

Use of kefir-derived lactic acid bacteria for the preparation of a fermented soy drink with increased estrogenic activity

Running Title: Kefir LABs to ferment soy drink

Giacomo Mantegazza¹, Alessandro Dalla Via¹, Armando Licata¹, Robin Duncan¹, Claudio Gardana¹, Giorgio Gargari¹, Cristina Alamprese¹, Stefania Arioli¹, Valentina Taverniti¹, Matti Karp², and Simone Guglielmetti^{1,*}

¹ Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy

² Materials Science and Environmental Engineering, Bio and Circular Economy, Tampere University, Finland

*Corresponding author. Mailing address: Via Celoria 2, 20133, Milan, Italy. E-mail:

simone.guglielmetti@unimi.it. Tel: +39 02 503 19136.

Table S1. General information on the draft genomes of the four lactic acid bacterial strains isolated from the fermented soy drink. GC%, percent of guanosine + cytosine in the genome. N50 is the shortest contig length that needs to be included to cover 50% of the genome. L50 is the count of the smallest number of contigs whose length sum makes up half of genome size. CDS, coding sequences.

Bacterial strain	Size (bp)	GC%	N50	L50	N° scaffolds	N° CDS
<i>L. lactis</i> K03	2'474'618	35.1	114'671	7	363	2'684
<i>L. pseudomesenteroides</i> K05	2'257'707	38.9	118'283	7	332	2'420
<i>L. mesenteroides</i> K09	2'051'111	37.6	1'183'347	1	185	2'161
<i>L. kefir</i> K10	2'661'619	41.7	127'974	4	443	2'830

Table S2. Putative antibiotic resistance genes of bacterial strains isolated from fermented soy detected through the CARD's Resistance Gene Identifier software (A) and the AMRFinder search tool (B). RGI, resistance gene identifier. AMR, antimicrobial resistance. ARO, antibiotic resistance ontology. The genome of strain *Enterococcus faecalis* TK-P4B and *Bifidobacterium animalis* subsp. *lactis* BB-12 have been used as positive controls for AMR genes.

A

Strain	RGI Criteria	ARO Term	SNP	Detection Criteria	AMR Gene Family	Drug Class	Resistance Mechanism	% Identity of Matching Region	% Length of Reference Sequence
<i>Enterococcus faecalis</i> TK-P4B	Strict	<i>efrA</i>	/	protein homolog model	ATP-binding cassette (ABC) antibiotic efflux pump	macrolide antibiotic, fluoroquinolone antibiotic, rifamycin antibiotic	antibiotic efflux	99.83	99.48
	Strict	<i>dfpE</i>	/	protein homolog model	trimethoprim resistant dihydrofolate reductase <i>dfp</i>	diaminopyrimidine antibiotic	antibiotic target replacement	98.78	100
	Strict	<i>lsaA</i>	/	protein homolog model	lsa-type ABC-F protein	lincosamide antibiotic, streptogramin antibiotic, streptogramin A antibiotic, streptogramin B antibiotic, pleuromutilin antibiotic	antibiotic target protection	97.79	100
	Strict	<i>emeA</i>	/	protein homolog model	multidrug and toxic compound extrusion (MATE) transporter	disinfecting agents and antiseptics	antibiotic efflux	97.71	97.71
	Strict	<i>efrB</i>	/	protein homolog model	ATP-binding cassette (ABC) antibiotic efflux pump	macrolide antibiotic, fluoroquinolone antibiotic, rifamycin antibiotic	antibiotic efflux	97.58	162.71
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BB-12	Strict	<i>tet</i> (W/N/W)	/	protein homolog model	tetracycline-resistant ribosomal protection protein	tetracycline antibiotic	antibiotic target protection	94.83	100
	Strict	<i>rpoB</i> mutants	/	protein homolog model	rifamycin-resistant beta-subunit of RNA polymerase (<i>rpoB</i>)	rifamycin antibiotic	antibiotic target alteration/replacement	92.66	100
<i>Lactococcus lactis</i> K03		none							
<i>Leuconostoc pseudomesenteroides</i> K05		none							
<i>Leuconostoc mesenteroides</i> K09		none							
<i>Lentilactobacillus kefirii</i> K10		none							

B

	Contig id	Gene symbol	Sequence name	Element type	Class	Method	Target length	Reference sequence length	% Coverage of reference sequence	% Identity to reference sequence	Alignment length	Accession of closest sequence	Name of closest sequence
<i>Enterococcus faecalis</i> TK-P4B	NZ_KB944666.1	<i>lsa(A)</i>	ABC-F type ribosomal protection protein Lsa(A)	AMR	LINCOSAMIDE / STREPTOGRAMIN	BLASTX	498	498	100	99.6	498	WP_002398829.1	ABC-F type ribosomal protection protein Lsa(A)
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BB-12	NC_017216.2	<i>tet(W)</i>	tetracycline resistance ribosomal protection protein Tet(W)	AMR	TETRACYCLINE	EXACTX	639	639	100	100	639	WP_004217777.1	tetracycline resistance ribosomal protection protein Tet(W)
<i>Lactococcus lactis</i> K03		none											
<i>Leuconostoc pseudomesenteroides</i> K05		none											
<i>Leuconostoc mesenteroides</i> K09		none											
<i>Lentilactobacillus kefirii</i> K10		none											

Fig. S1. Activation of the *Saccharomyces cerevisiae* BMAEReluc/ER α reporter system for the measurement of the estrogenic activity by zearalenone. RLU, relative luminescence units. These data are from an experiment conducted in triplicate.

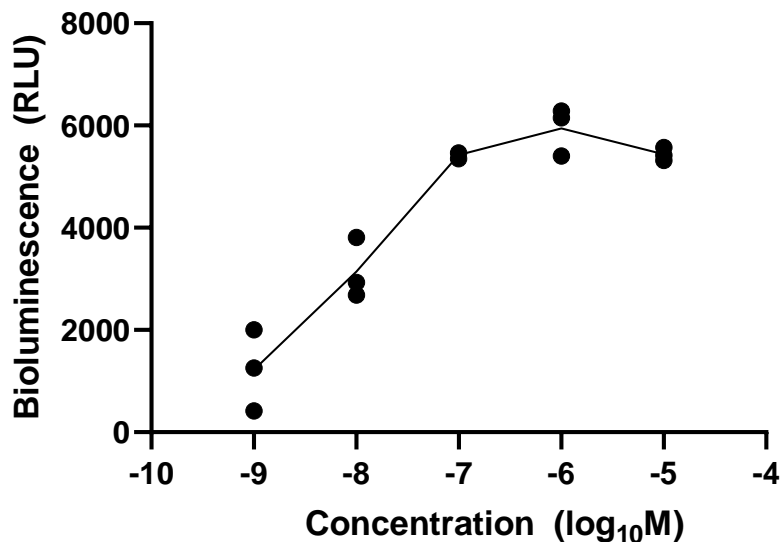


Fig. S2. Microbiological characterization of the fermented soy drink. Panels A-C, optical microscopy examination (1000X, under oil immersion) after methylene blue staining of the soy drink fermented with kefir grains obtained after two weeks of subculturing. Red and yellow arrows in panels A and B indicate rod-shaped cells and chains of bacterial cocci, respectively. Panel C shows an example of the clusters of rod-shaped cells sporadically found in the fermented soy drink. Scale bar, 10 μ m. Panel D, Colony morphology of the four strains on HHD differential agar medium. (a) *Lactococcus lactis* K03 (big white colonies, blue in the middle), (b) *Leuconostoc pseduomesenteroides* K05 (small blue colonies), (c) *Leuconostoc mesenteroides* K09 (medium-size white colonies) and (d) *L. kefir* K10 (small white/transparent colonies).

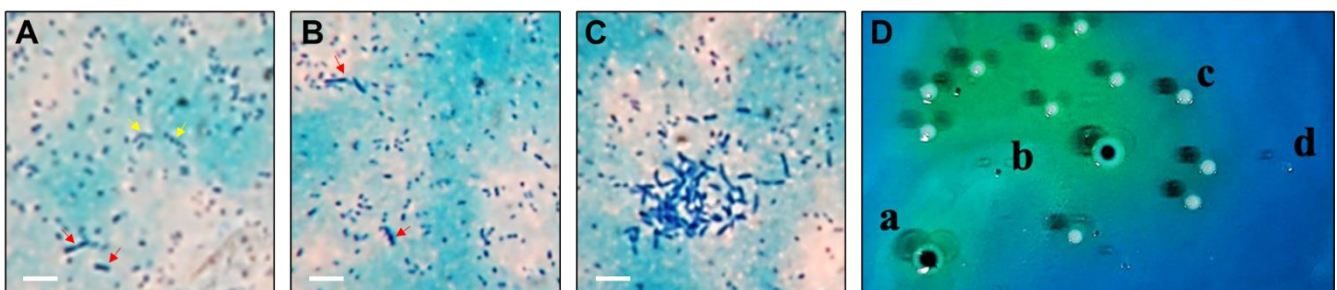


Fig. S3. Electrophoretic profiles (2% agarose gel) obtained through BOX-PCR genetic fingerprinting of bacterial isolates collected from M17 and MRS agar plates. The taxonomic identification of each genotypic group has been obtained by sequencing the 16S rRNA gene and is reported on the right. M, molecular-weight size marker (1 kb ladder).

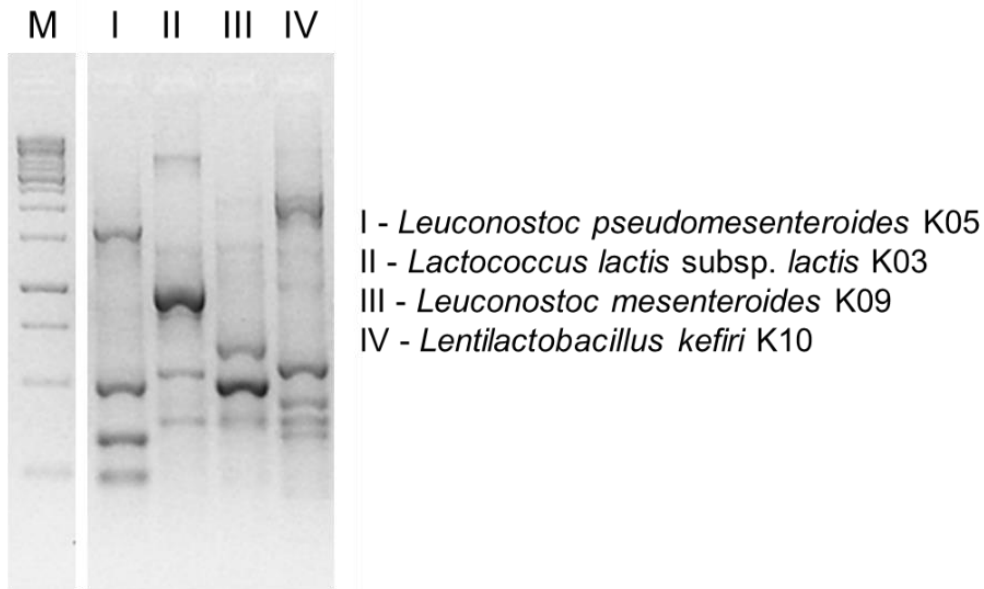


Fig. S4. Assessment of polysaccharide capsule production on Congo red (A) and Aniline blu (B) agar plates. K03, *Lactococcus lactis*; K05, *Leuconostoc pseudomesenteroides*; K09, *Leuconostoc pseudomesenteroides*.

