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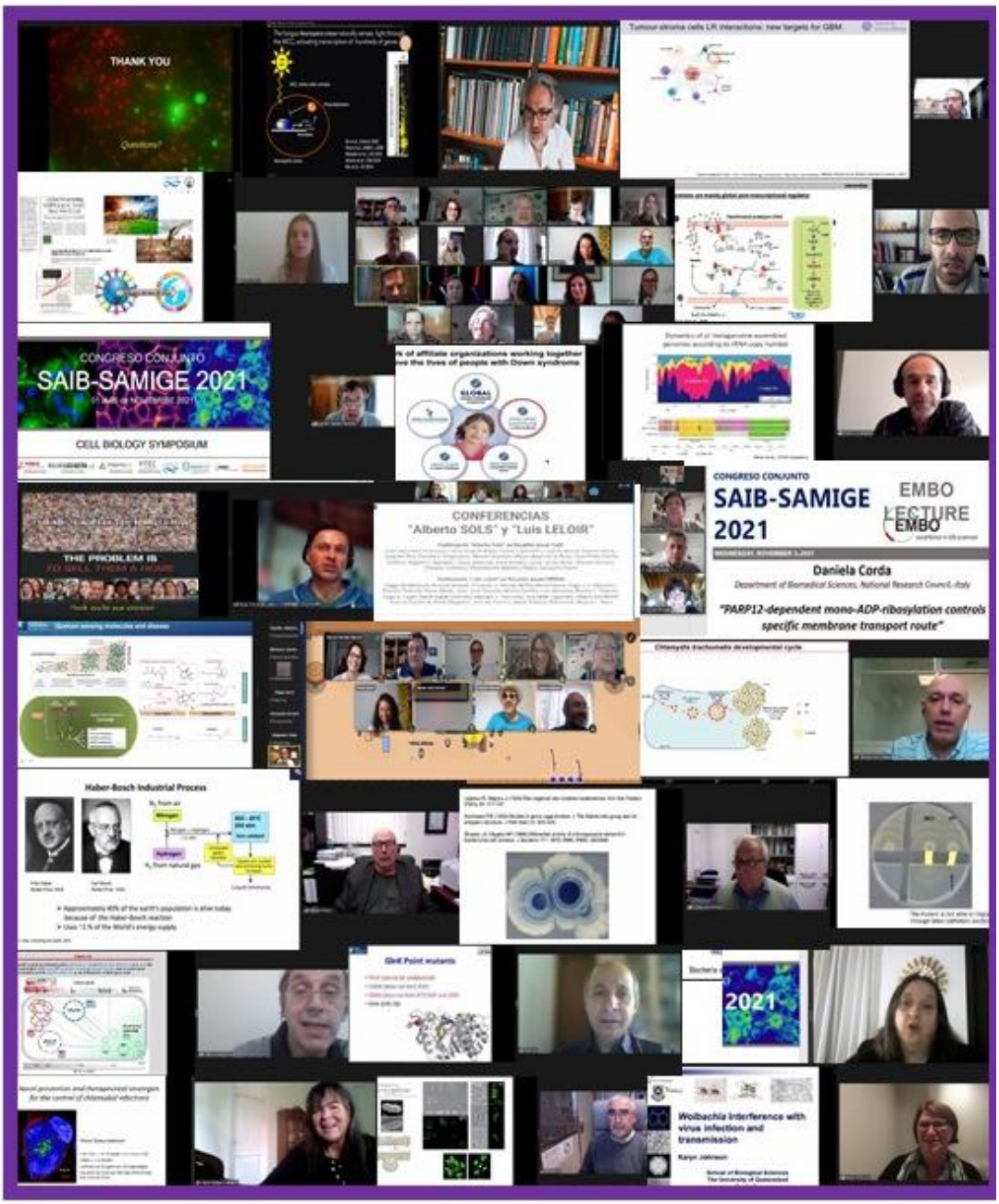
SAIB - SAMIGE Joint meeting
2021 *on line*



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

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STEC, and development of prophylactic bovine vaccines that controls bacterial colonization and consequently reduce the STEC shedding in the feces (pre-slaughter vaccines) is critical to avoid contamination of meat derivatives with STEC. Immunity based on antibodies directed against *E. coli* O157: H7 surface antigens (type III secretion system proteins and other membrane complexes) has been shown to interfere with intestinal colonization in cows, reducing bacterial fecal load feces. Thus, our vaccine preparation consists in a chimeric protein containing antigens Tir, Intimin, SpA and flagella (EITH7). The synthetic EITH7 gene was cloned into the host broad-range plasmid pBBR1MCS-4, which under the control of the Ptrc promoter drives the strong and constitutive expression of EITH7 antigen. Furthermore, since the EITH7 gene sequence was fused to the β-lactamase signal sequence, EITH7 was secreted into the bacterial periplasm where this protein can be purified easily, in a simple step by osmotic shock from *E. coli* BL21 strain. Periplasmic preparation was used to immunize mice and evaluate its antigenicity. The production of anti-EITH7 antibodies was evaluated after three antigenic doses by ELISA, observing an increase in the titer after the second immunization dose. These antibodies were evaluated in its neutralizing capacity to inhibit adhesion and pedestal formation of pedestals elicited by EPEC *E. coli* strain that shares with STEC an identical type three protein secretion system, Anti-EITH7 induced antibodies inhibit the interaction of these strains in an *in vitro* assay. Similarly, mice immunized with EITH7-enriched periplasmic fraction were able to control a challenge infection with *E. coli* O157: H7. These results indicate that our vaccine preparation based on the EITH7 antigen generates an optimal specific response without the requirement of any adjuvants, allowing the control of EDL933 experimental infection. Our future perspective is to immunize a small group of cattle with this preparation in order to and evaluate its effectivity as a pre-slaughter vaccine.

MI-P076-182
STUDY OF *Salmonella* Typhimurium ECOTIN GENE IN INTERACTION OF BACTERIA WITH GUT PROTEASES AND INTRACELLULAR LIFESTYLE IN MACROPHAGES

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Salmonella Typhimurium is a common pathogen associated to the development of acute diarrhea. The most usual way of infection is by eating contaminated food. There, *Salmonella* encounters the first line of defense in the lumen of our gastrointestinal tract (GI), where microorganisms, antigens and food are degraded in a nonspecific fashion by pH and gastric, pancreatic and biliary secretions. How protease inhibitors present in *Salmonella's* genome might contribute to survival in the gut proteolytic environment, stablish colonization and develop diarrhea is poorly understood. Ecotin is a gene present in many bacteria species encoding a protein, which has been shown to inhibit a wide range of proteases. In this work, we studied the growth of *Salmonella* Typhimurium wild type and ecotin knock-out strain (Δecotin) in presence of porcine pancreatin, we found that after incubation the replication of the Δecotin was attenuated when compared with the wild type strain. As pancreatin composition represents a mixture of proteases, we aimed to study them individually. We found that after incubation with porcine elastase the replication of Δecotin was attenuated. In both cases the complementation in trans with a plasmid encoding the ecotin gene restored the phenotype observed in the Δecotin to the wild type strain. Other important sources of proteases are the different cell types that *Salmonella* encounters while travelling the GI to finally establish the colonization, within these, macrophages are a preferential niche for the pathogen. Thus, we studied invasion and replication of the different strains in J774 murine macrophages. We found no differences in invasion but 4 h after the bacterial uptake, the replication of Δecotin was attenuated when compared to the wild type strain. This replication defect was also seen when doing a competitive 1:1 assay between Δecotin and the wild type strain in J774 murine macrophages. Taking all into account, these results indicate that ecotin may contribute to defending the bacteria against proteases in the GI tract and helping in the initial infection steps of macrophages.

MI-P077-189
LACTIC ACID BACTERIA REDUCED PRO-INFLAMMATORY CYTOKINES EXPRESSION AND OXIDATIVE STRESS ON BV-2 MICROGLIA CELLS STIMULATED WITH AMYLOID BETA OLIGOMERS

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Neuroinflammation and oxidative stress have been implicated as a common hallmark in some neurodegenerative diseases, including Alzheimer's disease (AD). Activation of microglia has been proposed to be one of the first steps in the onset of AD, generating neurotoxic compounds and pro-inflammatory cytokines. Lactic acid bacteria (LAB) are well known microorganisms and are widely studied for their various benefits to human health. Currently, there is an increasing interest in using these microorganisms as alternative therapies because of the role that gut microbiota seems to play in the pathogenesis of AD. In the present study, we examined the effects of three LAB strains on oxidative stress and inflammation-related gene expression on BV-2 microglial cells stimulated with β-amyloid oligomers ($oA\beta_{1-42}$). BV-2 cells were treated with 5 μM oAβ

and the effect of LAB was evaluated under three different conditions: using living bacteria, heat-inactivated bacteria, and bacterial conditioned media (BCM). After 8 hours of treatment, BV2 cells and supernatants were harvested separately. Total RNA was extracted from BV2 cells and the expression of TNF-α, IL-1β, IL-6, iNOS and SOD was examined by RT-qPCR. oAβ_{1-42} resulted in an increased expression of pro-inflammatory cytokines and oxidative stress in BV2 cells. Living and dead bacteria did not induced any significant changes in mRNA expression of the evaluated genes with respect to control groups. However, BCM from *Enterococcus mundtii* CRL 35, *Lactobacillus delbrueckii subsp. lactis* CRL 581 and *Levilactobacillus brevis* CRL 2013 significantly reduced IL-1β and IL-6 expression. Additionally, TNF-α expression was down-regulated on BV-2 cells treated with BCM from CRL 35. No significant differences were found in iNOS and SOD expression. Finally, total antioxidant activity of all the supernatant from BV-2 cells treated with BCM was measured using both the ABTS decolorization and the CUPRAC assays. We found that all the supernatants from microglia cells treated with BCM were capable of reducing $ABTS^{+}$ cations and only treatment with BCM from CRL 35 reduced cupric ions, indicating a significant antioxidant activity. Our results show that conditioned media from *E. mundtii* CRL 35, *L. delbrueckii subsp. lactis* CRL 581 and *L. brevis* CRL 2013 have the ability to reduced inflammatory and oxidative stress markers produced by beta-amyloid oligomers *in vitro*. We are currently examining the mechanisms and LAB metabolites implicated in these effects.

MI-P078-228
VOLATILE COMPOUNDS-MEDIATED PLANT GROWTH MODULATION BY *Microbacterium* sp. strain 15III

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The present study was conducted to advance in the characterization of the plant growth-promotion properties of a native strain of *Microbacterium* sp. strain 15III, isolated earlier from a microalgal non-axenic culture. We observed previously a dose-dependent modulation of wheat seeds germination and sprouts growth form promotion (lower dose) to strong inhibition (higher dose) by inoculation of *Microbacterium* cells. Inoculation of wheat seedlings with this bacterium promoted leaves and roots dry weigh, and leaves length and chlorophyll content. In this study, to evaluate whether growth promotion would be at least mediated by volatile compounds, we conducted similar experiments in which the Petri dishes also contained a smaller dish containing *Microbacterium* sp. at different densities onto LB medium. The results suggested that the previously observed wheat growth-promotion could be mediated by volatile compound released by the bacterium. To further analyze whether this effect could be a general effect on plant-growth modulation, similar experiments were conducted with *Arabidopsis thaliana*, as a plant distantly related to wheat, and also a convenient experimental model. In these experiments, *Microbaterium* sp. exerted a similar dose-dependent seedlings growth modulation from stimulation to strong inhibition at higher bacterial densities. Similar experiments using chambered Petri dishes to isolate plants from bacteria, showed a similar dose-dependent plant growth modulation. Asymmetric placement of seedlings and bacteria in the dishes also showed a gradual effect according to the relative distance between the seedlings and the bacterial inoculation spots. *A. thaliana* seedlings exposed to this bacterium's volatile compounds showed a dose-dependent more branched root-architecture and a significant increase in the number of root hairs. A preliminary gas chromatography coupled to mass spectrometry (GC-MS) analysis revealed the identity of the most abundant volatile compounds as small nitro-sulfur compounds such as dimethyl trisulphide and imidazolthione, which are strong candidates to be involved in the plant-growth modulating properties of *Microbacterium* sp. strain 15III. After immersion of *A. thaliana* flowers with a suspension of *Microbacterium* cells, the bacterium was consistently recovered from surface disinfected seeds and remained cultivable. It appeared that immersion in a higher cell density produced higher bacterial titles in the disinfected seeds. These results suggest a possible facultative endophytic life-style, and tolerance to the dehydrating conditions during seed development.

MI-P079-267
ADAPTATION OF *Pseudomonas aeruginosa* TO THE INTRACELLULAR MILIEU OF EUKARYOTIC CELLS

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Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the airways of cystic fibrosis (CF) patients. Major traits such as a biofilm mode of growth and hypermutability, are considered to constitute a source for adaptive phenotypes and causes of the increased tolerance and resistance of *P. aeruginosa*. Another mechanism through which pathogens are capable of evading the immune response, as well as exposure to some antibiotics, is the ability to thrive in the intracellular environment of the eukaryotic cell. However, the relevance of this mechanism in the ability of *P. aeruginosa* to persist in CF chronic infections is poorly explored. Here we performed a long-term evolution experiment with hypermutator and wt strains of *P. aeruginosa* by carrying out successive reinfection assays, which consisted in using intracellular bacterial cells, recovered after antibiotic exclusion assays from A549 lung epithelial cells, as the inoculum for the next round of infection. A549 cells were lysed to recover intracellular bacterial cells in each infection assay to measure invasiveness (t0), or