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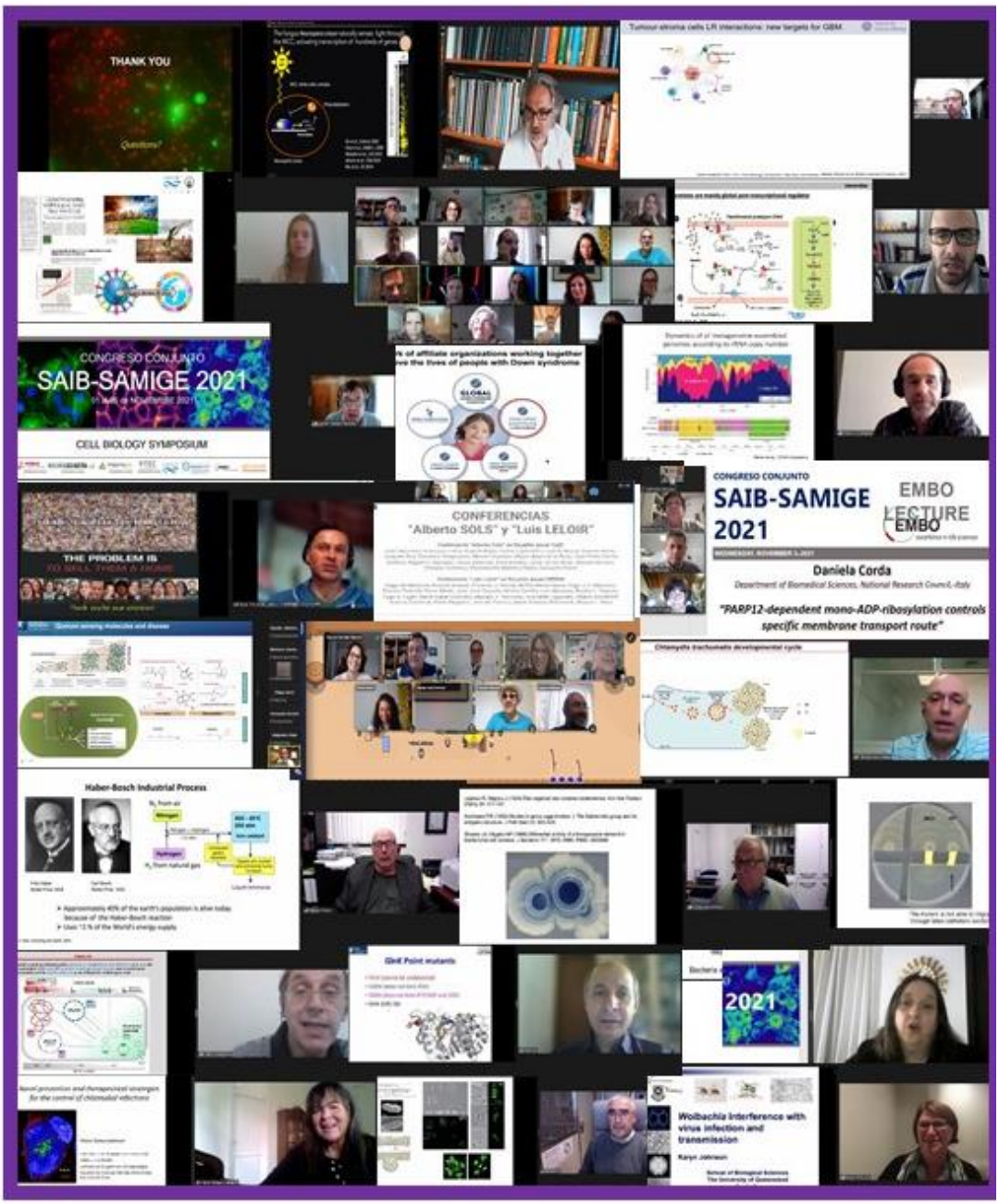
SAIB - SAMIGE Joint meeting
2021 *on line*



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

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17.57% (637 of 3669). The analyses of prevalence and genotyping showed that 82 positive samples (12.87%) belonged to 16-HPV, 32 samples (5.02%) were positive for 18-HPV and 446 for other HR-genotypes (70.02%). Interestingly, 77 samples (12.09%) were positive for more than one genotype, being 52 samples (8.16%) positive for panel 16-HPV+other, 16 (2,51%) for 18-HPV+other, 6 samples (0.94%) for 16-HPV+18-HPV, and 3 samples (0.47%) were positive for 16-HPV+18-HPV+other. The knowledge of the prevalence and genotyping of HPV in women from San Luis shows the dimension of the problem at the local level and is important to plan and develop prevention and treatment strategies by the government agencies to prevent and contain the disease in the provincial territory.

MI-P135-289
SUCROSE METABOLISM IN *Nitrosomonas europaea*

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The synthesis of sucrose (Suc) is carried out by the combined action of Suc-6P synthase (SucPSase, EC 2.4.1.14) and Suc-6P phosphatase (SucPase, EC 3.1.3.24). *Nitrosomonas europaea* is an ammonium oxidizing bacterium and is classified as a chemolithoautotroph organism. *N. europaea* can grow either autotrophic or heterotrophically when the carbon source is CO_2 or fructose, respectively. We found that *N. europaea* has a sequence coding for an ~80 kDa protein highly homologous to SucPSase type II (possessing both SucPSase and SucPase domains). Our previous results showed that the SucPSase type II displayed low SucPSase (0.065 U/mg) and SucPase (0.012 U/mg) activities. Conversely, the SucPSase and SucPase domains displayed activities of 0.33 and 30 U/mg, respectively, when separately expressed. Immunodetection assays against the SucPase domain in crude extracts from *N. europaea* grown with fructose as sole carbon source showed that the SucPSase type II protein is expressed in its complete and low-active form. Since the CO_2 present in the air can be considered as a limiting substrate, we developed a device to grow cells with constant air bubbling using an aeration pump. We observed a notable increase in OD (2-fold higher) compared to the condition without air supplementation. When we performed immunodetection on extracts from cells grown under well-aerated, chemolithoautotrophic conditions, we detected the separated, highly-active domains. Results indicate that the enzyme is present in its low-activity, complete form (~80 kDa) when the bacterium grows heterotrophically, whereas SucPSase and SucPase domains are separated under chemolithoautotrophic conditions. Curiously, we found a gene that codes for a serine peptidase of the S8 family in the genome of *N. europaea*. This protease would cut the enzyme at the linker of both domains, giving rise to the most active forms of the enzyme. Based on these results, we hypothesize that sucrose metabolism in *N. europaea* could be regulated by proteolysis, a fast response to environmental changes.

MI-P136-290
CHEMOTAXIS AND WSP-LIKE PATHWAYS AFFECT BIOFILM FORMATION IN *Halomonas titanicae* KHS3

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Halomonas titanicae KHS3 is a moderately halophilic bacteria isolated from seawater of Mar del Plata harbour. During the analysis of its genomic sequence, two chemosensory clusters were identified. Cluster 1 includes genes and organization similar to those of the canonical *Escherichia coli* chemotaxis gene cluster and is involved in *Halomonas* chemotaxis. Cluster 2 encodes a Wsp-like pathway, whose genes and organization resemble those of the homonymous *Pseudomonas aeruginosa* cluster. In this pathway, a chemoreceptor-controlled histidine kinase activates a diguanylate cyclase (DGC) by phosphorylation, and the downstream response includes higher levels of biofilm. In this work, the participation of both chemosensory pathways in motility and biofilm formation was analyzed. Cluster 1 function was disrupted by a deletion in its histidine kinase gene, *che*A1 (*che*1- mutant). The wsp-like pathway was targeted in two different ways. On one hand, Htc10 (cluster 2 chemoreceptor) was inactivated by a plasmid insertion (*che*2- mutant). On the other, the methylesterase gene *che*B2 was deleted in order to assess the effect of an overmethylation (and presumably over-activation) of the pathway on the phenotype (*che*2++ mutant). Both *che*1- and *che*2++ mutants showed a significantly exacerbated biofilm formation when compared to wild-type strain when using the crystal violet assay. However, only the *che*2++ cells had a wrinkly aspect in agar medium, suggesting that the increased ability to form biofilm of the two strains was due to different mechanisms. Chemotaxis behavior, as assessed in soft agar plates, was severely affected in both hyperbiofilm mutants. However, when compared by video tracking analysis using SMT software, the motility of *che*1- mutant was indistinguishable from the wild-type strain, whereas *che*2++ showed a remarkable decrease in the number of motile cells. Substrate adherence after a short centrifugation was significantly increased in *che*2++ cells, and long-term biofilm assays also showed increased persistence of adhered cells in this mutant strain. Likewise, Congo Red staining of macrocolonies revealed an increased production of exopolysaccharides in this strain. All these features are consistent with a role of cluster 2 in biofilm formation as described for the *Pseudomonas* wsp pathway. Consistently, the *che*2-mutant showed a reduced ability to form biofilm under the same circumstances. The hyperbiofilm phenotype of the *che*1- mutant remains intriguing: complementation with very low levels of CheA1 restores the wild-type biofilm behavior even though chemotaxis is not fully restored. Up to now, we cannot find the mechanism underlying the

increased biofilm in the absence of the chemotaxis kinase. Disruption of the cluster 2 chemoreceptor gene in the *che*1- mutant will help to elucidate whether or not the hyperbiofilm phenotype is dependent on the presence of cluster 2.

MI-P137-292
HTC10, THE CHEMORECEPTOR OF THE WSP-LIKE PATHWAY IN *Halomonas titanicae* KHS3

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The marine bacterium *Halomonas titanicae* KHS3 encodes a cluster of chemotaxis-like genes that exhibits certain peculiarities highly reminiscent of the *Pseudomonas* Wsp system. Besides including a diguanylate cyclase (DGC) with a receiver domain that is presumably activated by a chemoreceptor-controlled histidine kinase, it includes two CheW coupling proteins, an extra tetratricopeptide (TPR) domain at the C-terminus of CheR2 methyltransferase, and an extra receiver domain at the C-terminus of the CheA2 kinase. In *Pseudomonas*, the Wsp pathway is involved in biofilm formation through an increment of the c-di-GMP levels. However, molecular details about how the pathway is controlled are still missing and its activation has only been observed in response to growth on solid surfaces. We were interested in characterizing the role of Htc10, the chemoreceptor encoded within the Wsp-like cluster in *H. titanicae* KHS3, in the control of the pathway. The overexpression of the methyltransferase CheR2 resulted in a significantly increased ability to form biofilm, suggesting that a higher level of methylation of the receptor correlates with an activation of the pathway that result in higher levels of c-di-GMP. Consistent with that, a mutant with an interrupted *htc10* gene did not show any increase in biofilm upon overexpression of CheR2. In the search for stimuli that control the pathway, the ligand binding domain of Htc10 (LBDHtc10) was expressed with a 6X-histidine tag, purified, and subjected to thermal shift assays against a library of compounds. The purine derivatives guanine and hypoxanthine shifted the melting temperature (Tm) of LBDHtc10 by more than 10°C. The LBDHtc10 was crystallized in the presence of hypoxanthine and guanine, and analyzed by X-ray diffraction. The structure could not be solved by molecular replacement, thus, a new crystallization in the presence of selenomethionine was carried out, and the diffraction data from the obtained crystals were used for experimental phasing. Those data were used to solve the structure of the ligand binding domain from a crystal at 2.1 Å resolution. LBDHtc10 has a double Cache structure, and the ligand binds in the distal pocket. Contacts between the residues Y125, N161, D163 and N177 and the ligand were identified. Proteins carrying the mutations Y125F or N161A/D163A were expressed and subjected to thermal shift assays in the presence or absence of hypoxanthine. In the absence of any ligand, the Tm of the three recombinant proteins was around 45°C, indicating that the mutations did not alter the folding in a drastic way. In contrast, whereas the presence of 10 µM of hypoxanthine caused a shift of 15ºC in the Tm of the wild-type protein, it hardly changed the Tm of the two tested mutants. We have demonstrated the involvement of Htc10 signaling on biofilm formation, and its specific recognition of purine derivatives. Ongoing studies are aimed to understand the role of ligands on biofilm control.

MI-P138-296
VALIDATION OF A MULTIPLEX TaqMan qPCR SYSTEM FOR DETECTION OF *Salmonella* spp.

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Salmonella enterica is one of the major bacterial agents that cause foodborne infections in humans all over the world. Traditional *Salmonella* detection methods are based on cultures using selective media and characterization of suspicious colonies by biochemical and serological tests. These methods are generally time-consuming. The TaqMan system was designed as a multiplex reaction to simultaneously detect three molecular markers of *Salmonella* spp. The target sequences for *Salmonella* spp. were one housekeeping gene and two genes that encode for virulence factors. The validation assays for multiplex TaqMan system was carried out by real time PCR using different collection strains. The strains were: *Salmonella* Choleraesuis (ATCC 4931), *Salmonella* Typhi (CECT 4594), *Salmonella* Typhimurium LT2 (ATCC 15277), *Salmonella enterica* subsp *arizonae* (CECT 4395, 4396), *Shigella disenterea*e (CECT 584), *Shigella sonnei* (CECT 457), *Escherichia coli* (50365 NCTC), *Escherichia coli* O157:H (NCTC 12900), *Serratia marcescens* (ATCC 14041, 13880), *Vibrio campbellii* (ATCC 25920), *Enterobacter sakazakii* (ATCC 29544), *Yersinia enterocolitica* (CECT 500), *Listeria monocytogenes* (CECT 4032), *Staphylococcus aureus* (ATCC 9144). Additionally, a total of 141 *Salmonella* spp clinical strains were isolated during the 2018 outbreak in Salta-Argentina, 16 *Salmonella* spp. and 12 *Escherichia coli* strains isolated from Laboratory food hygiene inspection and control laboratory from Spain, were tested. The limit of detection (LoD) was determined using the standard curve of *Salmonella* Typhimurium ATCC 14028. The viable counts (CFU/mL) of cell suspension was determined by plating 100 µL of each dilution on S-S Agar (Britania) in triplicate and were incubated at 37°C for 24 h. The detection system TaqMan qPCR multiplex has 100% inclusivity and exclusivity, all *Salmonella* strains used were accurately detected. The method did not report any false-positive results. After standardization, the efficiency of the TaqMan qPCR reaction was> 98%, with a dynamic range of 7 orders (R^2= 0.98) for all molecular markers. The cut off assumed values of > 43.67 (Ct) for the