational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Candida orthopsilosis is a human fungal pathogen responsible for a wide spectrum of symptomatic infections. Azole antifungals are extensively used to treat *Candida*-related mycoses. Although evidence suggests that *C. orthopsilosis* is mainly susceptible to azoles, fluconazole resistant clinical isolates have recently been described. We identified a A395T mutation in the coding sequence of *CoERG11* resulting in a non-synonymous amino acid substitution in azole-resistant isolates only. This study evaluated the contribution of Y132F substitution in the development of azole resistance in *C. orthopsilosis*. A fluconazole susceptible isolate was used as a genomic background for a *SAT1* flipper driven transformation aimed at integrating a copy of *CoERG11* coding sequence bearing the A395T mutation. Mutant clones were tested for their azole susceptibility and for heterozygosity at the hot spot locus. Heterozygous mutant strains showed a resistant phenotype to all azoles tested. These findings provide the first evidence that CoErg11 Y132F substitution confers multi-azole resistance to *C. orthopsilosis*.

A29. Towards the characterisation of the metagenomic functional unknown fraction

C. Vanni[1], P. L. Buttigieg[2], F. O. Glöckner[1, 3], A. Fernandez-Guerra[1, 3, 4]
[1]Microbial Genomics and Bioinformatics Research Group, Max Planck Institute for Marine Microbiology, Bremen, Germany; [2]HGF-MPG Group for Deep Sea Ecology and Technology, Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany; [3]Jacobs University Bremen gGmbH, Bremen, Germany; [4]Oxford e-Research Centre (OeRC), University of Oxford, Oxford, UK

Metagenomic surveys of marine environments have generated terabases of sequencing data, allowing deep insight into this realm's microbial ecology. However, 40-60% of genes in these data sets are of unknown function and are usually ignored. Mapping this functionally uncharacterised space has the potential to accelerate discovery of new functions and help deepen our understanding of the role of microbes in the World Ocean. We built a bioinformatic pipeline to explore and structure this large pool of unknown genes. Firstly, the pipeline partitions Open Reading Frame (ORF) data sets into ORFs with known function and those of unknown function found in i) cultured organisms and ii) only in environmental metagenomes. Secondly, the pipeline clusters ORFs based on their sequence composition, allowing us to identify related unknowns. We applied our pipeline to data compiled from the Global Ocean Survey, TARA Oceans Expedition, Ocean Sampling Day, and Malaspina projects. Our pipeline structured the ~160 million ORFs in this data set into ~2.6 million clusters, with > 50% of these composed of unknowns. While this map of the unknown is immediately useful for accelerating functional characterisation, we are now linking unknowns to their ecological roles to further guide experimentalists with ecosystem-level context.

A30. The small protein TrpM modulates morpho-physiological differentiation in *Streptomyces coelicolor*

A. Vassallo[1], E. Palazzotto[1], A. Lanza[1], L. Botta[2], G. Renzone[3], R. Scaffaro[2], A. Scaloni[3], G. Gallo[1,4], A. M. Puglia[1]
[1]Laboratory of Molecular Microbiology and Biotechnology, Department of Biological Chemical and Pharmaceutical Sciences and Technology (STEBICEF), University of Palermo, Viale delle Scienze Ed. 16, 90128, Palermo, Italy; [2]Department of Civil, Environmental, Aerospace, Materials Engineering, University of Palermo, Viale delle Scienze, 90128, Palermo, Italy;

[3]Proteomics & Mass Spectrometry Laboratory, ISPAAM, National Research Council, Naples, Italy; [4]ATeN Center, University of Palermo, Palermo, Italy

TrpM, a small protein of 63 amino acids, is encoded by a gene of the *trpCMBA* locus involved in tryptophan biosynthesis in the model actinomycete *Streptomyces coelicolor*. Indeed, the *trpM* knock-out mutant strain is characterized by a delayed growth on minimal medium, smaller aerial hyphae, and reduction of both spore and antibiotic actinorhodin production in comparison with the wild-type strain. These observations are in agreement with proteomic analyses which highlighted a role for TrpM in controlling i) tryptophan production through precursor availability and, thus ii) bacterial growth and morpho-physiological differentiation.

To further elucidate the role of TrpM, a *S. coelicolor trpM* knock-in mutant was constructed through *E. coli-S. coelicolor* interspecific conjugation using the pIJ8600::*trpM* integrative plasmid. The *trpM* knock-in mutant strain, grown on minimal medium, is characterized by a faster differentiation and an increased actinorhodin production yield in comparison to the control strain carrying pIJ8600 vector. Moreover, differential proteomic analysis is ongoing in order to identify differentially represented proteins and their biochemical and molecular functions.

Altogether these data further confirm the role of TrpM in modulating morpho-physiological differentiation of *S. coelicolor* expanding our knowledge on the cellular functions of bacterial small proteins.

A31. Understanding the role of pINV integration in *Shigella flexeneri* and in enteroinvasive *E. coli* virulence

A. Zennaro[1], M. L. Di Martino[2], M. Stirpe[1], D. Mazel[3], B. Colonna[1], G. Prosseda[1]
[1]Istituto Pasteur Italia, Sapienza Università di Roma, Roma (IT); [2]University of Uppsala, Uppsala (S); [3]Institut Pasteur, Paris, (FR)

In *Shigella* and in enteroinvasive *E. coli* (EIEC), the etiological agents of bacillar dysentery, the virulence genes are located on a large plasmid, pINV. The expression of the virulence genes is regulated by multiple environmental stimuli through a regulatory cascade involving proteins and sRNAs encoded by both pINV and the chromosome. It is well known that in some cases pINV is able to integrate into the host chromosome and that integration results in silencing of all pINV-encoded virulence genes even under permissive conditions. Here we report that integration in EIEC occurs into a specific IS element (ISEc11) and that the four different ISEc 11 copies present in the EIEC chromosome are competent for integration. Moreover, to understand whether the integration gives rise to an avirulent phenotype also in *Shigella*, we induced pINV integration into different chromosomal loci using a specific prophage integrase. The results indicate that the localization of the virulence genes is relevant for the optimization of their expression.

Session B - Environmental and industrial microbiology

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B11. Effect of efflux pumps inhibition on *Pseudomonas aeruginosa* transciptome and virulence

G. Rampioni[1], C. Ramachandran Pillai[2], F. Longo[1], R. Bondì[1], V. Baldelli[1], M. Messina[1], F. Imperi[3], P. Visca[1], L. Leoni[1]

[1]Department of Science, University Roma Tre, Rome, Italy; [2]Inter University Centre for Bioscience, Kannur University, Palayad, Kerala, India; [3]Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Rome, Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Rome, Italy.

Efflux pumps of the resistance-nodulation-cell-division (RND) family increase antibiotic resistance in many bacterial pathogens and are considered candidate targets for the development of antibiotic adjuvants. RND pumps have also been proposed to contribute to bacterial infection in animals and plants, implying that efflux pump inhibitors (EPIs) could also act as anti-virulence drugs. Nevertheless, EPIs are usually investigated for their properties as antibiotic adjuvants, while their potential anti-virulence activity is seldom taken into account.

Here we show that RND efflux pumps contribute to *Pseudomonas aeruginosa* PAO1 pathogenicity in an insect model of infection and that the well-characterized EPI Phe-Arg-β-Naphthylamide (PAβN) is able to reduce the virulence of this pathogen both *in vitro* and *in vivo*. The protection exerted by PAβN from *P. aeruginosa* PAO1 infection *in vivo* correlates with the down-regulation of key virulence genes, as revealed by transcriptomic and/or phenotypic analyses. However, the PAβN-mediated repression of virulence-related traits is strain dependent, as assessed in a collection of *P. aeruginosa* clinical isolates.

Given that efflux pump inhibition has an impact on *P. aeruginosa* virulence, anti-virulence properties of EPIs are worthy to be explored, taking into account the strain-specificity of their activity.

B12. Effect of co-substrate on old landfill leachate treatment with a selected fungal strain

A. Bardi[a], Q. Yuan[b], G. Siracusa[a], I. Chicca[a], M. Islam[b], F. Spennati[c], V. Tigini[d], S. Di Gregorio[a], D.B. Levin[e], G. Petroni[a], G. Munz[c]

[a] Department of Biology, University of Pisa, Via Luca Ghini 13, 56126, Pisa, Italy; [b]Department of Civil Engineering, University of Manitoba, 15 Gillson Street, R3T 5V6, Winnipeg, Canada; [c]Department of Civil and Environmental Engineering, University of Florence, Via S. Marta 3, 50139, Florence, Italy; [d]Department of Life Sciences and Systems Biology, University of Turin, viale Mattioli 25, 10125, Torino, Italy; [e]Department of Biosystems Engineering, University of Manitoba, 75A Chancellor Circle, R3T 5V6, Winnipeg, Canada

Conventional wastewater treatment technologies are ineffective for the remediation of highly polluted wastewaters, such as old LandFill Leachate (LFL). Therefore, innovative, sustainable approaches to achieve satisfactory removal of old LFL recalcitrant fraction are needed. This study focused on old LFL treatment with a selected fungal strain, *Bjerkandera adusta* MUT 2295, through batch and continuous tests, using packed-bed bioreactors. To optimize the overall process performance, diverse types of co-substrates were used, including milled cellulose from beverage cups waste material, malt extract and glucose. The production of the extracellular enzyme Manganese-dependent Peroxidase (MnP) was assayed, in batch tests, as a function of a) milled cellulose concentration, b) leachate initial Chemical Oxygen Demand (COD) and Soluble COD (sCOD), and c) co-substrate type. Bioreactors, operated in non-sterile conditions, were

dosed with an initial start-up of glucose in Rg, while cellulose was added in Rc. An additional glucose dosage was provided in both reactors after 75 and 53 operating days, in Rg and Rc, respectively. Co-substrate dosages were associated with significant increases in COD and sCOD removal, suggesting that co-substrate step dosing may enhance process performance. The highest COD and sCOD removals were i) 63% and 53% in Rg and ii) 54 % and 51% in Rc.

B13. Functional characterization of a bacterial collection isolated from a thermal spring

M. Benedet, R. Pedron, V. Valentini, C. Zucal, A. Esposito, F. Cappelleti, D. Confalonieri, O. Jousson

Centre for Integrative Biology, Università degli Studi di Trento, Italy

The characterization of new microorganisms and their metabolites should lead to new medical and biotechnological applications, despite the increasing ability of de novo synthesized molecules to address specific needs. In this scenario we are investigating the functional properties of a bacterial collection of >200 strains isolated from the thermal spring of Comano Terme (Trentino, Italy), which has a proven effect for the treatment of inflammatory skin diseases such as psoriasis and atopic dermatitis. Since anti-inflammatory and keratinolytic agents may help in the topical treatment of these disorders, we screened the bacterial collection for: i) bacterial lysates able to decrease the inflammation of human epithelial keratinocytes stimulated by IL-17A and IFN-gamma pro-inflammatory cytokines; ii) bacterial strains with secreted alpha-keratin proteolytic activity; iii) bacterial strains with anti-bacterial activity. A number of bio-active strains were isolated from the three screens and will be further characterized in view of potential biomedical or industrial applications.

B14. Chitinases from metagenome as pest biocontrol agents

F. Berini[1,2], A. Montali[1], C. Katz[3], G. Tettamanti[1], M. Casartelli[4], F. Marinelli[1,2]

[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese Italy; [2]The Protein Factory research center, Politecnico di Milano and University of Insubria, Milan Italy; [3]MIGAL – Galilee Research Institute, Kiryat Shmona Israel; [4]Department of Biosciences, University of Milano, Milan Italy

The growing reluctance to use hazardous pesticides in agriculture has encouraged the search of alternative environmentally-friendly practices for controlling plant diseases. Among them, biocontrol, *i.e.* the employment of natural agents for suppressing plant pathogens with minimal harm for the environment, holds a great promise, and can be used by itself or as part of an integrated pest management (IPM) program. Chitinases, enzymes able to degrade chitin – a polysaccharide absent in plants, but with essential structural roles in insects, fungi and nematodes – represent optimal candidates for the development of IPM strategies using cocktails of microbial hydrolytic enzymes[1]. Indeed, combining existing pesticides or novel natural products with these enzymes that favour their action allowing a better penetration into the target, may dramatically reduce the use of toxic chemicals for crop protection.

We have recently purified two novel chitinases from metagenomics of phytopathogen-suppressive soils[2,3]. In the frame of the MAECI-funded CHITOBIOCONTROL project, we are currently testing their *in vitro* and *in vivo* potential as biocontrol agents against insect pests and phytopathogenic fungi.

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[3]Cretoiu MS *et al.* Appl. Microbiol. Biotechnol. 2015, 99(19):8199-215.

B15. Snow microbiome of mountain environments in southern Italy

A. Bucci, P. Monaco, F. Divino, G. Naclerio
Department of Biosciences and Territory, University of Molise, Contrada Fonte Lappone, 86090 Pesche (IS)

Microorganisms inhabiting the cryosphere have been increasingly studied over the past twenty years to gain an understanding of the ecology of extreme environments. Snow and ice cover over 108 km2 of the Earth's surface and have been regarded as extreme environments because of their low temperatures, high UV irradiation, low nutrients and low water availability.

The main purpose of this research was to analyze and compare the microbial communities of the snow collected in two different locations of Capracotta, a village in the Molise region (Italy), after a snowfall record that occurred on 5-6 March 2015 (256 cm of snow fell in 24 hr).

Microbial communities were investigated by using next-generation sequencing techniques (NGS). In addition, the effectiveness of some "statistical indices" was evaluated in order to summarize the innumerable information derived from the use of high-throughput sequencing technologies, both for comparison between different microbiomes and to assess the biodiversity within each microbial community.

The statistical indices introduced for the first time in this experimental work proved to be an effective tool and allowed a careful and timely analysis of the microbial communities investigated, which showed significant differences in terms of biodiversity, composition and distribution of the species present.

B16. Physiology and genetics of *Rhodococcus aetherivorans* BCP1 response to Arsenic

A. Firrincieli[1], A. Presentato[2], M. Petruccioli[1], D. Zannoni[3], M. Cappelletti[3]
[1]Department for the Innovation in Biological Systems, Agro-Food and Forestry (DIBAF), University of Tuscia; [2]Department of Biotechnology, University of Verona; [3]Department of Pharmacy and Biotechnology (FaBiT), University of Bologna

Arsenic ranks among the priority metals that are of public health significance. In the environment, the metalloid arsenic mainly exists under two forms: the arsenite [As(III)] and arsenate [As(V)]; the former being more toxic due to its high mobility and stability. Bacteria have developed multiple strategies for arsenic detoxification.

Rhodococcus aetherivorans BCP1 is able to cometabolize chlorinated compounds, mineralize a wide range of hydrocarbons[1], resist different stress conditions[2] and convert tellurite and selenite into less toxic forms[3], making this strain an ideal candidate for microbial biotechnology applications. In this study, we assessed the ability of BCP1 to tolerate high concentrations of As(V) during its growth under aerobic conditions. Furthermore, different aspects regarding the arsenic homeostasis and the response of BCP1 to As(V) were investigated: (i) the different capability to convert As(V) into As(III) depending on the initial concentration of arsenate; (ii) the arsenic biosorption; (iii) the effect of arsenic on polyphosphate granule formation and (iv) the genetic/genomic aspects involved in arsenic detoxification. Finally, the detection of electron-dense nanoparticles after the incubation with As(V) suggested the ability of BCP1 strain to generate As-based nanostructures.

[1]Cappelletti et al. (2011) Appl. Environ. Microbiology
[2]Cappelletti et al. (2016) Res. Microbiol.
[3]Presentato et al. (2016) Microb. Cell Factor.

B17. Blue biotechnology: oil bioremediation using hydrocarbon-degrading bacteria immobilized on biodegradable membranes

V. Catania[1], F. Lopresti[2], S. Cappello[3], R. Scaffaro[2], P. Quatrini[1]
[1]Dip.to Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), University of Palermo, Viale delle Scienze edif. 16 90128 Palermo, Italy.; [2]Department of Civil, Environmental, Aerospace and Materials Engineering University of Palermo,Viale delle Scienze, ed. 6, 90128 Palermo, Italy; [3]Istituto per l'Ambiente Marino Costiero (IAMC) – CNR U.O.S. of Messina, Spianata San Raineri, 86 – 98121 Messina, Italy.

A novel bioremediation system to clean up oil contaminated water was developed combining hydrocarbon (HC) degrading bacteria immobilized and polylactic acid (PLA) or polycaprolactone (PCL) membranes prepared by electrospinning. The bioremediation efficiency was tested on crude oil using highly performant HC degrading bacterial strains isolated from marine and soil environments. The membrane morphology, the microbial adhesion and proliferation were evaluated using scanning electron microscopy (SEM). The SEM analysis highlighted that the fibers of the electrospun mats were in nanoscale with a similar diameter size distribution. The electrospun membranes exhibited high oil absorption capacity (q): approximately q = 40 g/g for PLA and q = 20 g/g for PCL. The bacterial strains were able to attach to the PLA and PCL membranes after 48h, reaching high proliferation and biofilm formation within the whole structure in 5 days. The biodegradation efficiency of the bacteria-membrane systems was tested by GC-FID analysis and compared with planktonic cells after 5 and 10 days incubation. The bacterial immobilization is a promoting factor for biodegradation and a new tool to be developed for bioremediation of aquatic systems.

B18. Bacteria from extreme environments: analysis of bacterial communities from the Acquarossa River (Viterbo, Italy)

C. Chiellini[1], C. Fagorzi[1], E. Miceli[1], G. Bacci[1], G. Bianconi[2], F. Canganella[2], L. Presta[1], R. Fani[1]
[1]Dep. Biology, University of Florence (Italy); [2]Department for Innovation in Biological, Agrofood, and Forest Systems, University of Tuscia – Viterbo (Italy)

The Acquarossa river (Viterbo, Italy) is an extreme environment due to its high iron and arsenic concentrations. Red and black biofilms grow on the rock surfaces along the river course, maintaining a defined borderline. Samples of black and red biofilms were collected on July 2016 to characterize the bacterial communities inhabiting epilithons. Culturable bacterial communities analysis revealed a dominance of *Acinetobacter* sp. (56%) in black epilithon, and a dominance of *Pseudomonas* sp. in red epilithon (53%). NGS analysis partially confirmed these data reporting a different microbial assemblage in different biofilm types. *Acinetobacter* strains (77 out of 191) and *Pseudomonas* strains (44 out of 191) were divided respectively into 12 and 19 RAPD haplotypes; in both cases, none of the detected haplotype was shared between red and black epilithon suggesting that the community's structure is different in the two biofilms. Cross-streaking experiments revealed that strains of a given genus don't have any inhibitory activity both vs other strains from the same samples and strains from different samples.

Resistance patterns towards heavy metals and antibiotics revealed different phenotypic characteristics of the strains, suggesting that two distinct bacterial communities characterize the two kinds of epilithic biofilms along the whole river course.

B19. Recombinant production of difficult proteins at zero degrees in *Pseudoalteromonas haloplanktis* TAC125

C. Lauro, A. Colarusso, E. Parrilli, M. L. Tutino
Department of Chemical Sciences, Federico II University, Naples

The Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 (*P. haloplanktis* TAC125) is a model organism as a non-conventional system for production of recombinant proteins, especially of human origin. To further explore the biotechnological ability of *P. haloplanktis* TAC125, we developed a synthetic medium, containing D-gluconate and L-glutamate (GG), which allows the bacterium to grow even at subzero temperatures. *P. haloplanktis* TAC125 growing in GG medium at low temperature displays growth kinetic parameters which confirm its spectacular adaptation to cold environment and subzero lifestyle. Moreover, in this contribution, we report the setup of a finely regulated gene expression system inducible by D-galactose to produce recombinant protein in GG synthetic medium at temperatures as low as −2.5 °C. Thanks to the combination of the novel medium and the new expression system, we obtained for the first time the full-length production of a human recombinant protein at 0°C, thus providing an innovative strategy for the recombinant production of difficult to express proteins.

B20. Identification of anti-virulence FDA-approved drugs targeting the *pqs* quorum sensing system of *Pseudomonas aeruginosa*

F. D'Angelo[1], V. Baldelli[1], N. Halliday[2], F. Polticelli[1], P. Williams[2], P. Visca[1], L. Leoni[1], G. Rampioni[1]
[1]Department of Science, University Roma Tre, Rome, Italy; [2]Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK

The *pqs* quorum sensing (QS) system of *Pseudomonas aeruginosa* relies on 2-alkyl-4-quinolones (AQs) as signal molecules. Since this QS system positively controls the expression of multiple virulence determinants, it is considered a potential target for the development of anti-virulence drugs.

In this study a library of drugs has been screened by using a co-culture system in which bioluminescence emitted by an AQs-biosensor strain depends upon AQs produced by *P. aeruginosa* wild type. This reporter system, in principle, allows identification of molecules targeting the *pqs* signaling cascade at multiple levels, including signal synthesis, import/export or reception.

Three hits specifically inhibiting the *pqs* QS signaling system have been identified. *In silico* molecular docking and phenotypic characterization of *ad hoc* engineered strains indicate that all the newly identified inhibitors hamper the *pqs* system by targeting the transcriptional regulator PqsR. These molecules inhibited the expression of *pqs*-controlled virulence traits in *P. aeruginosa*, such as pyocyanin production and swarming motility. Further phenotypic analyses have shown the ability of the most promising *pqs*-inhibitor to reduce biofilm formation in *P. aeruginosa,* to protect *Galleria mellonella* larvae from *P. aeruginosa* infection and to hamper the *pqs* QS system in several clinical isolates from cystic fibrosis patients.

B21. Computation of structure-specific parameters to perform biofilm structure patterns.

L. M. De Plano[1], M. G. Rizzo[1], D. Franco[1], M. Caratozzolo[1], M. Frasca[2], L. Fortuna[2], S. P. P. Guglielmino[1]
[1]Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università degli Studi di Messina; [2]Dipartimento di Dipartimento di Ingegneria Elettrica Elettronica e

Informatica Università degli Studi di Catania

In our previous works was found, for the first time, that once a threshold cell number is retained on the surface, microbial retention patterns are formed following a power law, i.e. not stochastic. Moreover, the overall spatial patterns of microbial retention observed in different substrates are similar for the all investigated cell types. Furthermore, the drastic modification of the free energy surface does not affect this spatial organization. Finally, the experimental retention patterns have been well simulated by a general agent-based model, confirming that the typical fractal distribution of retained cells is the result of a self-organization process.

In this work we analyzed the dynamic growth of the P. aeruginosa biofilm in conditions of rich culture and in nutrient limitation. Adhesion dynamics, at different time, were microscopically analyzed and the obtained images were processed for a quantitative evaluation of adhering cell number and for a fractal dimension analysis. Finally, based on dynamic growth of the biofilm, a 3D model was performed by NetLOGO simulator. The data obtained suggest that the biofilm three-dimensional evolution was superimposable on the initial fractal pattern of bacterial retention on the surface, regardless of the active replication of the bacterial population.

B22. Characterization of new probiotic strains for their role as antimicrobial agents

De Giani, M. Boccarusso, P. Di Gennaro
Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy.

Beneficial effects of probiotics are leading to increasing research of new bacterial strains and new probiotic-formulations enhancing overall health and boosting the immune system, and balancing of the intestinal microbiota. One of the beneficial aspects of probiotics concerns their capacity to inhibit the growth of pathogen bacteria suggesting their possible role as antimicrobial agents in functional foods and dietary supplements. In this study novel probiotic strains, belonging to *Lactobacillus* and *Bifidobacteria* genera, were identified and characterized for their ability to inhibit the growth of *P. aeruginosa*, *E. coli, E. faecalis, S. aureus, and C. albicans*. Among the selected strains, we identified two strains of *L. plantarum* and *L. rhamnosus* that exhibited a strong antimicrobial activity against their antagonists. Results showed the capacity of these two strains to produce in the cultural medium antibacterial compounds. These compounds were analysed and characterized; they showed to be small peptides bacteriocin-like. Furthermore, these peptides will be characterized for their intrinsic properties and their possible effect against pathogens and human intestinal cells.

B23. The mycoremediation approach to restore an historically contaminated dredged sediments: the Bioresnova project

S. Becarelli[1,2], G. Siracusa[1], I. Chicca[1], M. Ruffini Castiglione[1], R. Lorenzi[1], G. Petroni[1], A. Gentini[3], G. Masini[3], S. Di Gregorio[1]
[1]Department of Biology, University of Pisa; [2]BD, Biodigressioni srl, Pisa; [3]Teseco Spa, Pisa

The mycoremediation is a biotechnological approaches in which fungi (ascomycetes and/or basidiomycetes) are applied in combination to chemico-physical treatments to remove contamination from solid matrices. A new fungal strain was isolated from contaminated dredged sediments, massively grown and re-inoculated in the original matrix obtaining a significant depletion of the contamination by Total Petroleum Hydrocarbon contamination (TPH). TPHs are toxic compounds and are one of the main pollutants that cause serious risks for public/human health (Andreoni and Gianfreda, 2007). *Vicia faba*, a higher plant, was exploited as indicator of the quality of the treated sediments and used for the evaluation of the eco-safety of the final product. In this study, the ability to deplete the contamination associated to TPH was studied in

a meso-scale experimentation. The experimentation was conducted to evaluate the capacity of an ascomycete strain isolated from the dredged sediment to deplete the UCM associated to the profile contamination of the polluted sediments. The goal of this work was the removal of TPHs contamination from sediments, being mainly focused on the reduction of the UCM toxicity, in order to restore safe environmental status of the matrix.

Acknowledgments

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B24. Hunting for new bacteriophages: isolation, characterisation and future perspectives

A.-B. Klatt[1], A. Trampuz[1,2], M. Di Luca[1,3]

[1]Berlin-Brandenburg Centrum für Regenerative Therapien, Charité Universitätsmedizin Berlin, Germany; [2]Centrum für Muskuloskeletale Chirurgie, Charité Universitätsmedizin Berlin, Germany; [3]NEST, Istituto Nanoscienze - Consiglio Nazionale delle Ricerche, Pisa, Italy

In recent years, a renewed interest arose in the potential applications of bacteriophages, beyond phage therapy in fields such bioremediation, biocontrol of foodborne pathogens, and as anti-biofilm agents. In all these cases, access to a comprehensive stock of different phages covering a broad bacterial spectrum is crucial. The aim of this study was to screen human and environmental sources for the presence of lytic phages active against selected bacterial strains. Saliva samples, sewage wastewater and river water were screened for the presence of bacteriophages. Bacteriophage, isolated from each source, were characterised by transmission electron microscopy (TEM). The activity of bacteriophages against *Escherichia coli* and methicillin resistant *Staphylococcus aureus* (MRSA) biofilms grown on glass beads was evaluated by highly sensitive isothermal microcalorimetry. Bacteriophages active against *E. coli* strains were isolated from all the sources screened, whereas bacteriophages specific for MRSA could only be isolated from saliva. Morphological TEM analyses suggested that the phages belonged to distinct families. Interestingly, selected bacteriophages were found to be active against MRSA and *E. coli* biofilms. Sequencing analysis will be performed to complete the characterization of the newly isolated bacteriophages.

B25. Anaerobic toluene degradation by *Cupriavidus metallidurans* CH34 in Bioelectrochemical Systems

A. Espinoza[1,2], M. Daghio[1], M. Seeger[2], A. Franzetti[1]

[1]Department of Earth and Environmental Sciences – University of Milano-Bicocca, Piazza della Scienza 1, 20126, Milano, Italy; [2]Department of Chemistry & Center of Biotechnology, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso, Chile

Bioelectrochemistry is a technology based on the connection between microbes (named as exoelectrogens) and electrodes. A promising field of application is the bioelectrochemical remediation, an effective strategy in environments where the absence of suitable electron acceptors limits classic bioremediation. Bioelectrochemical remediation of hydrocarbons with pure strains and microbial communities has been reported. However, only few exoelectrogenic hydrocarbonoclastic bacteria have been characterized so far and most researches have primarily focused on testing the hydrocarbonoclastic capacities of already known exoelectrogenic strains. In this study we took a different approach, and we aimed at studying the exoelectrogenic activity of *Cupriavidus metallidurans* CH34, a model metal-resistant strain, whose hydrocarbonoclastic capacities have been already reported. The capacity to degrade toluene under anaerobic conditions and the exoelectrogenic capacity of *Cupriavidus metallidurans* CH34 was determined. We

demonstrated for the first time that strain CH34 is able to degrade toluene under denitrifying conditions and the removal of this pollutant in MEC was assessed. Toluene degradation was linked to current production, showing current peaks after every toluene respike (maximum current density 48 mA/m^2).

B26. *Streptomyces coelicolor* extracellular vesicles

T. Faddetta[1,2], S. Castelli[1], F. Amato[3], C. Miccichè[3], G. Nasillo[2], G. Buscarino[2,3], D. Chillura Martino[1,2], S. Agnello[2,3], A. Palumbo Piccionello[1], G. Gallo[1,2], A. M. Puglia[1]

[1]Department of Biological Chemical and Pharmaceutical Sciences and Technology (STEBICEF), University of Palermo; [2]Advanced Technologies Network (ATeN) Center, University of Palermo, Palermo, Italy; [3]Department of Physics and Chemistry, University of Palermo

Bacterial extracellular vesicles represent a bacterial secretion system for both macromolecules and small metabolites. Streptomycetes, Gram-positive filamentous bacteria, produce an enormous repertoire of biologically active metabolites and enzymes which are secreted from mycelial cells. The biological role of nanovesicles in these bacteria is poorly investigated. Extracellular vesicles production and their protein content were analysed in the model strain *Streptomyces coelicolor*, whose extracellular vesicles were isolated from exudate droplets, formed on agar-plate cultivations. However, the production of extracellular vesicles in *S. coelicolor* liquid-medium cultivations is not proven yet. Therefore, a density gradient ultracentrifugation-based protocol was applied with the aim to isolate and purify extracellular vesicles from *S. coelicolor* spent-medium. In the expected fractions the presence of nano-sized particles compatible with extracellular vesicles was revealed by Transmission Electron Microscopy and Atomic-Force Microscopy. Moreover, electrophoretic analyses revealed the presence of proteins, DNA and RNA as well as Raman microscopy highlighted the presence of the antibiotic actinorhodin.

This study may reveal the importance of extracellular vesicles in the physiology of S. coelicolor and may also have important biotechnological implications.

B27. Diversity and structure of the bacterial community of the Kandalaksha Bay (White Sea, Russia) estuarine system

S. Gorrasi[1], C. Pesciaroli[1,2], P. Barghini[1], M. Fenice[1,3]

[1]Dipartimento di Scienze Ecologiche e Biologiche and [3]Laboratorio di Microbiologia Marina Applicata, (Conisma), Università degli Studi della Tuscia, Largo dell'Università, snc - 01100 Viterbo, Italy. [2]Dipartimento di Biotecnologie e Bioscienze, Università degli studi di Milano Bicocca, Piazza dell'Ateneo Nuovo, 1 - 20126, Milano, Italy

Kandalaksha Bay is an estuarine system located at the Arctic Polar Circle within the White Sea basin (Russia). This peculiar sub-extreme marine environment, combining features of temperate and Arctic seas, shows uncommon hydrodynamics due to broad sea level differences during tides, causing intense water mixing, and seasonal high runoff of freshwater (from rivers and precipitations). Temperature may fall to -40°C during the harsh and long winter, while in summer it could reach 30°C (average 15-20°C); its broad fluctuations are recorded principally in the littoral zone. Kandalaksha Bay bacterial communities are almost unknown and the few studies available supply a partial information often limited to specific groups. In this work, seawater bacterial communities were studied by 454-pyrosequencing to obtain detailed information on their structure and diversity. Proteobacteria is the most predominant phylum in all samples: its abundance (53%-96%) showed a direct correlation with depth and γ-Proteobacteria was the principal community fraction. No correlation with depth was detected for the remaining phyla. Bacterioidetes and Cyanobacteria were also abundant, in particular on sea surface samples collected close to the coast and offshore, respectively. At the genus level, biodiversity strongly

decreased at the highest depth with evident predominance (65%) of *Halomonas*. Communities' functional organization showed very high dominance (*Fo*: 82-97%)

B28. Cyclodextrin-based nano-devices for improved antibiotic activity

R. M. Fontana[1], L. Barbara[1], N. Milano[1], P. Lo Meo[2,3], G. Gallo[1,3]

[1]Laboratory of Molecular Microbiology and Biotechnology, STEBICEF Dept., University of Palermo, Italy; [2]Laboratory of Catalytic Nanomaterials, STEBICEF Dept., University of Palermo, Italy; [3]ATeN Center, University of Palermo, Italy

Cyclodextrins, cyclic oligosaccharides possessing a hydrophobic cavity, are able to bind reversibly a wide range of organic compounds. This ability, coupled with the substantial lack of cytotoxicity to human cells, makes cyclodextrins useful for the production of innovative and smart drug carriers.

The aim of this study is the development of two different cyclodextrin-based nano-devices used for antibiotic loading and antimicrobial efficacy improvement. Thus, polyaminocyclodextrin-silver nanoparticles (Pac-Ag NPs) and cyclodextrin-calixarene nanosponges (Cy-Cal NSs) were obtained. Both systems have been shown possessing the ability to function as carriers for ampicillin and tetracycline antibiotics. Moreover, our investigations revealed that Pac-Ag NPs have an intrinsic antibacterial activity and exert a synergic interaction with ampicillin against both Gram-negative and Gram-positive bacterial strains, including *Pseudomonas aeruginosa* and *Staphylococcus* aureus as well as an ampicillin resistant Escherichia coli. On the other hand, a pH-dependent release of antibiotic was observed for tetracyclin-loaded Cy-Cal NS system; in addiction, preliminary investigations revealed an increasing of antibacterial efficacy against *E. coli*.

Therefore, the two cyclodextrin-based devices are promising antibiotic carriers, showing antibacterial activity improvement and controlled drug-release, and will be subjected to future investigations.

B29. Filamentation as evolutive strategy in *Pseudomonas mediterranea*

D. Franco[1], M. G. Rizzo[1], L. M. De Plano[1], G. Crea[1], F. Moscato[1], M. S. Nicolò[1], S. P. P. Guglielmino[1]

[1]Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università degli Studi di Messina

The bacteria filamentation is a typical elongated shape cell and occurs through inhibition of cell division. For a variety of opportunistic pathogens, the filamentous morphology has been shown to provide survival advantages during exposure to environmental stresses.

It is known that phytopathogenic *Pseudomonas* strains use acidic amino acids and their amides contained in the radical exudates as sole carbon and nitrogen source, supporting a rapid growth.

Pseudomonas mediterranea causes tomato pith necrosis, yet it is a soil inhabitant bacteria capable of multiplying in the root system as it is a good competitor against other organisms. Previously we showed that *P. mediterranea* uses glutamine as good carbon source and its uptake in co-metabolism is not subjected to catabolite repression.

In this work, we determined which nutritional components as amino acids, sugar or fatty acid induced filamentous forms in *P. mediterranea*. In particular, glutamine, glycerol, glucose and sodium octanoate both as single carbon source and in co-metabolism were tested.

Our results showed that only glutamine led to filamentous forms. Furthermore, the addition of second carbon sources in co-metabolism maintained the filamentous forms.

This study provides insights into the filament-inducing conditions and indicates that the

formation of filaments could potentially be utilized by *P. mediterranea* as a survival strategy in its habitat.

B30. Diversity and role of Aerobic Anoxygenic Phototrophs on glacier surfaces.

A. Franzetti[1], F. Pittino[1], C. Ferrario[1], I. Tagliaferri[1], I. Gandolfi[1], G. Bestetti[1], R. S. Azzoni[2], G. Diolaiuti[2], S. Villa[1], R. Ambrosini[1]

[1]Dept. of Earth and Environmental Sciences (DISAT) - University of Milano-Bicocca, Milano, ITALY; [2]"A. Desio" Dept. of Earth Sciences, Università degli Studi di Milano, Milano, ITALY

Aerobic Anoxygenic Phototrophs (AAPs) are obligate heterotrophic phototrophs whose presence has been recently documented in many environments. However, only recently the diversity and distribution of AAPs have been studied in glacialized areas and even less is known about the role of these bacteria in such environments.

We used available whole metagenomic sequence data to investigate the role of AAPs in supraglacial sediments (cryoconite) from Forni (Italian Alps) glacier. We also investigated biodegradative processes by microcosm experiments simulating a cryoconite hole system conducted in situ on the Forni glacier, which was exposed to the organophosphorus insecticide chlorpyrifos (CPF), a xenobiotic tracer that accumulates on glaciers.

Sequencing data showed that AAPs are abundant and significantly contribute to carbon and energy input of the glaciers being involved in light energy harvesting reactions, organic carbon incorporation and carbon monoxide oxidation. Consistently, in situ microcosms revealed that abundant populations of AAPs belonging to *Burkholderiales* are involved in the biodegradation of CPF, which resulted to be the most relevant process leading to the removal of CPF from glaciers.

The high metabolic versatility of supraglacial AAPs might explain the colonisation success of *Burkholderiales* in cold environments.

B31. Bacterial and fungal diversity of phyllospheric communities hosted by different urban tree species

I. Gandolfi[1], G. Ottoboni[1], I. Rossi[1], S. Molinetti[1], C. Casagrande[2], E. Ceci[2], S. Covino[2], R. Ambrosini[1], E. Padoa-Schioppa[1], M. Papacchini[3], B. Sebstiani[2], D. Cappelletti[2], E. Federici[2], A. Franzetti[1]

[1]Dept. of Earth and Environmental Sciences, University of Milano-Bicocca (Milan, Italy); [2]Dipartimento Di Chimica, Biologia e Biotecnologie, Università degli Studi di Perugia (Perugia, Italy); [3]INAIL, Dipartimento Innovazioni Tecnologiche e Sicurezza degli Impianti, Prodotti ed Insediamenti Antropici (Rome, Italy)

Plant-associated bacteria and fungi have been suggested to play a role in air pollution mitigation, especially in urban areas, by degrading atmospheric hydrocarbons. However, phyllospheric microbial communities are highly variable depending on several factors, e.g. tree species, leaf age, environmental conditions. In this work, temporal variability of epiphytic bacterial and fungal communities hosted by leaves of different tree species (*Cedrus deodara, Magnolia grandiflora, Quercus ilex, Platanus x acerifolia, Tilia x vulgaris)*, located in urban parks in Milan and Terni, was assessed. Bacterial and fungal communities were taxonomically characterized by Illumina high throughput sequencing of the hypervariable regions V5-V6 of 16S rRNA gene and of the ITS1-ITS2 regions, respectively. Culturable bacterial strains, both epiphytic and endophytic, were isolated on different culture media and tested for biodegradation abilities towards PAHs. Moreover, the presence of naphthalene dioxygenase genes was assessed by qPCR. Results revealed that tree species and seasonality acted as a strong driver on biodiversity of microbial communities, particularly in deciduous plants. Approximatively

15% of the isolated strains were able to degrade naphthalene and/or phenanthrene in laboratory cultures. It was therefore hypothesized that phyllospheric populations hosted by urban trees might play a role in the mitigation of hydrocarbon air pollution.

B32. The temperature of sporulation affects structure and function of *Bacillus subtilis* spores

R. Isticato, M. Lanzilli, G. Donadio, L. Baccigalupi, E. Ricca
Department of Biology, University of Naples Federico II, Naples, Italy

Bacterial spore formers are commonly and abundantly isolated from soil, water and air samples and are also found in large numbers associated with other organisms. Such diverse habitats are characterized by extremely different chemical and physical conditions, raising the question of whether spores produced at different conditions are identical or differ in terms of structure and function. We used Bacillus subtilis spores produced at 25°C and 37°C but of similar age to analyze and compare their coat structure, their resistance properties and germination efficiency. We observed that spores produced at 25°C were more hydrophobic than those produced at 37°C but contained less DPA and were less heat-resistant.

The different hydrophobicity induced us to analyze in more details the protein composition of the spore surface of 25°C and 37°C spores. We observed that morphogenetic protein CotH and CotH-dependent coat proteins were more abundantly present in the coat of 25°C than 37°C spores and that cotH null mutant spores were more damaged when produced at 25°C than at 37°C.

Our results suggest that CotH plays a more relevant role at 25°C than at 37°C and reveal a complex interplay between the function of the morphogenetic proteins and environmental factors during spore formation.

B33. Contribution of DIC-fixing prokaryotes to the global carbon balance in Mediterranean Sea

V. La Cono, F. Crisafi, G. Ruggeri, F. Smedile, G. La Spada, R. Denaro, M. Yakimov
Institute for Coastal Marine Environment, CNR, Spianata S. Raineri 86, 98122 Messina, Italy

The dark meso- and bathypelagic realms represent the largest marine subsystems comprising almost three quarters of the global oceanic volume and contain more than 98% of the global dissolved inorganic carbon (DIC). Despite that, this environment is by far one of the least explored ecosystems on Earth, and very little data are available about microbial life and biogeochemical processes in this ocean's 'inner space'. Here we report on measurements of microbial DI14C fixation rates, heterotrophic production and respiration in the oxygenated meso- and bathypelagic realms (below 200m) of Mediterranean Sea. Seven stations were sampled along a longitudinal transect from 08°W to 26°E following the path of the Atlantic Water, which enters the Mediterranean through the Strait of Gibraltar. For comparative reasons, the assimilation of [14C]-bicarbonate in the dark (ABD) was additionally estimated in sub- and anoxic transition zones, covering seven deep-sea hypersaline anoxic lakes. In this regional analysis, we demonstrate that ABD substantially contributes to the global carbon cycle and to the organic carbon demand of the deep-sea microbial food-web. Furthermore, following the methodological practice of successive isolation of marine DIC-fixing thaumarchaea and proteobacteria, we obtained three DIC-fixing and nitrifying bathypelagic enrichments.

B34. Development and evaluation of GeneCARD-FISH assays for *in situ* detection and quantification of bacteria carrying antibiotic resistance genes

M. L. Luprano[1], B. Matturro[1], S. Rossetti[1], E. Grohmann[2], C. Levantesi[1]
[1] Water Research Institute, CNR, Via Salaria Km 29.600, 00015 Monterotondo, Italy; [2] Faculty of Life Sciences and Technology, Beuth University of Applied Sciences, Berlin, Germany

GeneCARD-FISH is an innovative *in situ* hybridization-based assay, developed for the detection and enumeration of bacterial cells carrying key functional genes. Thus, it was selected for quantification of environmental bacteria carrying antibiotic resistance genes (ARGs). Other techniques commonly applied for antimicrobial resistance monitoring, such as qPCR and cultivation-based methods, only provide a measure of ARG gene copy numbers and of the cultivable fraction of antibiotic resistant bacteria, respectively. Two GeneCARD-FISH assays were developed for the specific detection of bacteria carrying *ermB* and *sul1* genes that confer resistance to erythromycin and sulphonamides, respectively. The assays were optimized on pure cultures as positive controls, *sul1*-carrying *E.coli* and *ermB*-carrying *Enterococcus*, and applied for the enumeration of target genes in water samples. Detection efficiencies of 90% ± 9% and of 95.0 ± 4% were obtained, for *sul1* and *ermB* positive controls respectively showing the efficiency of GeneCARD-FISH at the selected hybridization conditions. Optimized GeneCARD-FISH protocols were successfully applied for the quantification of *ermB*- and *sul1*-carrying bacteria in water with different levels of contamination. Finally, in comparison to qPCR data, results suggest that the possible presence of bind extracellular DNA in sample rich of particulate matters might largely influence the determination of target gene abundance in contaminated water samples.

B35. Unveiling the gut microbiota composition and functionality associated with constipation through metagenomic analyses

L. Mancabelli[1], C. Milani[1], G. A. Lugli[1], F. Turroni[1], M. Mangifesta[1,2], A. Viappiani[2], A. Ticinesi[3], A. Nouvenne[3], T. Meschi[3], D. van Sinderen[4], M. Ventura[1]
Laboratory of Probiogenomics, Department of Chemistry, Life Sciences, and Environmental Sustainability, University of Parma, Parma, Italy[1]; GenProbio srl, Parma, Italy[2]; Department of Medicine and Surgery, University of Parma, Italy; Dipartimento Medico-Geriatrico-Riabilitativo, Azienda Ospedaliero-Universitaria di Parma, Italy[3]; APC Microbiome Institute and School of Microbiology, Bioscience Institute, National University of Ireland, Cork, Ireland[4]

Functional constipation (FC) is a gastrointestinal disorder with a high prevalence among the general population and having a negative impact on the quality of life of affected individuals. The precise causes of FC are still unknown and are most likely multifactorial. Growing evidence indicates that alterations of the gut microbiota composition contribute to constipation symptoms. Nevertheless, many discrepancies exist in literature and no clear link between FC and gut microbiota composition has as yet been identified. In this study, we performed 16S rRNA-based microbial profiling analysis of stool samples from FC individuals and compared their microbial profiles with those of healthy subjects (HS). Notably, the gut microbiota of FC individuals was shown to be depleted of members belonging to *Bacteroides*, *Roseburia* and *Coprococcus 3*. Furthermore, the metabolic capabilities of the gut microbiomes were evaluated through shotgun metagenomics, indicating that HS are enriched in pathways involved in carbohydrate, fatty acid and lipid metabolism as compared to FC. In contrast, the microbiomes corresponding to FC were shown to exhibit high abundance of genes involved in hydrogen production, methanogenesis and glycerol degradation. The identified differences in bacterial composition and metabolic capabilities may play an important role in development of FC symptoms.

B36. MCT13: a newly discovered agarase-producing *Sphingomonas* sp. isolated from freshwaters

P. Marmo[1], M. M. D'Andrea[2], F. Casu[3], G. Di Lallo[1], L. Migliore[1], M. C. Thaller[1]
[1]Department of Biology, University of Rome "Tor Vergata", 00133 Rome, Italy; [2]Department of Medical Biotechnologies, University of Sienna, 53100 Sienna, Italy; [3]School of Biological Sciences, The University of Auckland, 3A Symonds Street, Auckland, New Zealand.

The yellow-pigmented Gram-negative MCT13 strain was isolated from a drainage ditch within a disused system of constructed wetlands, flowing through uncultivated land. Growth on agar media was associated with clearing and pitting around the colonies, a feature never reported so far among sphingomonads. This trait, together with the unusual biochemical profile, let us to hypothesize that MCT13 belonged to a novel species.

The 16S rDNA sequence shared the highest (98%) identity with uncultured sphingomonads.

High Throughput DNA Sequencing confirmed the presence of putative agarase-like enzymes, and allowed to perform an Average Nucleotide Identity analysis, by comparing MCT13 with other *Sphingomonas* sp. deposited in the INSDC databases. The closest homolog (78.3%) is *Sphingomonas koreensis* NBRC_16723. As the species delineation cut-off is <95%, the hypothesis that MCT13 belongs to a novel species in the genus *Sphingomonas* is supported. The ability to degrade agar, together with the lack of known antibiotic resistances other than the genus-related resistance to streptomycin, make *Sphingomonas* sp. MCT13 a potentially interesting strain for the degradation of complex carbohydrates.

B37. Conversion of sugar beet pulp into lipids by *Lipomyces starkeyi* for biodiesel production

F. Martani[1], M. Torchio[1], D. Ami[1], A. Natalello[1], M. Lotti[1], P. Branduardi[1], D. Porro[1]
[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy

The depletion of fossil reserves and the increasing demand of energy by a continually growing world population are boosting the development of production processes based on the bioconversion of renewable resources. Among them, plant-based raw materials and side-stream wastes are promising to reduce our dependency on petroleum and to contribute to the development of circular bioeconomy. In this scenario, the BEETOUT (Sugar BEEt biorefinery for the inTegrated prOduction of biofUel and polyesTers) project, funded by Fondazione Cariplo, aims at the microbial conversion of beet pulp wastes derived from sugar industry into triacylglycerols (TAGs), which are subsequently transformed into biodiesel and bioplastics. To this purpose, the oleaginous yeast *L. starkeyi* has been selected for its ability to accumulate up to 70% of cell biomass as lipids under defined culture conditions.

Here, we show the strategies adopted to support high lipid accumulation by *L. starkeyi* cultured on beet pulp hydrolysates and to convert TAGs into biodiesel thanks to an enzyme-driven reaction.

B38. Single-step conversion of xylan into xylose by thermoacidophilic enzymes adsorbed on *Bacillus subtilis* spores

R. Mattossovich[1] , R. Iacono[2] , B. Cobucci-Ponzano[2] , R. Isticato[1] , G. Cangiano[1], M. Moracci[1,2], E. Ricca[1]
[1]Dept of Biology, Federico II University of Naples; [2]Institute of Biosciences and BioResources, CNR,Naples

The *Bacillus subtilis* spores has long been used to display antigens and enzymes. We used a non-recombinant approach to independently display on the spore surface two thermoacidophilic enzymes, an endo-xylanase of *Alicyclobacillus acidocaldarius (*GH10-XA) and a xylosidase of

Thermotoga thermarum (GH3-XT) involved in the multi-step degradation of xylan.

We report that both purified enzymes were independently and efficiently adsorbed on purified spores of *B.subtilis.* The adsorption was tight, both enzymes retained their specific activity upon displays on the spore surface and were more stable than the free enzymes to conditions of low Ph and hight temperature. When spores displaying either GH10-XA or GH3-XT were mixed together, xylan was efficiently used as a substrate and free xylose produced more efficiently than by the two free enzymes not adsorbed to the spores.

Our results indicate that the single-step release of free xylose from xylan can be accomplished by mixing spores displaying the two required enzymes. The efficiency of the process, the pH and the temperature stability of the adsorbed enzymes, and the well documented robustness of spores of *B.subtilis* propose the spore as a suitable platform to displays enzymes for single as well as multistep reactions.

B39. *In silico* identification and *in vivo* validation of potential quorum sensing inhibitors

F. D'Angelo, E. Di Muzio, M. Mellini, S. Ferrillo, L. Leoni, G. Rampioni, F. Polticelli
Department of Science, University Roma Tre, Rome, Italy

Transcriptional regulators and effector proteins of the quorum sensing (QS) communication system govern the expression of virulence determinants in the opportunistic human pathogen *P. aeruginosa,* hence they are considered promising targets for the development of anti-virulence drugs. Virtual screening approaches allow fast and cost-effective selection of target ligands among vast libraries of bioactive molecules, so accelerating the drug-discovery process. On these bases, an *in silico* screening campaign has been undertaken to identify novel drugs targeting the QS transcriptional regulators LasR and PqsR, or the QS effector protein PqsE of *P. aeruginosa*.

An *in silico* library of 1,400 FDA-approved drugs has been screened by molecular docking, and ten hits showing the highest binding affinity to each target protein have been selected. Wet-lab experiments have been performed by means of *ad hoc* engineered biosensor strains to validate the functionality of the best hits to decrease LasR, PqsR and PqsE activity in *P. aeruginosa*. Phenotypic analyses have been performed to evaluate the impact of the most promising hits on the expression of *P. aeruginosa* QS-controlled virulence determinants.

B40. Identification of FDA-approved anti-virulence drugs targeting PqsE

V. Baldelli, F. D'Angelo, V. Pavoncello, P. Visca, G. Rampioni, L. Leoni
Department of Science, University Roma Tre, Rome, Italy

In *Pseudomonas aeruginosa* the quorum sensing effector protein PqsE is required for the expression of a large array of virulence factors, and *pqsE* mutation results in decreased virulence in plant and animal infection models. Therefore, PqsE is a candidate target for the development of *P. aeruginosa* anti-virulence drugs.

In this study, a library of FDA-approved drugs has been screened by using a purpose-built bioluminescent *P. aeruginosa* biosensor in which light emission is expected to increase in the presence of PqsE inhibitors.

The screening campaign led to the identification of three drugs increasing bioluminescence in the biosensor and decreasing the expression of PqsE-dependent virulence traits in *P. aeruginosa* wild type (*i.e.* pyocyanin production and swarming motility).

Preliminary analyses showed that the most promising PqsE inhibitor (named PqsE-I2) does not affect PqsE-repressed phenotypes in a *P. aeruginosa pqsE* mutant strain, supporting target specificity. Moreover, Real Time PCR analysis showed that PqsE-I2 does not decrease the mRNA levels of the *pqsE* gene, thus suggesting interference with PqsE at the post-transcriptional level.

Analyses are ongoing to elucidate the mechanism of action of PqsE-I2, and to assess its effect on *P. aeruginosa* pathogenic potential both *in vitro* and *in vivo*.

B41. Biocontamination of surfaces and waters inside the international space station

E. Perrin[1], G. Bacci[1], A. Mengoni[1], F. Canganella[2], G. Bianconi[2], L. Garrelly[3], S. Amalfitano[4], C. Levantesi[4], S. Rossetti[4], S. Perero[5], M. Ferraris[5], R. Fani[1]

[1]Department of Biology, University of Florence, Via Madonna del Piano 6, 50019, Sesto Fiorentino (Fi), Italy; [2]Department of Biological, Agrofood and Forestry sciences (DIBAF), via C. de Lellis, 01100 Viterbo, Italy; [3]GL-Biocontrol, Cap Alpha, Avenue de l'Europe, 34830 Clapiers, France; [4]IRSA --Istituto di Ricerca Sulle Acque - Water Research Institute, CNR -- Consiglio Nazionale delle Ricerche - National Research Council of Italy, Area della Ricerca Roma 1 Via Salaria Km 29.300, 00015 Monterotondo - Roma, Italy; [5]Polytechnic of Turin, Department of Applied Science and Technology (DISAT), C.so Duca degli Abruzzi 24, 10129 Torino

Space exploration requires the development of methods for preventing, monitoring and controlling biocontamination within human confined environments. Space and terrestrial monitoring as well as prevention/mitigation methods are currently working separately, rather than in synergy. The Horizon 2020 Biowyse project foresees development and demonstration of an integrated biocontamination control system for water and humid areas. Previous experiments provided valuable information for the Biowyse project. Viable ISS (financially sponsored by ASI and supported by NASA) study involves the evaluation of the microbial biofilm development on space materials. Samples included in Viable ISS are composed of both metallic and textile space materials, that are placed both inside and outside of four foam lined Nomex bags, each one subjected to a different pre-treatment procedure. These bags were exposed inside International Space Station from 2011 to 2016. Vials with samples of potable water were also included. Different methodologies were used for the determination of the bacterial load (ATP-metry, Flow-Cytometry, qPCR). The composition of the microbial communities was determined through 16S rDNA high-throughput sequencing. A low bacterial load was found on all surface samples and on potable water. Surface samples were mainly colonized by member of Gammaproteobacteria (mainly *Enterobacteriaceae*), while Alphaproteobacteria (*Caulobacterales*) colonized water samples.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 687447

B42. Use of crude glycerol for the microbial production of lipids for fish feed formulation

C. Pesciaroli, D. Ami, A. Salvetti, M. Labra, P. Branduardi

Department of Biotechnology and Biosciences, University of Milano-Bicocca, 20126 Milan, Italy

The global demand of fish products is growing fast (3.2% growth on year basis). Consequently, aquaculture market is increasing too. One of the major limitation is the supply of Fish Meal (FM) and Fish Oil (FO), which are the main components of fish feed, and whose price is consequently rising. In this context, the final aim of the MYSUSHI project (Microalgae and Yeasts SUStainable fermentation for HIgh quality fish feed formulation) is to replace FO with Microbial Oil (MO). MO is produced by a biotechnological process that involves the ability of oleaginous microorganisms, such as microalgae and yeasts, to convert crude glycerol from the biodiesel industry into nutraceutics, mainly long chain fatty acids (such as DHA) and carotenoids. Therefore, the project follows the logic of Circular Bioeconomy, valorizing and

up-grading an industrial waste that is also a potential pollutant. Here we report the optimization of the fermentation processes together with analysis of lipids accumulation by flow cytometry and FTIR. GC analyses were performed for completing and confirming the quali-quantitative profiles. The final aim will be the formulation of novel fish feed to be tested in aquaculture, following and monitoring different physiological parameters together with biomarkers.

B43. Mycobiota in arsenic-contaminated mine sites

M. Petruccioli, L. Pesciaroli, M. Felli, A. D'Annibale, S. Crognale
DIBAF, University of Tuscia, Via San Camillo De Lellis, Viterbo (Italy)

Large quantities of heavy metals are released by gold mining activities, causing widespread contamination of the ecosystem, and harboring microorganisms well adapted to the presence of multiple stress factors. The present study was aimed to explore fungal community structure in As and Hg historically polluted soils by using Illumina metabarcoding. Moreover, the attention was focused on the possible mechanisms adopted by fungal isolates in As mitigation. The metagenomic analysis evidenced a fungal community diversity not related to the concentrations of inorganic toxicants and an increasing fungal richness in presence of the highest contamination level. Although the taxonomic profiles showed the dominance of Zygomycota, the cultivable dependent approach permitted to isolate, besides the presence of *Mucor* and *Mortierella* spp., very high As resistant strains belonging to *Penicillium* and *Trichoderma* genera. In As exposed cultures, the main mechanism by which As was removed by fungal isolates involved volatilization the highest extent of which was found for *P. janthinellum* (31.2%).

B44. Time-resolved transcriptomics and constraint-based modelling identify system-level metabolic features and overexpression targets to increase spiramycin production in *Streptomyces ambofaciens*

E. Pinatel[1] , M. Fondi[2] , A. Talà[3] , F. Damiano[3] , C. Consolandi[1], B. Mattorre[2], D. Fico[4] , G. E. De Benedetto[4] , L. Siculella[3] , G. De Bellis[1], P. Alifano[3], C. Peano[1]
[1]Institute of Biomedical Technologies, National Research Council, Segrate, Italy,; [2]Department of Biology, University of Florence, Florence, Italy; [3]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; [4]Laboratory of Analytical and Isotopic Mass Spectrometry, Department of Cultural Heritage, University of Salento, Lecce, Italy

The production of industrially relevant secondary metabolites, like antibiotics, requires several metabolic intermediates which are indispensable also for the biosynthetic processes leading to bacterial growth. In silico modelling and omic-approaches can provide system level comprehension of bacterial metabolism, identifying new focused strategies to obtain industrially relevant engineered strains.

We applied these techniques to model *Streptomyces ambofaciens*, an industrial producer of antibiotics, anti-fungal and iron-chelating compounds. To reconstruct the metabolic model we improved *S. ambofaciens* genome annotation with the information of several databases for gene/protein function prediction, manually refining it to include spyramicin, antimycin, stambomycin and congocidine production pathways. The integration of time-resolved transcriptomic data with Flux Balance Analysis was then adopted to highlight the effects of gene expression changes on the overall metabolic adjustments occurring during *S. ambofaciens* growth curve. At the same time Flux Scanning based on Enforced Objective Flux (FSEOF) analysis was performed to identify new targets for spiramycin production increase. FSEOF predictions were experimentally validated manipulating the ethylmalonyl-CoA metabolic node, providing evidence that spiramycin productivity is increased by enhancing the carbon flow through this pathway and

demonstrating that the metabolic model herein proposed represents a solid platform for the exploitation of *S. ambofaciens* biotechnological potential.

B45. Role of bacteria in pollutants biodegradation in Alpine cryoconite holes

F. Pittino[1], C. Ferrario[1], R. S. Azzoni[2], I. Tagliaferri[1], I. Gandolfi[1], G. Bestetti[1], G. Diolaiuti[2], A. Franzetti[1], R. Ambrosini[1], S. Villa[1]
[1]Università degli Studi di Milano-Bicocca, Department of Earth and Environmental Sciences; [2]Università degli Studi di Milano, Department of Earth Sciences 'Ardito Desio'

Pollutants released into the atmosphere are subjected to medium and long range transport. These compounds can reach also remote regions apparently uncontaminated. One of these regions are glaciers, which represent surprisingly rich ecosystems. Indeed, they host on their surface peculiar microhabitats named cryoconite holes: small depressions full of melting water and sediment on the bottom inhabited by metabolically active microbial communities. Recent studies found the presence of currently used pesticides such as chlorpyrifos and terbuthylazine in Forni glacier (Italian Alps) melting water and cryoconite. Our studies aimed at investigating the biodegradation of pollutants in cryoconite holes. To this end, microcosm *in situ* experiments were setup to evaluate the ability of bacteria to degrade these contaminants during the ablation season. These microcosms intended to simulate cryoconite holes under both light and dark conditions to evaluate also the photolysis effect. Chemical analysis allowed evaluating the decrease of pollutants, while Illumina high throughput sequencing of the 16S rRNA gene assessed temporal variations in microbial communities. Moreover, qPCR quantifications were performed for both 16S rRNA gene and genes involved in the degradation of specific pollutants. The results showed that bacteria in cryoconite holes are determinant for pollutants removal from glaciers and revealed novel insight into the role of supraglacial bacterial communities.

B46. Exploitation of the strictly aerobic *Rhodococcus aetherivorans* BCP1 strain for the production of Selenium- and Tellurium-nanostructures

A. Presentato[1], E. Piacenza[1], M. Anikovskiy[2], M. Cappelletti[3], D. Zannoni[3], R. J. Turner[1]
[1]Microbial Biochemistry Laboratory, Department of Biological Sciences, University of Calgary, 2500 University Dr. NW, Calgary, AB T2N 1N4, Canada; [2]Department of Chemistry, University of Calgary, 2500 University Dr. NW, Calgary, AB T2N 1N4, Canada; [3]Unit of General and Applied Microbiology, Department of Pharmacy and Biotechnology, Via Irnerio 42, Bologna, 40126, Italy

Tellurite (TeO_3^{2-}) and selenite (SeO_3^{2-}) are hazardous and toxic oxyanions for living organisms. However, microorganisms that tolerate and bioconvert these oxyanions to the less toxic and available form of elemental tellurium (Te^0) and selenium (Se^0) are seen as ideal candidates not only for bioremediation purposes, but also as novel cell factories to produce valuable metalloid-nanostructures. Here, *Rhodococcus aetherivorans* BCP1's tolerance and consumption of both TeO_3^{2-} and SeO_3^{2-} have been investigated, along with the production and characterization of metalloid-nanomaterials in the form of Se/Te-nanorods and Se-nanoparticles. BCP1 displayed a Minimal Inhibitory Concentration towards TeO_3^{2-} and/or SeO_3^{2-} of 11.2 and 500 mM, respectively, in rich medium under oxic conditions. Generally, pre-adapted BCP1 cells bioconverted a higher amount of oxyanions, regardless the initial concentrations of TeO_3^{2-} (0.4 or 2 mM) and/or SeO_3^{2-} (0.5 or 2 mM). Transmission Electron Microscopy and Energy-Dispersive X-Ray Spectroscopy showed stable and non-aggregated metalloid-nanomaterials surrounded by an organic coating, whose ability to self-assemble in the nanoscale was assessed through Dynamic Light Scattering. Finally, Zeta potential analyses revealed negative surface charges for both metalloid-nanomaterials and organic coating. We hypothesize that the biogenic Se/Te-

nanostructures stability might be achieved through electro-steric interactions occurring between metalloid-nanomaterials and the surrounding organic coating surrounding.

B47. Microbial communities and novel taxa in « Continental Smokers » of northern Greece

P. Quatrini[1], M. Tagliavia[1,3], A. L. Gagliano[2], M. Tolone[4], V. Catania[1], W. D'Alessandro[2]
[1]Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF) Università degli Studi di Palermo; [2]Istituto Nazionale di Geofisica e Vulcanologia (INGV) – sezione di Palermo; [3]Institute for Coastal Marine Environment of the National Research Council (IAMC-CNR) Torretta Granitola - Campobello di Mazara (TP); [4]Dipartimento di Scienze Agrarie Alimentari e Forestali (SAAF) - Università degli Studi di Palermo

Analogously to the ocean-bottom black/white smokers, "continental smokers" have recently been defined as sites of mantle degassing through continental crust. The goal of this research, carried out within the Deep Carbon Observatory Community, is to investigate the role of deeply-sourced fluids in niche ecosystem differentiation in European continental smokers. A 16S rRNA gene survey of microbial communities was carried out on 11 geothermal manifestations of northern Greece, all showing a distinct contribution of mantle fluids (R/Ra up to 1.2) that allow to classify them as continental smokers. The selected sites cover a wide range of temperatures (15-77 °C), pH (6.11-8.46), Eh (-289 – 40 mV), salinities (TDS 0.4-38 g/L) and show significant differences in energy sources for microbial life, like H_2 (up to 0.8 µmol/L), CH_4 (up to 400 µmol/L), NH_4 (up to 112 µmol/L), sulphide (up to 103 µmol/L), Fe (up to 130 mmol/L). Illumina sequencing revealed negligible presence of Archaea and dominance of Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. Different bacterial taxa dominate depending on physico-chemical features: Deinococcus-Thermus dominates the hottest sites while chemoautotrophic genera (*Sulfurovum* and *Sulfurimonas*) are abundant in the most reducing H_2S-rich waters. Signatures of deep-sea vent microbial ecosystems were detected in most assemblages, together with novel taxa.

B48. Production of phenoxy-substituted poly(3-hydroxyalkanoates) (PHA) by *Pseudomonas mediterranea*

M. G. Rizzo[1], L. M. De Plano[1], D. Franco[1], M. S. Nicolò[1], A. Ballistreri[2], G. Impallomeni[3], S. P. P. Guglielmino[1]
[1]Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università degli Studi di Messina; [2]Dipartimento di Scienze del Farmaco, Università degli Studi di Catania; [3]Istituto per i Polimeri, Compositi e Biomateriali, Consiglio Nazionale delle Ricerche, Catania

Medium-chain length poly(3-hydroxyalkanoates) (mcl-PHAs) with functional groups can be used to bind bioactive compounds. However, some precursors, containing a phenyl group, are not readily utilized by bacteria when used as sole carbon source.

Co-feeding strategies are used to promote both substrates uptake and PHA accumulation. Conversely, the simultaneous use of two different sources may induce variations in the monomer composition.

In previous studies we showed that glutamine in co-metabolism promotes the uptake of second carbon sources and increases PHA yield in *P. mediterranea*.

The aim of this work was to verify whether glutamine in co-metabolism with 11-phenoxyundecanoic acid could promote PHA accumulation without changes in monomer composition. The structural characterization of the polymers was performed through MALDI-TOF MS and NMR analyses. Glutamine/11-phenoxyundecanoic acid-fed cultures (GLN-

11POU) were compared to sodium octanoate/11-phenoxyundecanoic acid-fed cultures (SO-11POU) at the same molar carbon concentration.

The results showed that glutamine did not alter the PHA monomer composition. Proton signal areas indicated that 100% of monomers with aromatic substitutions were present in GLN-11POU mcl-PHA, while 52% of monomers in SO-11POU mcl-PHA. This could be applied to large-scale processes for the production of mcl-PHA with active chemical groups aimed at obtaining more advanced Drug Delivery Systems.

B49. Environmental persistence and risk assessment of *Trichoderma atroviride* strains

F. Luziatelli, M. Tronati, A. G. Ficca, M. Ruzzi
DIBAF-University of Tuscia Viterbo Italy

Trichoderma is a well-studied fungal genus that currently consists of more than 200 molecularly defined species, some of these, as *Trichoderma atroviride*, are widely used in horticulture crops for its biostimulant and biocontrol activity. Persistence and multiplication in soil of *T. atroviride* strains is affected by a variety of environmental factors including pH, temperature, soil composition, biogeography and plant host species. In the present study, we used a fast laboratory test to predict the behaviour of *T. atroviride* in natural environments. Using different media and varying the initial pH and incubation temperature, we evaluated radial growth, spore formation and strain persistence under conditions simulating natural environments. Using this approach we could demonstrated that incubation at non-permissive temperature (0-7°C or 37-40°C) affected spore formation and the radial growth, but was not detrimental to cell viability. The optimal pH for cell growth is temperature-dependent and varies from 4 (30°C) to 6 (25°C). In *vitro* analysis of the differential cytotoxicity of *T. atroviride*'s toxins/secondary metabolites in neoplastic versus proliferating normal cells was also undertaken and no cytotoxic activity was evident on the all tested systems. These findings suggest that the exposure to *T. atroviride*-based products does not appear to pose health risk for operators workers, residents and bystanders.

B50. Biphenyl modulates the expression and function of respiratory oxidases in the polychlorinated-biphenyls degrader *Pseudomonas pseudoalcaligenes* KF707

F. Sandri[1], S. Fedi[1], M. Cappelletti[1], F. Daldal[2], D. Zannoni[1]
[1]Departement of Pharmacy and Biotechnology, University of Bologna; [2]Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Pseudomonas pseudoalcaligens, KF707 strain, is a soil polychlorinated biphenyls (PCBs) degrader which is able to grow aerobically in the presence of various toxic aromatic compounds. KF707 represents a promising tool for bioremediation applications, and the study of the modulation of its respiratory chain in the presence of different carbon sources, can lead to a better understanding of its use in bioremediation procedures. Five terminal respiratory oxidases were identified in KF707 by genomic and functional analyses: two *c(c)aa*$_3$-type oxidases (Caa$_3$ and Ccaa$_3$), two isoforms of *cbb*$_3$-type oxidases (Cbb$_3$-1 and Cbb$_3$-2) and one *bd*-type cyanide-insensitive quinol oxidase (CIO). While the functional role of both Cbb$_3$-1 and Cbb$_3$-2 is prevalent in glucose grown cells, the Caa$_3$ oxidase is over-expressed when biphenyl is used as the sole carbon source; conversely, the CIO oxidase is over-expressed when the Cbb$_3$-1 oxidase is deleted in both glucose and biphenyl grown cells. By studying: protein expression, oxidase activities in vivo and isolated membranes, KF707 growth curves in both W.T. and mutants, we concluded that *cbb*$_3$ and *caa*$_3$ type oxidases are modulated in cells grown in biphenyl which is the co-metabolite needed for the activation of the PCBs-degradation pathway.

B51. Quantitative and qualitative profile of *Tuber mesentericum* microbiome, an edible black truffle

D. Sateriale[1], R. Colicchio[2], C. Pagliuca[2], E. Scaglione[2], E. Varricchio[1], P. Salvatore[2,3], C. Pagliarulo[1]

[1]Department of Science and Technology, Sannio University, Benevento, BN, Italy, [2]Department of Molecular Medicine and Medical Biotechnology, Federico II University, Napoli, NA, Italy, [3]CEINGE, Advanced Biotechnologies s.c.ar.l., Napoli, NA, Italy

Truffles (*Tuber* spp.) are ascomycete hypogeous fungi which establish ectomycorrhizal symbiotic relationship with plant roots in order to accomplish their life cycle.

Truffle fruiting bodies are highly appreciated for their distinctive taste and aroma, which are partially derived from the presence of microorganisms. Indeed, the inner and the outer parts of truffles are colonized by a complex microbial community composed of bacteria, yeasts and occasionally of filamentous fungi, selected from soil microorganisms.

Although truffles are distributed worldwide, only a few species are edible. Among these, *Tuber mesentericum*, a black truffle, represent an important economic resource for Southern Italy, the major diffusion area.

The aim of this study has been to characterize quantitatively and qualitatively the microbiological profile of this particular truffle species by culture-dependent techniques.

Preliminary results showed the presence of an high amount of aerobic mesophilic microorganisms. Anaerobic bacteria and yeasts were also found; in particular, anaerobic species represent the prevalent microflora of truffle's gleba.

Identification tests highlighted the presence of microbial species common to other black and white truffles, some important for mycelia growth, fruit bodies development and for determining the typical organoleptic properties. However, identification data show also species that could represent an exclusive microbiological feature of *Tuber mesentericum.*

B52. Hydrogen oxidation coupled to sulfur reduction: an ancient respiratory pathway wide spread between deep-sea vent bacteria

F. Smedile[1], A. Grosche[1], S. Patwardhan[1], D. Giovannelli[1,2], B. Jelen[1], C. Vetriani[1]

[1]Department of Biochemistry and Microbiology and Department of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ 08901, USA; [2]Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo 152-8551, Japan

Among deep-sea ecosystems, hydrothermal vents represent "relic" environments that resemble the early Earth. The genomes of modern vent microorganisms carry both ancestral and recently acquired genes providing excellent models to reconstruct how microbial metabolism co-evolved with our planet.

Nautiliaceae (class *Epsilonproteobacteria*) and *Desulfurobacteraceae* (class *Aquificae*) are two key groups of bacteria in marine geothermal habitats. Although these bacteria are phylogenetically distant and colonize different temperature niches, they share key central metabolic characteristics: both fix CO_2 and obtain energy coupling the oxidation of H_2 to the reduction of elemental sulfur and/or nitrate. While nitrate reduction has been investigated, the sulfur reduction pathways have yet to be fully resolved.

Focusing on the genomes of *Cetia pacifica, Nautilia profundicola and Caminibacter mediatlanticus (Nautiliaceae),* as well as the genomes of *Thermovibrio ammonificans* strains HB-1 and HB-5*, Desulfurobacterium thermolithotrophum* and *Phorcysia thermohydrogeniphila (Desulfurobacteraceae)*, we reported the distribution and phylogenetic reconstruction of genes encoding for polysulfide reductase in the *Nautiliaceae,* and the sulfhydrogenase II and the NAD/FAD-dependent reductase in the *Desulfurobacteraceae*. Comparative genomic analyses

were used to formulate hypotheses on how H_2 oxidation can be coupled with sulfur reduction. Supported by experimental work, these data will help to understand the interconnection between carbon fixation and respiratory pathways in this chemosynthetic-driven environment.

B53. The occurrence of mycorrhizal fungi in *Betula aetnensis* Raf. roots: from ecological role to conservation strategies

S. Sofia[1], E. Badalamenti[1], V. Catania[2], T. La Mantia[1], P. Quatrini[2]

[1]Department of Agricultural, Food and Forest Sciences – SAAF. University of Palermo; [2]Department of Biological Chemical and Pharmaceutical Sciences and Technologies – STEBICEF. University of Palermo

Betula aetnensis Raf. is an endemic tree species of particular conservation value. It only thrives in the north-eastern slopes of Mount Etna (Sicily), from 1200 to 2100 m a.s.l. This pioneer plant is able to begin primary succession on nutrient-poor and water-limited soils (C = 0.17%; N = 0.05 ‰; P_2O_5 = 4.1 ppm), where beneficial mycorrhizal fungi (MF) may play a crucial role. In order to investigate MF role in *B. aetnensis*, plant roots from natural sites and nursery grown specimens were analyzed for both ectomycorrhizal and endomycorrhizal structures. Typical structures of both symbiosis were detected by root staining and morphological observations. Ectomycorrhizae (EM) were more abundant in natural sites (≈88%) than in nursery (≈77%). Clear morphological differences in the EM root tips suggest the occurrence of different fungal species. About 50% of roots had arbuscular structures, both in natural habitats and nursery. The community structure of EM and AM fungal symbionts was characterized by DGGE analysis.

Mycorrhizal dependence trials are in progress to elucidate the relative importance of ecto- and endomycorrhizal symbionts for this endemism, whose conservation could be strongly linked to mutualistic associations established at root level.

B54. Chemical and microbiological characterization of a complex contaminated industrial area

T. Stella[1], A. Franzetti[1], I. Pietrini[2], F. de Ferra[3], G. Carpani[3], M. Marchesi[2], L. Alberti[2], D. Antonelli[4]

[1]University of Milano-Bicocca, Department of Earth and Environmental Sciences, Piazza della Scienza, 1, 20126 Milano (Italy); [2]Politecnico di Milano, Department of Civil and Environmental Engineering, Piazza Leonardo da Vinci 32, 20133 Milano (Italy); [3]Research Center for Non-Conventional Energy – Istituto Eni Donegani Environmental Technologies, Via Maritano 26, 20097 San Donato Milanese, Milano (Italy); [4]Eni Syndial S.p.A., Piazza Boldrini, 20097, San Donato Milanese, Milano (Italy)

The management and the remediation of large contaminated areas with multiple pollutant sources represent a big challenge to site owners. Therefore, a detailed chemical and microbiological site characterization is crucial to assess, at first, the potential of intrinsic remediation of the contaminated area and, then the feasibility to enhance specific biodegradation processes.

This study aimed at gathering data from a complex contaminated industrial area to evaluate the presence of potential degraders of chlorinated compounds and, thus to design an effective bioremediation strategy.

Contaminated groundwater was collected from a restricted area of the site. Chemical analyses of chlorinated ethenes, 1,2-dichloroethane (1,2-DCA), benzene, toluene, xylenes, ethylbenzene and chlorinated benzenes were performed. The structure of the microbial community was determined by Illumina Next-Generation Sequencing, whereas its functional profile was assessed by quantitative PCR of key genes encoding for enzymes involved in specific metabolisms.

Vinyl chloride (VC) and 1,2-DCA were found in most of the water samples at high

concentration as well as tetra- and tri-chlorinated ethenes. Illumina sequencing data showed a high bacterial diversity probably due to contamination heterogeneity. However, species belonging to *Burkholderiales* and *Rhodocyclales* orders were predominant in 1,2-DCA and VC-contaminated groundwater, respectively. Catabolic genes enconding for reductive dehalogenases (*rdh* and *vcr)* were detected in all samples.

B55. Use of 'scotta' as fermentation medium for the production of valuable chemicals

G. Zaccagnini, S. Tabacchioni, L. Chiarini
ENEA- Department of Sustainable Territorial and Production Systems, via Aguillarese 301, 00123 Rome, Italy

Whey and 'scotta', the liquids remaining after cheese and ricotta making, respectively, are no longer disposed of as wastes, but are considered valuable by-products because both contain high-value substances, which can be successfully recovered by different filtration technologies, or can be used as fermentation medium for the production of various economically valuable chemicals.

So far, compared to whey, 'scotta' has been poorly investigated as fermentation broth for the production of biofuels and high-value chemicals. In the present work, we evaluated the feasibility, on laboratory scale, of using scotta as fermentation broth for the production of hydrogen and 2,3-butanediol. Single and two-stage fermentation processes using activated sludge and an isolate of *Paenibacillus polymyxa* as inocula were set up. In the two-stage process, scotta was inoculated with activated sludge to boost hydrogen production. The effluent was used as culture medium for the production of 2,3-butanediol by *P. polymyxa* during stage 2.

Results from single stage fermentations indicate that 'scotta' is a good medium for the production of both hydrogen and 2,3-butanediol. Unfortunately, two-stage processes, which should theoretically guarantee a greater reduction in COD than single stage processes, failed to reach high production rates of both hydrogen and 2,3-butanediol.

B56. Genetic manipulation of spiramycin-producing strain *Streptomyces ambofaciens* ATCC 23877 by φC31 Att/Int system-based vectors results in a significant reduction of antibiotic production.

A. Talà[1], F. Damiano[1], C. Peano[2], E. Pinatel[2], M. Testini[1], M. Calcagnile[1], G. E. De Benedetto[3], D. Fico[3], M. Di Salvo[1], G. Gallo[4], A. Sutera[4], G. Renzone[5], A. Scaloni[5], A. M. Puglia[4], P. Alifano[1]
[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy, [2]Institute of Biomedical Technologies, National Research Council, Segrate, Italy, [3] Laboratory of Analytical and Isotopic Mass Spectrometry, Department of Cultural Heritage, University of Salento, Lecce, Italy; [4]Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO), University of Palermo V.le delle Scienze, Palermo, Italy; [5]Proteomics and Mass Spectrometry Laboratory, ISPAAM, National Research Council, Naples, Italy

The genus *Streptomyces* produces about two-thirds of naturally occurring antibiotics and many other biologically active secondary metabolites.

The genetic manipulation of Streptomycetes is often labor and time intensive due to their large genome and complex development. Much progress has been made to develop gene transfer methods useful to construct antibiotic-producing strains with improved properties.

The φC31 Att/Int system is an integration system that has been widely used to produce stable recombinants in Actinomycetes and its integration site is an *attB* site located in a pirin-like gene (*pirA)* of the bacterial chromosome.

In this study we demonstrate that the integration of φC31 DNA in *Streptomyces ambofaciens* ATCC 23877 chromosome results in a significantly reduced spiramycin production and causes

some phenotypic changes.

With the aim to get insight into the molecular mechanisms underlying these effects we have analyzed the transcriptome and proteome of the wild type strain and a derivative strain harboring pTYM-18 vector plasmid integrated into the *pirA* gene.

Evidence is provided that inactivation of the *pirA* gene by plasmid integration leads to dramatic gene expression changes with marked effects on central carbon and energy metabolism, high sensitivity to oxidative injury and repression of polyketide antibiotic production.

B57. *Actinoallomurus*, a genus with the potential for novel antimicrobials and bioactive molecules

A. Tocchetti, M. Iorio[1], J. C. Santos Cruz[2], C. Brunati[2], S. Maffioli[1,2], M. Sosio[1,2], S. Donadio[1,2]
[1]Naicons - Viale Ortles 22/4 - 20139 Milano - Italy; [2]Ktedogen - Viale Ortles 22/4 - 20139 Milano - Italy

The emergence of a dramatic landscape of infectious diseases in these past few years clearly calls for a constant and significant investment on drug discovery, as the spread of antibiotic resistance keep occurring by giving origin to more multi-drug resistant strains.

One possibility to enhance the antibacterial arsenal is to look into what Nature has been using to solve the inevitable phenomenon of antibiotic resistance. The idea is to screen the myriad of microbial species collected from different natural environments, giving priority to those less exploited but phylogenetically related to known producers.

The metabolic potential of the promising genus *Actinoallomurus* was accessed by a screening program based on the dereplication process of a small selected portion of Naicons' library (ca. 200 *Actinoallomurus* microbial strains). Small-scale fermentations were followed by cell-based bioactivity assays of the broths extracts. Positive samples were then subjected to a combination of chemical analyses and queries within a proprietary natural product database to determine the novelty of active compounds. The data obtained show that *Actinoallomurus* can produce a broad range of chemical classes, from lantipeptides to new hyperhalogenated angucyclines or new aromatic polyethers. Few examples of these will be presented.

Furthermore, genomic analysis of some interesting *Actinoallomurus* strains revealed the presence of a diverse set of clusters for secondary metabolites, corroborating the biosynthetic potentiality of this genus.

In conclusion, both cultivation screening and genomic analysis of *Actinoallomurus* supported this approach for the discovery of new interesting bioactive metabolites.

B58. Antimicrobial activity in TiO_2-based hybrid nanoparticles

A. Zanfardino[1], G. Vitiello[2], G. Luciani[2], M. Varcamonti[1]
[1]Department of Biology, University of Naples "Federico II"; [2]Department of Chemical, Materials and Production Engineering, University of Naples "Federico II"

Recently there has been increasing interest in organic–inorganic hybrids as alternatives for obtaining new multifunctional nanomaterials. This strategy has been exploited to produce eco-friendly hybrid nanostructures with antimicrobial activity even under visible light conditions. We used the eumelanins, negatively charged polymers, hydrophobic pigments of high molecular weight, widely present in mammalians, plants and microorganisms. Many studies have recently shown that melanins interfere with numerous host defense mechanisms: they are highly immunogenic and have anti-inflammatory properties, protecting organisms against UV-radiation and can induce microbial lysis. Titanium dioxide (TiO2, titania) is an inexpensive, environmentally friendly, nontoxic and photo-stable material, as well as a high performance catalyst in photo-oxidative processes. In this work we propose hybrid systems containing eumelanin –TiO2 having

potential antimicrobial activity. We investigated on possible antimicrobial action mechanism on model strain *Escherichia coli*. We propose that the formation of oxygen radicals such as $OH^{\cdot}$ and/or H2O2 can have an effect on bacterial surviving; the antimicrobial activity remains inside the nanoparticles (no effect in the nanomaterials supernatants), and the contact between nanoparticles aggregates and bacterial cells induces membrane damage and microbial death.

These materials are very intriguing as a platform to produce antimicrobial formulations for biomedical and food packaging applications.

B59. Expression of "rdar morphotype" by *Escherichia coli* from marine environment

L. Di Sante[1], A. Pugnaloni[2], B. Citterio[3], F. Biavasco[1], C. Vignaroli[1]

[1]Department of Life and Environmental Sciences and [2]Department of Molecular and Clinical Sciences, Polytechnic University of Marche, Ancona, Italy; [3]Department of Biomolecular Sciences sect. Toxicological, Hygiene, and Environmental, Sciences, University of Urbino "Carlo Bo" - Urbino, Italy

The "red, dry, and rough (*rdar*) morphotype" has been described in *Enterobacteriaceae* as a survival strategy in stressful conditions, outside the host. It is a distinctive phenotype characterized by production of an extracellular matrix, mainly comprising cellulose and curli fimbriae, resulting in a multicellular community. This is the first report of the ability of *Escherichia* cryptic clade isolates from the marine environment to express the *rdar* morphotype in response to low temperature, nutrient-poor conditions, and pyrimidine availability. All cryptic *Escherichia* clade strains (n=28) showed *rdar* colonies after 72h incubation on Congo Red agar, albeit with varied patterns, and were cellulose producers, forming fluorescent colonies on Calcofluor agar. On TEM examination they demonstrated morphological changes. In particular, bacterial cells grown at 24°C in carbon-poor medium were encased in a fibrous matrix unlike those grown in rich medium at 37°C. Strain preincubation in 0.25 mM uracil and growth at low temperature (24 and 10°C) enhanced matrix component biosynthesis, as demonstrated by the significant increase (up to 82% of isolates at 10°C) in strong biofilm producers. *rdar* morphotype expression by cryptic clades could account for their adaptation and persistence in natural environments.

B60. Gene redundancy for *o*-xylene degradation in *Rhodococcus opacus* R7

J. Zampolli[1], A. Di Canito[1], E. Collina[2], P. Di Gennaro[1]

[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy; [2]Department of Environmental Sciences, University of Milano-Bicocca, Milano, Italy

Rhodococcus opacus R7 has a remarkable ability to degrade a wide range of xenobiotics. It was isolated from a PAH-contaminated site and it is known for its ability to grow on *o*-xylene as sole carbon and energy source. The main objective of this work was to identify the R7 enzymes involved in the *o*-xylene degradation and to characterize the molecular mechanisms. Previous studies on R7 showed that 2,3-dimethylphenol was the first intermediate of the *o*-xylene metabolism, then converted into the 3,4-dimethylcatechol. This pathway has never been described before in *Rhodococcus* strains and it is also very uncommon in other bacteria. A preliminary annotation allowed to identify two main enzyme classes putatively involved in the*o*-xylene pathway: monooxygenases/hydroxylases and dioxygenases. Genes involved in alkylbenzene-degradation were identified in *R*. sp DK17 and similar sequences were found in R7 genome (*akb* gene cluster).

Phylogenetic analyses allowed to identify several monooxygenases/hydroxylases putatively involved in *o*-xylene degradation pathway, such as a BMM and several phenol hydroxylases.

The involvement of *akb* genes and monooxygenases/hydroxylases in *o*-xylene metabolism was investigated by RT-PCR, random mutant analysis and protein predictive models. Results

indicated that *akb* genes were induced by *o*-xylene, but also other monooxygenases/hydroxylases could be involved in *o*-xylene degradation.

Session C - Interactions between microbes/viruses and their hosts

[Continue from Oral session C. Click here to view the Poster abstracts from C1 to C10]

C11. The meningococcal HrpA protein: a multitasking secreted protein involved in host-pathogen interaction

P. Alifano, G. Pizzolante, A. Paiano, A. Talà, L. Cogli, G. Alfarano, M. Di Salvo, C. Bucci
Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy

In Neisseria meningitidis the HrpA /HrpB two-partner secretion (TPS) system has been implicated in many diverse functions that can hardly be traced back to a single mechanism. It has been involved not only in meningococcal competition as a toxin-antitoxin fratricide system, but also in adherence to epithelial cells, bacterial escape from the internalization vacuole into the cytoplasm and exit from infected epithelial cells, and biofilm formation on human bronchial epithelial cells. We hypothesized that the diverse functions of the HrpA/HrpB TPS system could be attributed to distinct domains of secreted HrpA. Here we show that, while the amino-terminal region of HrpA encompasses an established adhesin domain, the carboxy-terminal region, which contains a haemolysin / haemoagglutinin domain, acts as a manganese-dependent cell lysin. To elucidate the enigmatic function of the central region of HrpA, which does not contain any established functional domain, a yeast two-hybrid screening was used to identify putative HrpA-interacting host proteins. Results indicated that this HrpA region could interact with host proteins implicated in diverse processes including membrane trafficking, cytoskeleton organization, protein turnover, extracellular matrix assembly and blood coagulation. In particular, the interaction with human fibrinogen was here validated by Ni-NTA pull-down assays.

C12. *Lactobacillus gasseri* SF1183 protects the intestinal epithelium and prevents colitis symptoms *in vivo*

B. Di Luccia[1], A. Mazzoli[1], R. Cancelliere[1], R. Crescenzo[1], I. Ferrandino[1], A. Monaco [1], A. Bucci[2], G. Naclerio[2], S. Iossa[1], E. Ricca[1], L. Baccigalupi[1]
[1]Department of Biology, Federico II University of Naples, Italy; [2] Department of Biosciences and Territory, University of Molise, Pesche (Is), Italy

Lactobacillus gasseri SF1183 belongs to a subpopulation of human intestinal bacteria tightly associated to the ileal epithelium (Res. Microbiol. 160: 817-823, 2009). Previous *in vitro* studies with human intestinal cells indicated that SF1183 secretes molecule(s) that interferes with the cell cycle (PLoS ONE 8(7): e69102, 2013). Here we used a murine model of DSS(Dextran Sulfate Sodium)-induced colitis to test if SF1183 has a protective role *in vivo*.

Metabolic and immuno-hystochemical data indicated that SF1183-treatment strongly reduces inflammation and intestinal epithelial damages caused by DSS. A DGGE analysis showed that the DSS or the DSS/SF1183 treatments did not cause a strong alteration of the gut microbiota. A deeper analysis of the faecal bacterial population with a metagenomic approach showed that members of the *Robinsoniella* and *Ruminiclostridium* genera increased in the gut of DSS-treated animals respect to control and newly reduced following the treatment with *L. gasseri*. These results induced us to hypothesize a correlation between the relative abundance of members of these genera and the DSS-induced inflammation and to propose *L. gasseri* SF1183 as a new probiotic strain able to counteract the increase of bacteria linked to the development of intestinal inflammation and potentially useful for the treatment of intestinal diseases.

C13. Phage therapy against *Pseudomonas aeruginosa* infections in cystic fibrosis patients

M. Cafora[1], F. Forti[1], D. Roach[2], M. Pasini[1], F. Briani[1], D. Horner[1], L. Debarbieux[2], D. Ghisotti[1]
[1]Dep. of Biosciences, University of Milan, Italy; [2]Institut Pasteur, Paris, France

Pseudomonas aeruginosa is the most common pathogen found in the lung of cystic fibrosis patients (CF). The quality of life of CF patients largely depends on the success or failure of antibiotic treatment. Now, the alarming diffusion of isolates of *P. aeruginosa* multi-resistant to the antibiotics currently in use makes urgently need to develop alternative therapies. Phage therapy can be considered as a therapeutic alternative or a complementary treatment to antibiotics in curing lung infections in CF patients.

P. aeruginosa phages were isolated from sewage samples and characterized for their host range on a panel of 55 *P. aeruginosa* clinical strains isolated from CF patients. Six phages, with a different host range, were mixed in a cocktail and tested *in vitro* for their ability to kill *P. aeruginosa* and to destroy a 48h preformed *P. aeruginosa* biofilm. Electron microscopy indicated that 2 are Podoviridae and 4 Myoviridae. Genome analysis of the sequences indicated the absence of any undesirable gene, suggesting that the phages could be used without problems for human therapy. *In vivo* infection performed in two model systems, *Galleria mellonella* larvae and mouse, demonstrated a therapeutic effect of the phage cocktail against *P. aeruginosa* infections.

C14. Inhibition of *Staphylococcus aureus* biofilm on medical-grade silicone by different biosurfactant-based coating strategies

C. Ceresa[1], F. Ruini[5], F. Tessarolo[2,3], D. Maniglio[2], F. Piccoli[4], S. Eccheli[2], V. Chiono[5], I. Caola[4], G. Nollo[2,3], M. Rinaldi[1], G. Allegrone[1], L. Fracchia[1]
[1]Department of Pharmaceutical Sciences, Università del Piemonte Orientale "A. Avogadro", Novara, Italy; [2]Healthcare Research and Innovation Program (IRCS-FBK-PAT), Bruno Kessler Foundation, Trento, Italy; [3]Department of Industrial Engineering and BIOtech Center, University of Trento, Trento, Italy; [4]Department of Medicine Laboratory, Azienda Provinciale per i Servizi Sanitari di Trento, Trento, Italy; [5]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy

Microbial biosurfactants have recently emerged as a potential new generation of anti-adhesive and anti-biofilm agents for medical device coatings with enhanced biocompatibility.

The ability of the lipopeptide AC7 and of the rhamnolipid R89 (AC7BS, R89BS) to inhibit *Staphylococcus aureus* biofilm formation on silicone disks was evaluated by means of CV staining, MTT assays and scanning electron microscopy at different time-points. The biomaterial was functionalized following three different strategies: BS physical absorption, plasma treatment followed by BS absorption, plasma treatment followed by BS covalent binding.

All coating strategies promoted a significant inhibition of biofilm formation but treatments with R89BS resulted to be more effective. In particular, on R89BS covalent binding disks, biomass, metabolic activity and surface coverage of *S. aureus* biofilms were respectively reduced up to 98%, 93% and 80% at the last time-point (72h). Finally, an antibacterial action for R89BS was observed (MIC=0.06 mg/ml, SMIC=0.12 mg/ml), but not for AC7BS. No cytotoxic effect on eukaryotic cells was detected at concentrations up to 0.2 mg/ml and 0.5 mg/ml for R89BS and AC7BS respectively. In conclusion, plasma treatment prior to BS covalent binding seems to be a promising coating strategy for BSs inhibition of bacterial biofilm formation on medical-grade silicone.

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C15. Comparison of oral and intestinal microbiota and investigation of *Fusobacterium nucleatum* abundance in patients with colorectal cancer *vs* healthy controls: a pilot study

C. Fagorzi[1], G. Bacci[1], C. Chiellini[1], E. Russo[2], A. Taddei[4], R. Borrelli[2], M. Novella Ringressi[4], P. Bechi[4], A. Mengoni[1], A. Amedei[2, 3], R. Fani[1]

[1]Department of Biology, University of Florence, Sesto Fiorentino (Florence), Italy; [2]Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy; [3]Neuromusculoskeletal Department (Interdisciplinary Internal Medicine), Azienda Ospedaliera Universitaria Careggi (AOUC) Florence, Italy; [4] Department of Surgery and Translational Medicine, University of Florence, Florence, Italy

Human microbiota from three different compartments (saliva, feces, and cancer tissue) of 10 patients with colorectal cancer (CRC) *vs* 10 healthy controls (saliva and faeces) was investigated through NGS analysis. At the same time, *Fusobacterium nucleatum* abundance was evaluated through qPCR.

Healthy controls showed a different microbiota assemblage than CRC patients with different levels of *F. nucleatum*.

Firmicutes (39.18%), *Bacteroidetes* (30.36%), and *Proteobacteria* (10.65%) were the more abundant bacterial phyla detected in all samples. The enrichment of *Bacteriodetes* within fecal samples of CRC patients was observed, while *Firmicutes* were over-represented in the fecal samples of healthy controls. *F. nucleatum* in CRC patients was more abundant in saliva than in fecal samples.

Data obtained revealed both a different bacterial communities' composition between healthy and CRC subject and, at the same time, a differentiation in *F. nucleatum* abundance between the two groups of patients. Moreover, differences were observed among the three different analysed compartments too.

Further investigation will highlight the possible correlation between CRC and bacterial community, as well as the correlation between the presence of *F. nucleatum* and the clinical course of CRC patients.

C16. Role of CIP protein in GBS interaction with the complement system

S. Giussani[1,2], G. Pietrocola[2], P. Speziale[2], M. Fabbrini[1], I. Margarit[1]

[1]GSK Vaccines, Siena; [2]University of Pavia, Department of Molecular Medicine

Group B *Streptococcus* (GBS) is a Gram positive commensal bacterium that colonizes the human gastrointestinal and genitourinary tracts. Neonates and elderly people can become infected by this microorganism, and GBS is a primary cause of infant invasive disease that can result in sepsis, pneumonia and/or meningitis. GBS is characterized by a number of virulence factors that protect it from the host immune system, and in particular from complement deposition. Besides the capsular polysaccharide that covers the bacteria, thus hiding it from the innate immune system, other factors are able to protect GBS from complement deposition. A GBS protein, named CIP (Complement Inhibitory Protein) was proven to bind C4 and inhibit complement deposition via the lectin and classical pathways.

In this study we focused on the activity of CIP protein at the interface between the innate and acquired immunity, in order to describe a new mechanism by which GBS is able to evade the host immune system.

C17. Assessing the suitability of quorum sensing inhibition in cystic fibrosis therapy

G. Giallonardi[1], V. Lancia[1], E. Fiscarelli[2], F. Ungaro[3], F. Imperi[4], P. Visca[1], G. Rampioni[1], L. Leoni[1]

[1]Department of Science, University Roma Tre, Rome, Italy; [2]Bambino Gesù Hospital, Rome, Italy; [3]Department of Pharmacy, University of Naples Federico II, Naples, Italy; [4]Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Rome, Italy.

The *Pseudomonas aeruginosa* quorum sensing (QS) system is considered a good target for anti-virulence drugs. However, the suitability of anti-QS therapy for the treatment of *P. aeruginosa* infections in cystic fibrosis (CF) patients is under debate. Indeed, *P. aeruginosa* strains producing low levels of virulence factors or QS-defective are frequently isolated from CF patients.

Here, a collection of 100 *P. aeruginosa* CF isolates has been tested to evaluate the suitability of anti-QS therapy for CF lung infection. In particular these strains have been characterized for: *i)* antibiotic susceptibility; *ii)* QS signal molecules production; *iii)* susceptibility to niclosamide (NCL), a strong QS and virulence inhibitor targeting the *N*-3-oxododecanoyl-homoserine lactone ($3OC_{12}$-HSL)-dependent QS system in the model strain PA14. Results have shown that 69% of the strains produce $3OC_{12}$-HSL and could be in principle susceptible to NCL. Nevertheless, NCL-mediated inhibition of $3OC_{12}$-HSL and virulence factors production is overall low and highly variable. Statistical analysis has shown no significant correlation between drug resistance, $3OC_{12}$-HSL production and NCL susceptibility.

Overall, this study highlights that the effect of anti-QS drugs is strain-dependent, indicating that each new molecule should be tested on a large collection of clinical strains before further studies leading to translation to CF therapy.

C18. The US21 protein of human Cytomegalovirus is a viral ion channel that regulates intracellular Ca^{2+} homeostasis and apoptosis

A. Luganini, F. Galati, F. Galliano, G. Di Nardo, A. Fiorio Pla, G. Gribaudo

Department of Life Sciences and Systems Biology, University of Turin

The Human Cytomegalovirus (HCMV) US12 gene family comprises a set of ten contiguous genes (US12-US21), with poorly characterized function. Whilst inactivation of individual US12 members does not affect viral replication in fibroblasts, we observed that disruption of US16, US18, US20, and US21 genes in the TR strains of HCMV prevented viral growth in endothelial cells. Here, we investigated the expression pattern of US21 protein and its ability to regulate intracellular Ca^{2+} homeostasis and apoptosis. We observed that pUS21 is a 7TMD protein which is expressed with a late kinetics and accumulates both in Golgi-derived vesicles and within ER-derived peripheral structures. Deletion or inactivation of US21 ORF determined a significant growth defect even in fibroblasts due to a reduced viral gene expression. Among the US12 ORFs, US21 shows the highest level of amino acids similarity with two cellular TMBIM proteins, the BI-1 and GAAP, which are both involved in regulation of Ca^{2+} homeostasis and apoptosis. Relevant to this, Ca^{2+} imaging experiments and apoptosis assays then revealed that pUS21 lowered the Ca^{2+} content of intracellular stores and inhibited the activation of caspases 3 and 7 upon staurosporine stimulation. Thus, pUS21 is suggested to counteract apoptosis and promote survival of infected cells.

C19. ROS activation and innate immunity in HSV-1 infection

F. Marino-Merlo[1], E. Papaianni[2,3], S. Grelli[4], S. Pedatella[5], M. De Nisco[5], C. Borner[6,7], A. Mastino[2,3]

[1]IRCCS Centro Neurolesi "Bonino-Pulejo", Messina; [2]Dipartimento di Scienze Chimiche,

Biologiche, Farmaceutiche ed Ambientali, Università di Messina, Messina; [3] Istituto di Farmacologia Traslazionale, CNR, Roma; [4]Dipartimento di Medicina Sperimentale e Chirurgia, Università di Roma "Tor Vergata", Roma; [5]Dipartimento di Scienze Chimiche, Università di Napoli Federico II; [6] Institute of Molecular Medicine and Cell Research; [7]Spemann Graduate School of Biology and Medicine (SGBM), Albert Ludwigs University of Freiburg, Freiburg, Germania

Primary infections and recurrences from herpes simplex virus type 1 (HSV-1) are usually associated with mild or no symptoms. However, in rare cases, HSV-1 infection causes severe complications at central nervous system level, mainly associated with immunological disorders. HSV-1 can infect a wide range of cell types, including adaptive and innate immunity cells but, normally, completes a replication cycle only in epithelial or neural cells. We have recently shown that the transcription factor NF-kB can act as cell permissiveness regulator of HSV-1 infection in monocytic cells. To better define mechanisms involved in this phenomenon and, particularly, the possible involvement of ROS, wild type U937 cells or U937 cells stably transfected with a DN IkB-mutant and non-conventional selenium-containing anti-oxidants, were utilized. The main results can be summarized as follows. The NF-kB-mediated restriction of HSV-1 infection in monocytic cells is not due to increased levels of apoptosis or to changes in the expression of a panel of known innate immunity mediators. HSV-1 infection induces an immediate ROS production in monocytic cells that can be efficiently countered by selenium antioxidants. Treatment with such compounds determines increased levels of HSV-1 replication in monocytic cells and a reduction of NF-kB activation induced by HSV-1.

C20. Endophytes from medicinal plants' seeds: exploring new reservoirs of bioactive molecules

E. Miceli[1], C. Chiellini[1], C. Fagorzi[1], G. Bacci[1], T. Faddetta[2], G. Gallo[2], M. Cardinale[3], R. Fani[1], A. M. Puglia[2]

[1]Dep. Biology, University of Florence (Italy); [2]STEBICEF Department, University of Palermo (Italy); [3]Institute of Applied Microbiology, Justus–Liebig–University Giessen (Germany)

Plant-associated microorganisms have recently gained more attention for their influence on plant health and biotechnological relevance. Bioactive molecules have been already isolated from plant-associated bacteria. Seed-borne bacterial endophytes have not been much explored though. Such endophytes are particularly important**,** since they can influence germination and be transmitted from generation to generation. Interestingly, seed endophytes from medicinal plants could influence the production of molecules with therapeutic properties.

Bacterial endophytic strains were extracted from surface-sterilized seeds of the medicinal plant *Echinacea purpurea* and their 16S rRNA genes were sequenced. The analysis revealed the predominance of *Paenibacillus* (55.8%) and *Pantoea* (39.5%) genera among isolates. Fluorescence *in situ* hybridization and confocal laser scanning microscopy (FISH–CLSM) revealed a consistent colonization of *Echinacea* seeds by microbial endophytes as well as epiphytes. Resistance to antibiotics revealed that Ciprofloxacin and Rifampicin are the most effective antibiotics, even at minimum concentrations (respectively 0.5 and 5 mg/l). Isolates were less sensitive to Tetracycline and Streptomycin instead.

Further investigation of *Echinacea* plants seed-borne endophytes may elucidate how such endophytes influence the therapeutic properties of the plant, and pave the way for the discovery of biotechnologically relevant compounds.

C21. Transcriptional profiling of the *Streptococcus pneumoniae* phage SpSL1 during the lytic and lysogenic cycle

R. Haigh, L. Furi, L. Crawford, A. Cremers, M. De Jonge, G. Rangel Pineros, M. Clokie, M. Oggioni

We have characterised the gene expression during lysogeny and the lytic cycle of the pneumococcal temperate bacteriophage SpSL1. Phage SpSL1 is a siphovirus with a linear dsDNA genome of 33,756 bp (GenBank NC_027396.1). The lytic cycle of the phage was defined by a one-step growth curve to be 200 minutes with an absorption time of 10 minutes and a burst size of 10. Gene expression by RNAseq was performed at 5, 10, 15, 30, 50 and 90 minutes and during the exponential phase of a strain containing an integrated copy of the prophage. Gene expression data define four transcriptional units. Three of which are expressed during the lytic cycle and include an early expression of replication associated genes, intermediate and late expression of packaging and morphogenesis associated genes and a late expression of nucleases. The lysogeny gene cluster is repressed during the lytic cycle, but well expressed when the phage is integrated in the bacterial genome. The detection of gene expression during lysogeny of the packaging and morphogenesis genes, including the tail fibre gene pblB, is of difficult interpretation as it cannot be defined if this expression derives from integrated phage or from a subpopulation of phages entering a lytic cycle. With respect to host gene expression during the lytic cycle only the three operons of the anaerobic ribonucleoside-triphosphate reductase regulon are upregulated, in accordance with their involvement in the anaerobic de novo synthesis of deoxyribonucleotides necessary for DNA production by SpSL1.

C22. Comparison of the vaginal microbiome and metabolome of healthy, bacterial vaginosis and *Chlamydia trachomatis*-infected women

C. Parolin[1], L. Laghi[2], C. Foschi[3], B. Giordani[1], V. Gaspari[3], A. D'Antuono[3], A. Marangoni[3], B. Vitali[1]

[1]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; [2]Department of Agro-Food Science and Technology, Centre of Foodomics, University of Bologna, Cesena, Italy; [3]Department of Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy

The human vaginal niche is generally dominated by *Lactobacillus* spp. which exert a protective role toward microbial dysbiosis and pathogen overgrowth through different mechanisms, including the secretion of antimicrobial compounds and the competition for substrates and adhesion sites. Alterations of the vaginal microbiota are found in bacterial vaginosis (BV). Lactobacilli have also been hypothesized to protect women from sexually transmitted diseases, including *Chlamydia trachomatis* (CT) infection. Both BV and CT infection can lead to severe sequelae, such as preterm delivery and tubal infertility.

In this study we analysed the composition of the vaginal microbiota and the metabolic profiles of healthy, BV-affected and CT-infected women. The microbial signature was determined by using a microarray-based tool (VaginArray) targeting the most representative vaginal bacterial groups, implemented with a qPCR for *Gardnerella vaginalis*. The vaginal metabolic profiles were assessed by ^{1}H-NMR.

The microbial signature of BV-affected women is clearly different from that of healthy subjects, CT-infected women are characterized by a microbiota similar to the healthy condition. The metabolomic approach evidenced that BV-samples are characterized by significant variations in organic acids, aminoacids, short chain fatty acids concentrations. CT-infected women metabolome resembles that of healthy subjects, nevertheless significant variations in biogene amines content were underlined.

C23. Role of the gut microbiota in the progression of sporadic colorectal adenoma-carcinoma sequence

G. Mori[1], B. S. Orena[1], S. Rampelli[2], C. Rengucci[3], G. DeMaio[3], F. Armanini[4], G. Barbieri[1], A. Passardi[5], A. Casadei Gardini[5], G. L. Frassineti[5], S. Gaiarsa[1], A. Albertini[1], G. N. Ranzani[1], N. Segata[4], P. Brigidi[2], D. Calistri[3], M. R. Pasca[1]

[1]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy; [2]Unit of Microbial Ecology of Health, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; [3]Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola (FC), Italy; [4]Centre for Integrative Biology (CIBIO), University of Trento, Trento, Italy; [5]Department of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola (FC), Italy.

Colorectal cancer (CRC) development is a multistep process by which healthy gut epithelium slowly develops into adenomas, which in turn progress into malignant carcinomas. The adenoma-carcinoma sequence suggests that specific mutations occur at specific stages, affecting genes and pathways involved in regulation of cell growth and differentiation. About 90% of CRC cases develop sporadically from a benign adenomatous polyp. The formation of intestinal preneoplastic lesions and CRC are associated with several risk factors, where microbiota dysbiosis is the most significant one.

In fact, gut microbiota, through several mechanisms, affects genomic instability and epithelial cell proliferation, eventually inducing CRC carcinogenesis.

In our study, the faecal microbiota of the adenoma-carcinoma sequence has been analysed and compared in all the different stages under investigation (healthy, hyperplastic polyps, low-risk adenomas, high-risk adenomas, adenocarcinomas). Moreover, a group of people with adenocarcinomas that underwent chemotherapy and/or radiotherapy has been taken in consideration in order to analyse how the treatments could influence the composition of gut microbiota.

In conclusion, we identified specific microbial associations during neoplastic progression in sporadic CRC that will be of help in the investigation of the gut microorganisms that predispose or protect a host from CRC development.

C24. HIV-1 recNEF$_{SF2}$ protein increases the exosome production in THP-1 differentiated with PMA

F. Giannessi[1], A. Aiello[1], Z. A. Percario[1], K. Fecchi[2], M. Sargiacomo[2], E. Affabris[1]

[1]Dept.of Science, University Roma Tre, Rome, Italy; [2]Dept. of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

The protein Nef of Human Immunodeficiency Virus (HIV) is a virulence factor that acts as an adaptor molecule inside the infected cell. It regulates viral production and induces immunoevasion. Importantly, it can also be transferred to uninfected cells through exosomes (Percario et al., 2015).

In this study we examined if the recombinant myristoylated HIV-1 Nef$_{SF2}$ was able to induce the production of exosomes in THP-1 cells differentiated with PMA. To this aim, we used commercially available BODIPY®-C16 fatty acid to label the cells. Upon addition to the cell culture, BODIPY-C16 fatty acid was rapidly incorporated and ultimately produced fluorescent exosomes and microvesicles that we examined and quantified as reported by Sargiacomo and colleagues (Coscia et al., Methods Mol Biol, 2016). We observed that recNef$_{SF2}$ increases by about twice the production of exosomes but not that of microvesicles and Nef is also incorporated into the exosome fraction. Further studies are in progress to analyse if the exosomes containing

recNef$_{SF2}$ are able to induce a pro-inflammatory response in differentiated THP-1 as it has been reported for the viral protein expressed endogenously during the HIV infection.

C25. Biodiversity of "non-model" *Rickettsiales* and their association with ciliates and other aquatic organisms: state of the art and future trends

G. Petroni
Department of Biology, University of Pisa, Via Luca Ghini 13, 56126, Pisa, Italy

Rickettsiales are an order of obligate intracellular bacteria inhabiting eukaryotes (i.e., they cannot proliferate in host cell-free media), belonging to the *Alphaproteobacteria* class. For a long time, knowledge on *Rickettsiales* was restricted almost exclusively to its pathogenic and medically relevant members belonging to the genera *Rickettsia*, *Anaplasma*, *Ehrlichia*, and to reproductive manipulators in insects of the genus *Wolbachia*. In the past few decades, thanks to molecular and phylogenetic data, the view on the evolutionary relationships within *Rickettsiales* significantly changed. The description of novel taxa of bacterial symbionts boosted with the turning of the century. Molecular biology techniques integrated in the full cycle rRNA approach represented the technical basis of this renaissance of study on obligatory intracellular bacteria. This approach brought our group to describe more than 10 new *Rickettsiales* genera in ciliates and, recently, to propose a taxonomic revision of the Order. In this context, a short overview of recent literature on the topic integrated with ongoing results from our group including genomic ones will be provided.

C26. Microbial interactions in *Tetraselmis suecica* phycosphere

E. Piampiano[1], F. Pini[1], F. Decorosi[1], R. Pastorelli[2], M.R. Tredici[1], N. Biondi[1], L. Giovannetti[1], C. Viti[1]
[1]Dipartimento di Scienze delle Produzioni Agro-alimentari e dell'Ambiente, Università di Firenze, Piazzale delle Cascine 24, 50144, Firenze, Italia; [2]Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per l'Agrobiologia e la Pedologia (CRA-ABP) via di Lanciola, 12, 50125 Firenze, Italia

The zone that surrounds microalgal cells is defined as phycosphere. Algae-bacteria interactions in the phycosphere can be neutral, positive or negative for algal growth and can vary depending on the main growth limiting factors. Besides, the same bacteria can either stimulate or inhibit algal growth. The identification of the bacterial species associated with microalgae and their influence on microalgal metabolism, is a key point. *Tetraselmis suecica* is a green marine microalga used in biotechnology, mainly as feed in aquaculture; moreover it is gaining importance for biofuel production. In this work, the bacterial community associated with cultures of *T. suecica* (strain F&M-M33) grown in different conditions (different seasons, conditions, and culture systems) were analyzed both by molecular and traditional techniques. A denaturing gradient electrophoresis gel (DGGE) approach was used to investigate the diversity of bacterial communities, analyzing three regions of 16S rDNA: bacterial diversity was better described using the V3-V4 region. Thanks to these results we performed an NGS metagenetic analysis. At the same time, 300 bacterial strains were isolated on Marine Agar medium, from *T. suecica* F&M-M33. The isolated bacteria were characterized for siderophore and indole-3-acetic acid production, to select strains able to actively stimulate microalgal growth.

C27. TFF1 regulation from acute to chronic inflammatory process during *Helicobacter* infection

M. Vllahu, R. Esposito, S. Morello, D. Eletto, A. Tosco, A. Porta
Department of Pharmacy, University of Salerno, Fisciano (SA), Italy

The human pathogen *Helicobacter pylori* causes a long-lasting chronic inflammation of gastric mucosa that is a strong risk factor for development of gastric cancer. TFF1 is a small secreted peptide of gastric mucosa which has an important role as protective agent against different gastric insults. Its role in *Helicobacter* infection is still not clear: TFF1 expression is induced in cellular models, but down-regulated in chronic infected patients, suggesting different mechanisms of regulation.

Different gastric cell models (which express or not TFF1) and C57BL/6 mice have been used to characterize the early phase of *Helicobacter* infection at different time points, trying to distinguish between acute to chronic phase of infection.

TFF1 expression level is inversely associated with inflammation status in all infected cells as well as in the early phase of infected mice. We demonstrated for the first time an up-regulation of TFF1 mRNA in the acute *Helicobacter* infection in *in vivo* models, which allows cells to reduce the bacterial damage, while it is gradually silenced during the chronic phase of infection, contributing to malignant phenotype development.

Our experiments suggest that TFF1 is differently regulated throughout the infection-associated inflammatory process from acute to chronic phase, playing an essential role as gastric protective agent in the acute *H. pylori* infection.

A deeper investigation of the molecular mechanisms underlying the colonization and survival of bacteria will help not only to improve current therapies but also to prevent the gastrointestinal disease.

C28. Characterization of the gut microbiota and related immune response in Multiple Sclerosis patients undergoing autologous hematopoietic stem cell transplantation

E. Russo[1], V. Di Pilato[1], F. Ricci[1], R. Borrelli[1], E. Niccolai[1], G. Nannini[1], A. Repice[2], C. Innocenti[2], GM. Rossolini[1], R. Saccardi[3], L. Massacesi[2], A. Amedei[1]
[1]Clinical and Experimental medicine department , University of Florence, Florence, Italy; [2]Neurological Department, University of Florence, Florence, Italy; [3]Hematology Department, University of Florence, Florence

Multiple sclerosis (MS) is the most common autoimmune disorder affecting the central nervous system. Recent animal studies showed that the interplay of the host immune system and the gut microbiota (and its metabolites as Short Chain Fatty Acids – SCFA-) can have a key role in the progression of demyelinating disease. Moreover, microbiota modulation can lead to either exacerbation or improvement of symptoms. Intense immunosuppression followed by autologous hematopoietic stem cell transplantation (aHSCT), aimed to restore the hemato-lymphopoietic system, reset the immune system and induces a prolonged tolerance toward self-antigens.

We propose a multifactorial approach:

- AIM 1: Metagenomic characterization of gut microbiota and SCFA from fecal specimens of aHSCT- MS *vs* Alemtuzumab –MS patients.
- AIM 2: Evaluation fecal/serum cytokines by Luminex technology
- AIM 3: Correlation of quality of the immune response and the composition of the gut microbiota (and SCFAs) with the clinical parameters of the MS patients.

Our preliminary results might suggest both a different bacterial structure and altered cytokine profile between aHSCT-MS patients *vs* standard therapy controls and patients with different diseases (such as colorectal cancer)

Our findings may provide the opportunity to new therapeutic or preventive strategies based on microbiota-targeted interventions aimed at restoring the intestinal ecosystem.

C29. A vancomycin/colistin-polypeptide conjugate (PAA-VC) for the treatment of biofilm associated infections

M. G. Cusimano, N. Mauro, G. Giammona, G. Cavallaro, D. Schillaci
Department of "Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche" (STEBICEF), University of Palermo, Via Archirafi, 32 90123 Palermo, Italy

Few therapeutic options simultaneously tackle *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two of the most relevant antibiotic-resistant pathogens. Moreover, the growth as biofilms is prevalent in chronic infections and their harmful effect is well-known.

In this study, a new molecular entity (PAA-VC), a synthetic polypeptidic structure, named poly(argilylaspartamide)aspartic acid (PAA), with vancomycin and colistin as side chains acting against multiple microbial targets, has been tested against planktonic and established biofilms of *S.aureus* ATCC 25923 and *P.aeruginosa* ATCC 15442 and its activity compared with the free single antibiotics.

PAA-VC conjugate is more active against planktonic strains than free drugs comparatively tested when we confront the MICs in terms of μM. The IC_{50} values of PAA-VC against established biofilms of *S.aureus* and *P.aeruginosa* have been respectively 4.9 μg/ml (0.07 μM) and 42.8 μg/ml (0.64 μM). PAA-VC is much more active than free antibiotics whose values are 100 μg/ml (69.0 μM) for vancomycin against staphylococcal biofilm and 303 μg/ml (252.57 μM) for colistin against pseudomonal biofilm.

We believe that the *in vitro* presented data, the biocompatibility, and other good features as the stability, an excellent bioavailability and a possible use at high-dosage, make PAA-VC a promising candidate for developing a broad spectrum antimicrobial macromolecule.

C30. Isolation and characterisation of vB_Kpn_F48, a novel bacteriophage for *Klebsiella pneumoniae*

N. Ciacci[1,2], M. M. D'Andrea[2,3], P. Marmo[1], E. Demattè[2], E. Vannuccini[4], P. Lupetti[4], GM. Rossolini[2,3,5,6], M. C. Thaller[1]
[1]Department of Biology, University of Rome "Tor Vergata", Rome, Italy; [2]Department of Medical Biotechnologies, University of Siena, Siena, Italy; [3]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [4]Department of Life Science, University of Siena, Siena, Italy; [5]Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy; [6]Don Carlo Gnocchi Foundation, Florence, Italy

Klebsiella pneumoniae carbapenemases (KPC)-producing *Klebsiella pneumoniae* represents a major clinical problem given the lack of available treatment options. In this work we have characterized one bacteriophage, named vB_Kpn_F48, with a lytic activity against KPC-positive *Klebsiella pneumoniae* (KPC-Kp) of Sequence Type (ST) 101. Transmission electron microscopy (TEM), burst size, host range and sensitivity to temperature and pH were used to characterize phage vB_Kpn_F48. Molecular characterization through High Throughput DNA Sequencing and direct sequencing by Sanger method was employed to define the phage genomic sequence, while genome annotation was performed by RAST analysis. Bacteriophage vB_Kpn_F48 was classified as a member of *Myoviridae*, order *Caudovirales*, on the basis of TEM analysis. Physiological characterization demonstrated that vB_Kpn_F48 is highly stable to both temperature and pH variations. A total of 283 possible coding regions have been detected by RAST analysis and a hypothetical function was assigned to 120 (42.4%). The obtained results suggest that vB_Kpn_F48 could represent a valid alternative therapeutic agent. In addition, the phage exhibits a number of properties indicative of a potential utility in phage therapy cocktails.

LIST OF PARTICIPANTS

A

Elisabetta Affabris
Dipartimento di Scienze
Università Roma Tre
@: elisabetta.affabris@uniroma3.it

Alessandra Aiello
Dipartimento di Scienze
Università Roma Tre
@: alessandra.aiello@uniroma3.it

Rosa Alduina
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: valeria.alduina@unipa.it

Pietro Alifano
Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali
Università del Salento
@: pietro.alifano@unisalento.it

Francesco Ardizzone
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: francesco.ardizzone02@unipa.it

Claudia Arenaccio
National Center for Global Health
Istituto Superiore di Sanità, Roma
@: claudia.arenaccio@iss.it

B

Loredana Baccigalupi
Dipartimento di Biologia
Università di Napoli "Federico II"
@: lorbacci@unina.it

Valerio Baldelli
Dipartimento di Scienze
Università Roma Tre
@: valerio.baldelli@uniroma3.it

Alessandra Bardi
Dipartimento di Biologia
Università di Pisa
@: alessandra.bardi@for.unipi.it

Frédéric Barras
Laboratoire de Chimie Bactérienne
CNRS, Aix Marseille Université, France
@: barras@imm.cnrs.fr

Simone Battaglia
Divisione di Immunologia, Trapianti e Malattie Infettive
Ospedale San Raffaele
@: s86.battaglia@tiscali.it

Andreas Baur
Department of Dermatology
University Hospital Erlangen, Germany
@: andreas.baur@uk-erlangen.de

Marco Bazzicalupo
Dipartimento di Biologia
Università di Firenze
@: marco.bazzicalupo@unifi.it

Mattia Benedet
Centro per la Biologia Integrata (CIBIO)
Università di Trento
@: mattia.benedet@unitn.it

Francesca Berini
Dipartimento di Biotecnologie e Scienze della Vita
Università dell'Insubria
@: f.berini@uninsubria.it

Giovanni Bertoni
Dipartimento di Bioscienze
Università di Milano
@: giovanni.bertoni@unimi.it

Annamaria Bevivino
Dipartimento Sostenibilità dei Sistemi Produttivi e Territoriali
ENEA Centro Ricerche Casaccia, Roma
@: annamaria.bevivino@enea.it

Francesca Biavasco
Dipartimento di Scienze della Vita e dell'Ambiente
Università Politecnica delle Marche
@: f.biavasco@univpm.it

Elisa Binda
Dipartimento di Biotecnologie e Scienze della Vita
Università dell'Insubria
@: elisa.binda@uninsubria.it

Jonas Blomberg
Department of Medical Sciences
Uppsala University, Sweden
@: jonas.blomberg@medsci.uu.se

Daria Bottai
Dipartimento di Biologia
Università di Pisa
@: daria.bottai@unipi.it

Emmanuelle Bouveret
Laboratoire d'Ingénierie des Systèmes Macromoléculaires
CNRS, Aix Marseille Université, France
@: bouveret@imm.cnrs.fr

Paola Branduardi
Dipartimento di Biotecnologie e Bioscienze
Università di Milano-Bicocca
@: paola.branduardi@unimib.it

Federica Briani
Dipartimento di Bioscienze
Università di Milano
@: federica.briani@unimi.it

Tarcisio Brignoli
GSK Vaccines, Siena
@: tarciscio.x.brignoli@gsk.com

Francesca Bruscolini
Dipartimento di Scienze Biomolecolari
Università di Urbino
@: francesca.bruscolini@uniurb.it

Antonio Bucci
Dipartimento di Bioscienze e Territorio
Università del Molise
@: antonio.bucci@unimol.it

Davide Bulgarelli
Plant Sciences
University of Dundee, Scotland, UK
@: d.bulgarelli@dundee.ac.uk

C

Marco Cafora
Dipartimento di Bioscienze
Università di Milano
@: marco.cafora@studenti.unimi.it

Martina Cappelletti
Dipartimento di Farmacia e Biotecnologie
Università di Bologna
@: martina.cappelletti2@unibo.it

Massimiliano Cardinale
Institute of Applied Microbiology
Justus-Liebig-University Giessen, Germany
@: massimiliano.cardinale@umwelt.uni-giessen.de

Eleonora Carota
Dip. per la Innovazione nei Sistemi Biologici, Agroalimentari e Forestali
Università della Tuscia
@: carota@unitus.it

Alessandro Cascioferro
Unité de Pathogénomique Mycobactérienne Intégrée
Institut Pasteur, Paris, France
@: alecascio@yahoo.it

Angela Casillo
Dipartimento di Scienze Chimiche
Università di Napoli "Federico II"
@: angela.casillo@unina.it

Valentina Catania
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: valentina.catania@unipa.it

Chiara Ceresa
Dipartimento di Scienze del Farmaco
Università del Piemonte Orientale "A. Avogadro"
@: chiara.ceresa@uniupo.it

Laurent Chiarelli
Dipartimento di Biologia e Biotecnologie
Università di Pavia
@: laurent.chiarelli@unipv.it

Carolina Chiellini
Dipartimento di Biologia
Università di Firenze
@: carolina.chiellini@unifi.it

Nagaia Ciacci
Dipartimento di Biologia
Università di Roma Tor Vergata
@: nagaia_ciacci@libero.it

Andrea Colarusso
Dipartimento di Scienze Chimiche
Università di Napoli "Federico II"
@: andrea.colarusso@unina.it

Bianca Colonna
Dipartimento di Biologia e Biotecnologie "C. Darwin"
Sapienza Università di Roma
@: bianca.colonna@uniroma1.it

Angelina Cordone
Dipartimento di Biologia
Università di Napoli "Federico II"
@: angelina.cordone@unina.it

Angela Corona
Dipartimento Scienze della Vita e dell'Ambiente
Università di Cagliari
@: angela.corona@unica.it

D

Matteo Daghio
Dipartimento di Scienze dell'Ambiente e della Terra
Università di Milano-Bicocca
@: matteo.daghio@unimib.it

Marco Maria D'Andrea
Dipartimento di Biotecnologie Mediche
Università di Siena
@: marco.dandrea@unisi.it

Francesca D'Angelo
Dipartimento di Scienze
Università Roma Tre
@: francesca.dangelo@uniroma3.it

Laura Maria De Plano
Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali
Università di Messina
@: ldeplano@unime.it

Barbara De Siena
Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche
Università della Campania "Luigi Vanvitelli"
@: barbara.desiena@unicampania.it

Alessandra Di Canito
Dipartimento di Biotecnologie e Bioscienze
Università di Milano-Bicocca
@: a.dicanito@campus.unimib.it

Patrizia Di Gennaro
Dipartimento di Biotecnologie e Bioscienze
Università di Milano-Bicocca
@: patrizia.digennaro@unimib.it

Simona Di Gregorio
Dipartimento di Biologia
Università di Pisa
@: simona.digregorio@unipi.it

Mariagrazia Di Luca
Berlin-Brandenburg Centrum für Regenerative Therapien
Charité Universitätsmedizin Berlin, Germany
@: mariagrazia.di-luca@charite.de

Maria Letizia Di Martino
Department of Medical Biochemistry and Microbiology
Uppsala University, Sweden
@: ml.dimartino@imbim.uu.se

Marco Di Salvo
Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali
Università del Salento
@: marco.disalvo@unisalento.it

George diCenzo
Department of Biology
McMaster University, Ontario, Canada
@: diceng@mcmaster.ca

Nora Djahnit
Environnement Marin
Institut des Sciences de la Mer et de l'Aménagement du Littoral
@: dja.nora12@gmail.com

Stefano Donadio
NAICONS Srl, Milano
@: sdonadio@naicons.com

Antonina Dolei
Dipartimento di Scienze Biomediche
Università di Sassari
@: doleivir@uniss.it

Sabrina Duranti
Dipartimento di Bioscienze
Università di Parma
@: sabrina.duranti@unipr.it

E

Anna Speranza Espinoza Tofalos
Dipartimento di Scienze dell'Ambiente e della Terra
Università di Milano-Bicocca
@: a.espinozatofalos@campus.unimib.it

Francesca Esposito
Dipartimento Scienze della Vita e dell'Ambiente
Università di Cagliari
@: francescaesposito@unica.it

F

Teresa Faddetta
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: teresa.faddetta@unipa.it

Camilla Fagorzi
Dipartimento di Biologia
Università di Firenze
@: camilla.fagorzi@unifi.it

Marilena Falcone
Dipartimento di Bioscienze
Università di Milano
@: marilena.falcone@unimi.it

Renato Fani
Dipartimento di Biologia
Università di Firenze
@: renato.fani@unifi.it

Elisa Fanunza
Dipartimento Scienze della Vita e dell'Ambiente
Università di Cagliari
@: elisafanunza@unica.it

Massimiliano Fenice
Dipartimento di Ecologia e Biologia
Università della Tuscia
@: fenice@unitus.it

Marco Fondi
Dipartimento di Biologia
Università di Firenze
@: marco.fondi@unifi.it

Rosa Maria Fontana
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: romariafontana@gmail.com

Angelo Fontana
Unità Chimica Bio-Organica
Istituto di Chimica Biomolecolare, CNR, Pozzuoli (NA)
@: afontana@icb.cnr.it

Francesca Forti
Dipartimento di Bioscienze
Università di Milano
@: francesca.forti@unimi.it

Alessandra Fortuna
Dipartimento di Scienze
Università Roma Tre
@: ale.fortuna@fastwebnet.it

Domenico Franco
Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali
Università di Messina
@: dfranco@unime.it

Andrea Franzetti
Dipartimento di Scienze dell'Ambiente e della Terra
Università di Milano-Bicocca
@: andrea.franzetti@unimib.it

G

Giuseppe Gallo
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: giuseppe.gallo@unipa.it

Isabella Gandolfi
Dipartimento di Scienze dell'Ambiente e della Terra
Università di Milano-Bicocca
@: isabella.gandolfi@unimib.it

Eleonora Gaspari
NAICONS Srl, Milano
@: egaspari@naicons.com

Daniele Ghezzi
Dipartimento di Farmacia e Biotecnologie
Università di Bologna
@: daniele.ghezzi3@unibo.it

Giulia Giallonardi
Dipartimento di Scienze
Università Roma Tre
@: giulia.giallonardi@uniroma3.it

Anna Giammanco
Dip. di Scienze per la Promozione della Salute e Materno Infantile "G. D'Alessandro"
Università di Palermo
@: anna.giammanco@unipa.it

Donato Giovannelli
Earth-Life Science Institute, Tokyo, Japan
@: donato.giovannelli@gmail.com

Luciana Giovannetti
Dip.di Scienze delle Produzioni Agroalimentari e dell'Ambiente
Università di Firenze
@: luciana.giovannetti@unifi.it

Stefania Giussani
GSK Vaccines, Siena
@: stefania.2.giussani@gsk.com

Susanna Gorrasi
Dipartimento di Ecologia e Biologia
Università della Tuscia
@: gorrasi@unitus.it

Milena Grossi
Dipartimento di Biologia e Biotecnologie "C. Darwin"
Sapienza Università di Roma
@: milena.grossi@uniroma1.it

H

Sarah Hijazi
Dipartimento di Scienze
Università Roma Tre
@: sarah.hijazi@uniroma3.it

I

Francesco Iannelli
Dipartimento di Biotecnologie Mediche
Università di Siena
@: francesco.iannelli@unisi.it

Francesco Imperi
Dipartimento di Biologia e Biotecnologie "C. Darwin"
Sapienza Università di Roma
@: francesco.imperi@uniroma1.it

Rachele Isticato
Dipartimento di Biologia
Università di Napoli "Federico II"
@: isticato@unina.it

J

Olivier Jousson
Centro per la Biologia Integrata (CIBIO)
Università di Trento
@: olivier.jousson@unitn.it

K

Meta Kuehn
Department of Biochemistry
Duke University, Durham, NC, USA
@: kuehn@duke.edu

L

Violetta La Cono
Istituto per l'Ambiente Marino Costiero
CNR, Messina
@: violetta.lacono@iamc.cnr.it

Emanuela Claudia La Marca
Dipartimento Scienze della Terra e del Mare
Università di Palermo
@: emanuelaclaudia.lamarca@unipa.it

Mariamichela Lanzilli
Dipartimento di Biologia
Università di Napoli "Federico II"
@: mariamichelalanzilli@gmail.com

Concetta Lauro
Dipartimento di Biologia
Università di Napoli "Federico II"
@: tittylauro@gmail.com

Livia Leoni
Dipartimento di Scienze
Università Roma Tre
@: livia.leoni@uniroma3.it

Alessandra Lo Sciuto
Dipartimento di Biologia e Biotecnologie "C. Darwin"
Sapienza Università di Roma
@: alessandra.losciuto@uniroma1.it

Sacha Lucchini
Dipartimento di Ingegneria Industriale
Università di Salerno
@: slucchini@unisa.it

Anna Luganini
Dipartimento di Scienze della Vita e Biologia dei Sistemi
Università di Torino
@: anna.luganini@unito.it

Gian Marco Luna
Istituto di Scienze Marine
CNR, Ancona
@: gianmarco.luna@ve.ismar.cnr.it

Maria Laura Luprano
Istituto di Ricerca sulle Acque, CNR, Roma
@: luprano@irsa.cnr.it

M

Raquel Maia
Microbiologia
Università di Napoli "Federico II"
@: raqueldiasmaia@outlook.com

Leonardo Mancabelli
Dipartimento di Scienze Chimiche della Vita e della Sostenibilità Ambientale
Università di Parma
@: leonardo.mancabelli@genprobio.com

Riccardo Manganelli
Dipartimento di Medicina Molecolare
Università di Padova
@: riccardo.manganelli@unipd.it

Marta Mangisfesta
Dipartimento di Scienze Chimiche della Vita e della Sostenibilità Ambientale
Università di Parma
@: marta.mangifesta@studenti.unipr.it

Elena Manini
Istituto di Scienze Marine
CNR, Ancona
@: e.manini@ismar.cnr.it

Flavia Marinelli
Dipartimento di Biotecnologie e Scienze della Vita
Università dell'Insubria
@: flavia.marinelli@uninsubria.it

Pasquale Marmo
Dipartimento di Biologia
Università di Roma Tor Vergata
@: pasquale.marmo@uniroma2.it

Francesca Martani
Dipartimento di Biotecnologie e Bioscienze
Università di Milano-Bicocca
@: francesca.martani@unimib.it

Antonio Mastino
Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali
Università di Messina
@: antonio.mastino@unime.it

Rosanna Mattossovich
Dipartimento di Biologia
Università di Napoli "Federico II"
@: rosannamattossovich@libero.it

Emilia Mauriello
Laboratoire de Chimie Bactérienne
CNRS, Aix Marseille Université, France
@: emauriello@imm.cnrs.fr

Diletta Mazzantini
Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia
Università di Pisa
@: diletta.mazzantini@med.unipi.it
dilettam89@gmail.com

Marnix Medema
Bioinformatics Group
Wageningen University, The Netherlands
@: marnix.medema@wur.nl

Marta Mellini
Dipartimento di Scienze
Università Roma Tre
@: martamellini@gmail.com

Alessio Mengoni
Dipartimento di Biologia
Università di Firenze
@: alessio.mengoni@unifi.it

Elisangela Miceli
Dipartimento di Biologia
Università di Firenze
@: elisangela.miceli@unifi.it

Soeren Molin
Novo Nordisk Foundation Center for Biosustainability
Technical University of Denmark
@: sm@bio.dtu.dk

Marco Moracci
Dipartimento di Biologia
Università di Napoli "Federico II"
@: marco.moracci@unina.it

Lidia Muscariello
Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche
Università della Campania "Luigi Vanvitelli"
@: lidia.muscariello@unicampania.it

N

Gino Naclerio
Dipartimento di Bioscienze e Territorio
Università del Molise
@: naclerio@unimol.it

O

Marco Rinaldo Oggioni
Department of Genetics
University of Leicester
@: mro5@leicester.ac.uk

P

Emilia Palazzotto
Novo Nordisk Foundation Center for Biosustainability
Technical University of Denmark
@: emipal@biosustain.dtu.dk

Carola Parolin
Dipartimento di Farmacia e Biotecnologie
Università di Bologna
@: carola.parolin@unibo.it

Maria Cristina Parolin
Dipartimento di Medicina Molecolare
Università di Padova
@: cristina.parolin@unipd.it

Maria Rosalia Pasca
Dipartimento di Biologia e Biotecnologie
Università di Pavia
@: mariarosalia.pasca@unipv.it

Martina Pasqua
Dipartimento di Biologia e Biotecnologie "C. Darwin"
Sapienza Università di Roma
@: martina.pasqua@uniroma1.it

Viola Pavoncello
Dipartimento di Scienze
Università Roma Tre
@: vio.pavoncello@stud.uniroma3.it

Renato Pedron
Centro per la Biologia Integrata (CIBIO)
Università di Trento
@: renato.pedron@unitn.it

Zulema Antonia Percario
Dipartimento di Scienze
Università Roma Tre
@: zulema.percario@uniroma3.it

Elena Perrin
Dipartimento di Biologia
Università di Firenze
@: elena.perrin@unifi.it

Chiara Pesciaroli
Dipartimento di Biotecnologie e Bioscienze
Università di Milano-Bicocca
@: chiara.pesciaroli@unimib.it

Giulio Petroni
Dipartimento di Biologia
Università di Pisa
@: giulio.petroni@unipi.it

Maurizio Petruccioli
Dip. per la Innovazione nei Sistemi Biologici, Agroalimentari e Forestali
Università della Tuscia
@: petrucci@unitus.it

Elisa Piampiano
Dip.di Scienze delle Produzioni Agroalimentari e dell'Ambiente
Università di Firenze
@: elisa.piampiano@unifi.it

Anna Pianetti
Dipartimento di Scienze Biomolecolari
Università di Urbino
@: anna.pianetti@uniurb.it

Elena Piffer
Centro per la Biologia Integrata (CIBIO)
Università di Trento
@: piffer.elena@gmail.com

Eva Pinatel
Istituto di Tecnologie Biomediche
CNR, Milano
@: eva.pinatel@itb.cnr.it

Mattia Pirolo
Dipartimento di Scienze
Università Roma Tre
@: mattia.pirolo@gmail.com

Francesca Pittino
Dipartimento di Scienze dell'Ambiente e della Terra
Università di Milano-Bicocca
@: f.pittino@campus.unimib.it

Mariagrazia Pizza
GSK Vaccines, Siena
@: mariagrazia.x.pizza@gsk.com

Amalia Porta
Dipartimento di Farmacia
Università di Salerno
@: aporta@unisa.it

Alessandro Presentato
Dipartimento di Biotecnologie
Università di Verona
@: alessandro.presentato@univr.it

Roberta Provvedi
Dipartimento di Biologia
Università di Padova
@: roberta.provvedi@unipd.it

Carla Pruzzo
Dipartimento di Scienze della Terra, dell'Ambiente e della Vita
Università di Genova
@: carla.pruzzo@unige.it

Anna Maria Puglia
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: a.maria.puglia@unipa.it

Q

Paola Quatrini
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: paola.quatrini@unipa.it

R

Giordano Rampioni
Dipartimento di Scienze
Università Roma Tre
@: giordano.rampioni@uniroma3.it

Matteo Raneri
Dipartimento di Bioscienze
Università di Milano
@: matteo.raneri@hotmail.it

Ezio Ricca
Dipartimento di Biologia
Università di Napoli "Federico II"
@: ericca@unina.it

Giovanna Riccardi
Dipartimento di Biologia e Biotecnologie
Università di Pavia
@: giovanna.riccardi@unipv.it

Maria Giovanna Rizzo
Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali
Università di Messina
@: mgiovanna.biologo@gmail.com

Edda Russo
Dipartimento di Medicina Sperimentale e Clinica
Università di Firenze
@: edda.russo@unifi.it

Maurizio Ruzzi
Dipartimento per la Innovazione nei Sistemi Biologici, Agroalimentari e Forestali
Università della Tuscia
@: ruzzi@unitus.it

S

Anella Saggese
Dipartimento di Biologia
Università di Napoli "Federico II"
@: ornellasaggese2010@libero.it

Federica Sandri
Dipartimento di Farmacia e Biotecnologie
Università di Bologna
@: federica.sandri4@unibo.it

Daniela Sateriale
Dipartimento di Scienze e Tecnologie
Università del Sannio
@: sateriale@unisannio.it

Michael Sauer
Department of Biotechnology
BOKU University of Vienna, Austria
@: michael.sauer@boku.ac.at

Domenico Schillaci
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: domenico.schillaci@unipa.it

Viola Camilla Scoffone
Dipartimento di Biologia e Biotecnologie
Università di Pavia
@: viola.scoffone@unipv.it

Cristiana Sigona
Dipartimento di Biologia
Università di Pisa
@: cristiana.sigona@gmail.com

Francesco Smedile
Department of Biochemistry and Microbiology
Department of Marine and Coastal Sciences
Rutgers University, New Brunswick, NJ, USA
@: smedile@marine.rutgers.edu

Serena Sofia
Dipartimento di Scienze Agrarie, Alimentari e Forestali
Università di Palermo
@: serenasofia.94@gmail.com

Margherita Sosio
NAICONS Srl, Milano
@: msosio@naicons.com

Tatiana Stella
Dipartimento di Scienze dell'Ambiente e della Terra
Università di Milano-Bicocca
@: tatiana.stella@unimib.it

Mariarita Stirpe
Dipartimento di Biologia e Biotecnologie "C. Darwin"
Sapienza Università di Roma
@: mariarita.stirpe@uniroma1.it

Alberto Sutera
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: alsute@alice.it

T

Silvia Tabacchioni
Dipartimento Sostenibilità dei Sistemi Produttivi e Territoriali
ENEA Centro Ricerche Casaccia, Roma
@: silvia.tabacchioni@enea.it

Adelfia Talà
Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali
Università del Salento
@: adelfia.tala@unisalento.it

Giovanni Tassistro
Dipartimento di Scienze della Terra, dell'Ambiente e della Vita
Università di Genova
@: giovanni.tassistro@edu.unige.it

Arianna Tavanti
Dipartimento di Biologia
Università di Pisa
@: arianna.tavanti@unipi.it

Maria Cristina Thaller
Dipartimento di Biologia
Università di Roma Tor Vergata
@: thaller@uniroma2.it

Arianna Tocchetti
NAICONS Srl, Milano
@: atocchetti@naicons.com

Alessandra Tosco
Dipartimento di Farmacia
Università di Salerno
@: tosco@unisa.it

Makrina Totsika
Institute of Health and Biomedical Innovation
Queensland University of Technology, Herston, Australia
@: makrina.totsika@qut.edu.au

Enzo Tramontano
Dipartimento Scienze della Vita e dell'Ambiente
Università di Cagliari
@: tramon@unica.it

Francesca Turroni
Dipartimento di Scienze Chimiche della Vita e della Sostenibilità Ambientale
Università di Parma
@: francesca.turroni@unipr.it

V

Chiara Vanni
Max Planck Institute for Marine Microbiology
University of Bremen, Germany
@: cvanni@mpi-bremen.de

Mario Varcamonti
Dipartimento di Biologia
Università di Napoli "Federico II"
@: varcamon@unina.it

Alberto Vassallo
Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche
Università di Palermo
@: alberto.vassallo@unipa.it

Marco Ventura
Dipartimento di Scienze Chimiche della Vita e della Sostenibilità Ambientale
Università di Parma
@: marco.ventura@unipr.it

Costantino Vetriani
Department of Biochemistry and Microbiology
Rutgers University, New Brunswick, NJ, USA
@: vetriani@marine.rutgers.edu

Luigi Vezzulli
Dipartimento di Scienze della Terra, dell'Ambiente e della Vita
Università di Genova
@: luigi.vezzulli@unige.it

Carla Vignaroli
Dipartimento di Scienze della Vita e dell'Ambiente
Università Politecnica delle Marche
@: c.vignaroli@univpm.it

Daniela Visaggio
Dipartimento di Scienze
Università Roma Tre
@: daniela.visaggio@uniroma3.it

Paolo Visca
Dipartimento di Scienze
Università Roma Tre
@: paolo.visca@uniroma3.it

Beatrice Vitali
Dipartimento di Farmacia e Biotecnologie
Università di Bologna
@: b.vitali@unibo.it

Carlo Viti
Dip.di Scienze delle Produzioni Agroalimentari e dell'Ambiente
Università di Firenze
@: carlo.viti@unifi.it

W

Gerhart Wagner
Department of Cell and Molecular Biology
Uppsala University, Sweden
@: Gerhart.Wagner@icm.uu.se

Y

Michail M. Yakimov
Istituto per l'Ambiente Marino Costiero
CNR, Messina
@: michail.yakimov@iamc.cnr.it

Z

Jessica Zampolli
Dipartimento di Biotecnologie e Bioscienze
Università di Milano-Bicocca
@: jessica.zampolli@unimib.it

Anna Zanfardino
Dipartimento di Biologia
Università di Napoli "Federico II"
@: anna.zanfardino@unina.it

Davide Zannoni
Dipartimento di Farmacia e Biotecnologie
Università di Bologna
@: davide.zannoni@unibo.it

Alessandro Zennaro
Dipartimento di Biologia e Biotecnologie "C. Darwin"
Sapienza Università di Roma
@: zennaro.alessandro89@gmail.com

AUTHOR INDEX

D

E

F

G

H

I

J

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L

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SIMGBM

Web site: **www.simgbm.it**

Email: **congresso@simgbm.it**