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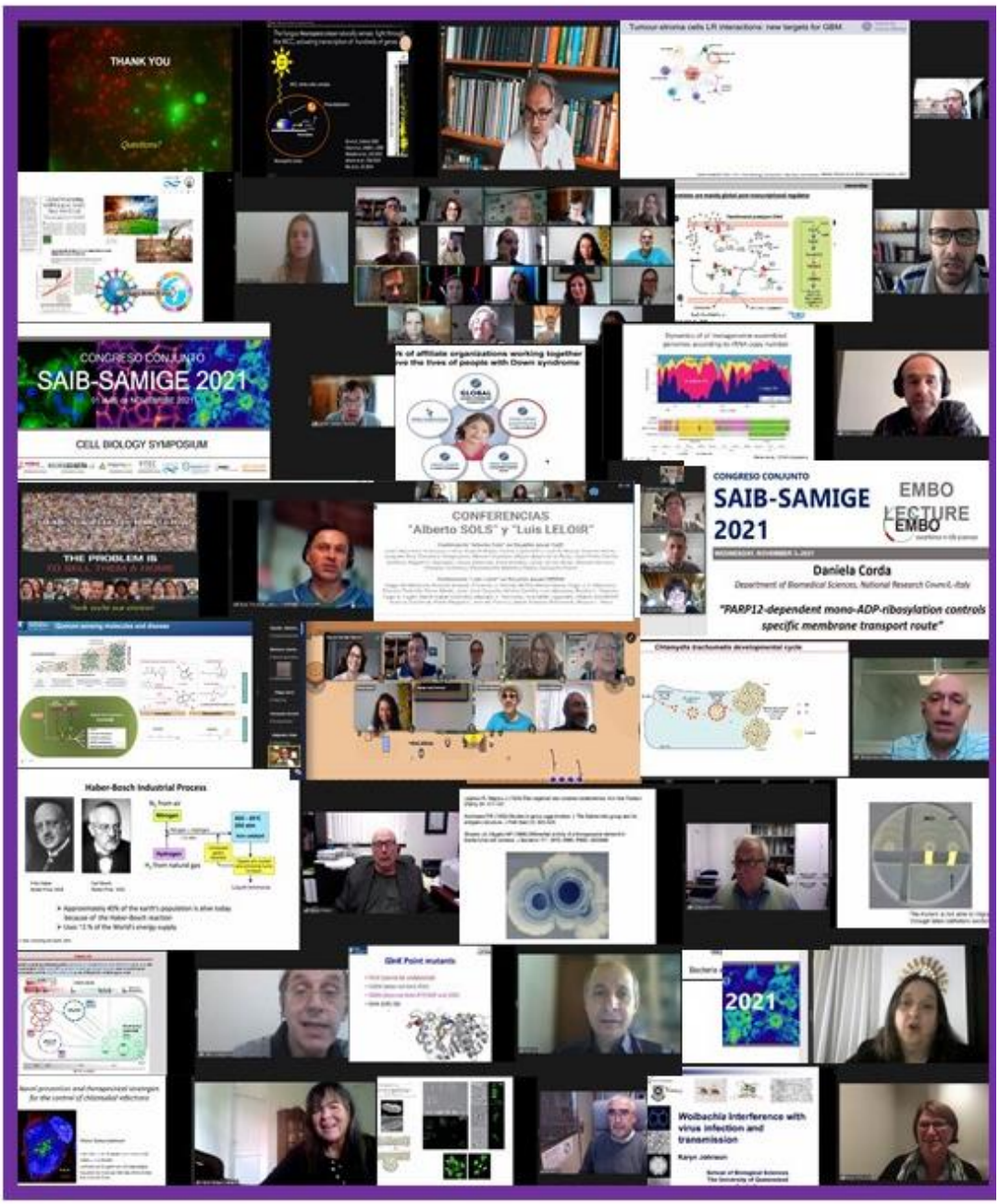
SAIB - SAMIGE Joint meeting
2021 *on line*



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LVII Annual Meeting of the Argentine Society for Biochemistry and Molecular Biology Research (SAIB)

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of the preservation techniques used, a quality control must be carried out that includes the evaluation of viability, purity, biochemical and molecular properties. These evaluations must be carried out at the beginning, after the conservation of the first batch, as well as after certain periods of time. The objective of this work was to reactivate 20 strains *Yersinia enterocolitica* cepariums conserved in the 1980s under two different preservation methods: lyophilization (LIO) and semi-solid medium (SS). The LIO strains were reactivated in tindalized skim milk and cultured at 25 ºC for 24 h, then were replicated in tryptic soy broth (TSB) + 0.6% yeast extract (STBY) and finally in brain heart broth (BHB). The strains conserved in SS medium were reactivated in STBY, picked up at BHB and finally at TSB at 25 ºC for 24 h, respectively. All strains, after reactivated, were seeded on Mac Conkey agar and biochemically identified to corroborate purity. Subsequently, they were ultrafreezed at -80 ° C in TSB + 20% glycerol in duplicate, and in tryptic soy SS medium in duplicate at 4 °C. Counting was not performed because the strains were very weak, and it was necessary to carry out the replicate cultures in nutritionally rich culture medium in order to prioritize survival over quantification. Of the 20 LIO strains, 5 were reactivated, representing 25% survival; meanwhile, from the strains conserved in SS medium, 14 strains could be reactivated, representing 70% survival. This demonstrates the importance of establishing periodic reactivation protocols and control of viability, in order to preserve every strain from the collection. In our study it was shown that conservation in SS medium gives better results than LIO, for long periods of conservation of *Y. enterocolitica* strains.

MI-P044-18
GENERATION AND CHARACTERIZATION OF *Haloferax volcanni* MUTANTS IN GENES WITH PREDICTED ROLES IN MOTILITY AND ELECTRON TRANSFER

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The halophilic archaeon *Haloferax volcanii* develops in a wide range of salinities (1.5-3.5 M NaCl) and, due to its ease of cultivation in the laboratory and the possibility of being genetically manipulated, it is used as a model organism for the study of archaeal biology. In the context of a comparative proteomics study of a rhomboid protease (RhoII) gene deletion mutant, we identified several proteins involved in metal homeostasis and cell surface structure/s assembly with differences in concentration and/or electrophoretic mobility in the protease deficient strain. Out of these, some were proteins which had not been previously characterized in *H. volcanii*, and that may constitute RhoII endogenous substrates. With the aim of understanding their physiological role, two of these annotated proteins (HVO_1153 and HVO_2150) were selected to generate and characterize the corresponding gene knock-out mutants. The hypothetical protein HVO_1153 primary sequence shows homology to adhesins and flagellins, and the *hvo_2150* gene encodes HcpG, a predicted small copper protein (similar to plastocyanin and azurin) that may participate in electron transport and/or act as a copper reservoir in the cell membrane of *H. volcanii.* Genes were removed from the wild type chromosome by the “pop-in / pop-out" method and the deletion in the null mutants was confirmed by PCR. The successful generation of the null mutant strains indicated that these genes are not essential for *H. volcanii* viability. The Δ*hvo_1153* mutant evidenced no differences in cell/colony morphology, cell adhesion to glass surfaces or growth in liquid medium at different conditions, when compared to the wild type strain. However, this mutant strain showed decreased motility in soft agar plates, in agreement with its predicted function in the databases. The Δ*hcpG* mutant did not exhibit deficiencies in growth in rich or minimal medium, when compared with the wild strain. Further phenotypic characterization of *H. volcanii* Δ*hcpG* is still ongoing. Altogether, the results presented in this work provide information regarding the role of two proteins which had not been previously characterized in *H. volcanii* and contribute to the general understanding of haloarchaeal physiology.
Funded by ANPCyT and UNMdP.

MI-P045-26
INTERSPECIES INTERACTIONS IN POLYMICROBIAL BIOFILMS INVOLVING *Enterococcus faecalis* AND *Escherichia coli* STRAINS ISOLATED FROM DIABETIC FOOT ULCERS

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Chronic infections of lower limbs in diabetic patients, denominated diabetic foot ulcers, show complex mixtures of bacterial species that are established in biofilms and are difficult to eradicate with conventional antibiotic therapies. Previous studies evidenced that the most prevalent species in these types of infections are *Enterococcus faecalis, Escherichia coli, Morganella morganii, Proteus mirabilis* and *Staphylococcus aureus.* Here, the interactions between *E. faecalis* and *E. coli* in biofilms were studied. The mono and polymicrobial (1:1 ratio) *E. faecalis* and *E. coli* clinical isolates biofilm formation were evaluated at 37°C in a culture medium that simulates the environment of the foot wound (45% tryptic soy broth, 50% bovine plasma and 5% lysed horse red blood cells). Biofilms assays were performed in two settings: collagen-coated multi-well plates and agar plates (macrocolony assay). For biofilm formation in collagen-coated plates, bacteria were allowed to attach to the surface for

3 h and then culture media was renewed every day. Biofilm biomass was measured by crystal violet assay (A_{595nm}) and quantification of cultivable cells was performed by enumeration of colony forming units (CFU) after collagenase treatment followed by mechanical biofilm disruption. Results obtained indicated that the two strains were able to establish mono and polymicrobial biofilms, showing a similar time-dependent increase of biomass (A_{595nm} values for biofilms at day three were: monomicrobial *E. faecalis* 8.19 ± 2.16, monomicrobial *E. coli* 10.14 ± 1.97, and polymicrobial *E. faecalis/ E. coli* 9.15 ± 2.17). Noteworthy, CFU counts showed a statical significant 7.5-fold lower *E. faecalis* adhesion to the surface in polymicrobial *E. faecalis/ E. coli* than in monomicrobial biofilms (Log_{10} CFU at 3h: 6.45 ± 0.31 *vs* 7.34 ± 0.29, respectively). However, after two days of biofilm development, no differences in *E. faecalis* cell numbers were observed between mono and polymicrobial biofilms. Regarding *E. coli*, similar viable cell numbers were found in mono and polymicrobial biofilms at all time-points assayed (3 h to 3 d). Cell-free supernatants from one-day-old *E. coli* biofilms did not produce a significant inhibition of *E. faecalis* attachment to the surface. On the other hand, mono and polymicrobial macrocolonies were developed for one day and cultivable cell numbers were enumerated by CFU counts. *E. faecalis* showed 44-fold higher bacterial numbers in polymicrobial than in monomicrobial macrocolonies (Log_{10} CFU per colony: 7.10 ± 0.35 *vs* 5.51 ± 0.30, respectively), however no differences in *E. coli* cell numbers were detected when poly and monomicrobial macrocolonies were compared. Altogether, these results show that *E. faecalis* and *E. coli* can coexist in biofilms, with *E. coli* partially inhibiting *E. faecalis* adhesion, but then favouring *E. faecalis* growth at latter biofilms stages.

MI-P046-27
1,8-CINEOLE AS AN ANTIMICROBIAL AND ANTIBIOFILM AGENT AGAINST MULTIDRUG RESISTANT *Klebsiella pneumoniae*

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Klebsiella pneumoniae is a common cause of antimicrobial-resistant opportunistic infections in hospitalized patients, including urinary tract infections. The emergence of multidrug-resistant (MDR) strains producing extended-spectrum β-lactamases (ESBL) and/or carbapenemases, in combination with the capacity to produce biofilm has created additional problems in providing adequate antibiotic treatment of urinary tract infections. Biofilms are complex bacterial communities adhered to biotic or abiotic surfaces that are surrounded by an extracellular matrix composed of exopolysaccharides, proteins and nucleic acids that give them differential phenotypic properties associated with greater resistance to antibiotics. 1,8-cineole, one of the main components of *Rosmarinus officinalis* volatile oil, has shown antimicrobial activity against non-MDR Gram negative bacteria (including *K. pneumoniae*) during planktonic growth. Here, we evaluated the antimicrobial and antibiofilm activity of 1,8-cineole against planktonic and pre-formed mature biofilms of non-MDR and MDR ESBL-producing *K. pneumoniae* clinical strains isolated from urinary tract infections. Killing curves were performed in planktonic cultures by adding 1% (v/v) 1,8-cineole for 5-180 min and counting viable cells (cfu/ml). Results showed variable decrease of *K. pneumoniae* viability (ranging from 0.5 to 4 log reduction) after phytochemical treatment, not related to their antibiotic resistance profile. Regarding biofilms, all tested strains formed robust biomass after 48 h, as determined by crystal violet staining ($Abs_{595nm} > 1$). One-hour treatment with 1% (v/v) 1,8-cineole partially disrupted biofilm biomasses (34 to 62% reduction in crystal violet staining). Additionally, a variable decrease in cell viability (between 0,5 and 4 log reduction of ufc/cm^2) was observed by viable cell counting, regardless if they were or not MDR. Two MDR ESBL-producing *K. pneumoniae* strains, presenting different susceptibility to 1,8-cineole, were chosen to study their extracellular matrix in biofilms by confocal laser scanning microscopy after calcofluor white staining. Noteworthy, differences in the extracellular matrix structure were observed between strains, that could account for differences in 1,8-cineole susceptibility. Altogether, our results show that some antibiotic-sensitive and MDR ESBL-producing *K. pneumoniae* isolates were sensitive to 1,8-cineole exposure and support the efficacy of 1,8-cineole as a potential antimicrobial agent for the treatment of planktonic and biofilm-associated infections caused by MDR *K. pneumoniae*.

MI-P047-28
REDUNDANT GENES ENCODING POTASSIUM TRANSPORTER SYSTEMS GUARANTEE THE SURVIVAL OF *Enterococcus faecalis* IN LOW POTASSIUM MEDIUM UNDER STRESS CONDITIONS

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A natural commensal member of the human gut flora that belongs to the group of lactic acid bacteria, *Enterococcus faecalis* is also a clinically important opportunistic pathogen. Despite its controversial profile, *E. faecalis* is part of food products, either due to contamination or as part of starter, adjunct or non-starter cultures. A distinct trait in the physiology of these bacteria is the ability to persist and thrive in harsh environments, that include heat, acid, oxidative and hyperosmotic stress. This tolerance to stress conditions involves the rapid movement of three critical ions: proton (H^+), sodium (Na^+), and potassium (K^+). In *E. faecalis* the activity of the proton F0F1ATPase and the sodium Na V-type ATPase under acidic or alkaline conditions, respectively are well established. However, little is known about the K^+ metabolism. In this study, an initial survey was done on K^+ uptake in *E. faecalis*. The mining of *E. faecalis* genome revealed the presence of the putative K^+ transporters Kup, KimA,