Código de Resumen: FM-001

Sección: Fisiología Microbiana

Modalidad: Oral

IMPACT OF THE *Rosmarinus officinalis* 1,8-CINEOLE ESSENTIAL OIL ON THE STRUCTURE AND VIABILITY OF PREFORMED *Klebsiella pneumoniae* BIOFILM

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Microbial biofilms are bacterial communities embedded in a self-producing matrix that develop on both living and nonliving surfaces, which exhibited a high resistance to antibiotics and the host immune defenses. In recent years, compounds of plant origin have been proposed as new alternatives for controlling infections caused by bacteria with biofilm-forming capacity. This study addresses the antimicrobial effect of 1,8-cineole, a main constituents of *Rosmarinus officinalis* essential oil against *Klebsiella pneumoniae,* a pathogen that can causes infections associated with biofilms. Biofilms of *K. pneumoniae* clinical isolates were developed at 37 °C in M9 medium on polystyrene 96-well plates. After 24 h, biofilms were treated with increased concentrations of the main constituents of rosemary essential oil for additionally 24 h. Following incubation, culture supernatants were discarded, and biofilms were stained with crystal violet (CV) to determine biofilm biomass. Additionally, fluorescence microscopy and confocal scanning laser microscopy (CSLM) were used to study biofilm structure.Colony-forming units (CFU) were determined to assess bacteria viability by the broth microdilution technique. Results obtained after CV staining showed that 1,8-cineole, one of the main essential oil constituents of *Rosmarinus officinalis*, at 1-2% v/v was able to significantly reduce the biomass of 24-h preformed biofilm. Data obtained by CFU counting evidenced that this treatment with 1,8-cineole allowed a reduction in bacterial viability (approximately 2 logs reduction) compared to the untreated control. Besides, we investigate the effect of 1,8-cineole on the structure of the 24-h preformed biofilm using the variant of a clinical *K. pneumoniae* isolate transformed with a plasmid expressing the green fluorescent protein GFP by CLSM. Bacterial samples treated with 1% and 2% v/v of 1,8-cineole showed dispersed layer of bacteria adhered to the surface of the coverslip and small multicellular aggregates, respect to the untreated control in which a regular complex matrix was observed. Biomass quantification with Comstat software (ImageJ) showed that the plant compound at 1% and 2% v/v decreased *K. pneumoniae* biofilm biomass 4 and 11 times, respectively, compared to biofilms treated with 0.5% ethanol (vehicle). Our findings support the idea that 1,8-cineole shows a high potential for the prevention/treatment of infections associated with *K. pneumoniae* biofilms.

Código de Resumen: FM-002

Sección: Fisiología Microbiana

Modalidad: Oral

CHEMICAL CHARACTERIZATION OF ANTIFUNGAL LIPOPEPTIDES PRODUCED BY *Bacillus amyloliquefaciens* SL-6

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Bacillus lipopeptides, produced by non-ribosomal peptide synthetases, are promising antifungal compounds against a wide range of phytopathogenic agents. These metabolites are small molecules (900-1550 Da) that contain a cyclic structure of 7 to 10 amino acids and a beta-hydroxy fatty acid or beta-amino fatty acid of variable lengths. They have been grouped into three families known as surfactins, iturins and fengycins according to their structural characteristics. These secondary metabolites are secreted as a mixture of homologues and/or isoforms. The present study reports the isolation and chemical characterization of fengycins synthesized by *B. amyloliquefaciens* SL-6, active against fungal phytopathogens.

The SL-6 strain was grown in batch culture with orbital shaking at 200 rpm for 24 h at 30ºC, using Synthetic Mineral Broth. Lipopeptides in cell-free supernatant (CFS) were precipitated overnight, at 4°C by addition of $HCl_{(c)}$ up to pH 2; the post-centrifugation supernatant was discarded and the yellow pellet was extracted with methanol to ten-fold concentration (ME10x). Antifungal titers of CFS and ME10x were estimated against *Alternaria alternata* and *Botrytis cinerea* by two-fold serial dilution, assayed using well diffusion method. Thin layer chromatography (TLC) were performed to separate lipopeptides from ME10x, using chloroform/methanol/water (65:25:4 v/v/v) as chromatographic system. Bands were visualized with ninhydrin, water and UV light to calculate retention factors (Rfs). Then, bioautographic assays were carried out with both fungi. Bioactive lipopeptides were scratched from the silica plate and extracted with methanol for chemical characterization by nanoHPLC-ESI-MS/MS and UV-MALDI-TOF-TOF.

The antifungal activity against *B. cinerea* and *A. alternata* in CFS was 25 AU/ml and 400 AU/ml respectively; without significant loss of titers in ME10x (200 AU/ml and 3200 AU/ml). TLC visualization methods showed the migration pattern of fengycins (Rf~0.1-0.2), bacillomycin (Rf~0.3), iturin A (Rf~0.4) and surfactins (Rf~0.7-0.8). Although, TLC-bioautography indicated a unique inhibition zone at Rf~0.1-0.2 for both organisms. ESI and MALDI mass spectra revealed the presence of two main signals (m/z 1464.8, 1478.8) and four weaker ones (m/z 1435.8, 1449.8, 1491.8, 1506.8) corresponding to fengycins homologues ranging from C14 to C17; whereas three iturins were detected at much lower intensity signals (m/z 1044.5, 1058.5, 1072.7). By MS/MS, fragmentation patterns showed the pairs of ions m/z 1080, 966 and m/z 1108, 994; which are considered fingerprints of fengycin isoforms A and B, respectively.

Therefore, six different homologues of fengycins A (C14 to C17) and fengycin B (C16–C17) secreted by *B. amyloliquefaciens* SL-6 were the major antagonistic compounds against the phytopathogens *A. alternata* and *B. cinerea*.

Código de Resumen: FM-003

Sección: Fisiología Microbiana

Modalidad: Poster

BIOFILMS: INCREASED TOLERANCE TO PENICILLIN IN *Streptococcus uberis* ISOLATED FROM BOVINE MASTITIS

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Mastitis is the most common and expensive disease affecting dairy cows throughout the world. *Streptococcus uberis* is one of the most prevalent environmental pathogens, causing a significant proportion of subclinical and clinical intramammary infections. Penicillin is still one of the first-line β-lactam antibiotics for the prophylaxis and treatment of pathologies in animals, including mastitis. Therapeutic failures cannot always be explained by the occurrence of antibiotic resistance determinants. Biofilm formation is a mechanism that allows to pathogens to become persistent colonizers, resist clearance by the host immune system, and enhance their resistance to antibiotics. We recently reported that more of 90% *S. uberis* isolates are able to produce biofilms in broth supplemented with 0.5% glucose or 1% sucrose. The objective of this study was to evaluate a possible relationship between biofilm formation ability, fibronectin and laminin addition, and penicillin tolerance of 34 *S. uberis* isolates, both for biofilm-growing bacteria and for planktonic bacteria. Minimal inhibitory concentrations (MIC) of penicillin for all isolates were determined in Mueller Hinton Broth (MHB) according to the 2012 guidelines of the CLSI. Antimicrobial susceptibility of bacteria embedded in a 24 h biofilm, minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC) were determined using Calgary Biofilm Pin Lid Device. Laminin or fibronectin were supplemented at a concentration of 10 μg/Ml. The MIC values of penicillin of *S. uberis* planktonic cells varied between 0.09 and 0.83 μg/mL; hence all isolates were sensitive to penicillin. MBIC values varied between 0.25 to 4 μg/mL, whereas MBEC values ranging from 8 to 256 μg/mL. These results shows that biofilm-growing *S. uberis* cells required higher concentrations of the antibiotic than those needed to inhibit planktonic cells. Similar MBIC values of penicillin were obtained when *S. uberis* cells growing in THB supplemented or not with laminin or fibronectin, whereas the MBEC values increased 5 times when both proteins were added to culture medium in relation to the medium without proteins. To the best of our knowledge, this is the first report of enhanced tolerance to penicillin likely related to a higher production of biofilms estimulated by laminin or fibronectin, glycoproteins found in extracellular matrix. Therapeutic failures of penicillin to treat *S. uberis* infections may be due to biofilm formation. Culture techniques using Calgary Biofilm Device reveal the properties of biofilm growing bacteria and, antibiotic susceptibility testing therefore gives more accurate results than ordinary culture techniques, reflecting the increased tolerance of the bacteria living in biofilms. Further investigations to establish epidemiological cutoff values of penicillin more stringent seems mandatory for successful therapy of persistent infections caused by biofilm growing cells of *S. uberis*

Código de Resumen: FM-004

Sección: Fisiología Microbiana

Modalidad: Poster

FIBRONECTIN AND LAMININ INDUCES *IN VITRO* BIOFILM FORMATION BY *Streptococcus uberis*

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Streptococcus uberis is one of the most important environmental pathogens associated with clinical bovine mastitis, but such bacterial infections can evolve towards chronic and subclinical mastitis, with high cell counts and persistence of the bacteria in the mammary gland. They represent a serious economical problem for dairy farmers with reduction of milk production and quality. Biofilm is a structured microbial community of bacterial cells added and embedded in an extracellular polymeric matrix. Biofilm formation increases the resistance to both the host immune system and the antimicrobial agents, and therefore represents one of the most important survival mechanisms of bacteria persistently colonizing the extracellular niche. *S. uberis* is also known to adhere, and subsequently penetrate, and even survive within the epithelial cells of the mammary gland. The role of the high-molecular weight glycoproteins of the extracellular matrix as laminin and fibronectin in this adherence has been described, forming a bridge between the host cell and the bacterial pathogen that leads to adhesion and internalization in the mammary epithelial cell. The objective of this work was to evaluate the effect of fibronectin and laminin on the ability of *in vitro* biofilm production in 34 *S. uberis* isolates*. S. uberis in vitro* biofilm formation in Todd Hewitt broth (THB) was determined by growing of overnight cultures in THB and using crystal violet staining. Laminin or fibronectin were supplemented at a concentration of 10 µg/mL. Each isolate was tested in triplicate, and the assay was repeated two times. The following classification was used for the determination of *in vitro* biofilm formation: no biofilm production (ODs ≤ ODnc), weak biofilm production (ODnc < ODs ≤ 2 ODnc), moderate biofilm production (2 ODnc < ODs ≤ 4 ODnc) and strong biofilm production (4ODnc < ODs). It was observed that a high percentage (76.5%) of the isolates was classified as weak biofilm producers in THB, while moderate producers and non-biofilm producers were observed in 5.9% and 14.7%, respectively, of the isolates. A lower percentage (2.9%) of non-biofilm producer isolates was observed in the medium supplemented with laminin, while weak and moderate biofilm producers were detected in 50% and 38.2%, respectively, of the isolates. When THB was supplemented with fibronectin, all the *S. uberis* isolates showed *in vitro* biofilm-forming capacity. Fifty-three percent of them were classified as strong biofilm producers, while forty-seven percent were moderate and weak producers. To the best of our knowledge, this is the first report of *in vitro* biofilm formation of *S. uberis* induced by both fibronectin and laminin, being the first was more effective. Further investigations to understand the role of biofilm in the survival strategy of *S. uberis* and the complex relationships between biofilm formation and adhesion-supporting extracellular proteins are warranted

Código de Resumen: FM-005

Sección: Fisiología Microbiana

Modalidad: Poster

EPS II FROM *Sinorhizobium meliloti* AS AN ADHESIVE MOLECULE IN MULTI-SPECIES BACTERIAL AGGREGATES

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Sinorhizobium meliloti is a rhizobium able to establish a nitrogen fixing symbiosis with the *Medicago* legume. This bacterium produces a mix of exopolysaccharides (EPSs) identified as succinoglycan or EPS I and galactoglucan or EPS II. EPSs play a major role in the bacterial processes of cell aggregation, flocculation, and biofilm formation. Those physiological events are critical for the initial steps of the interaction mechanism between bacteria and eukaryotic hosts.

Bacterial aggregation is a highly specific process which involves interaction between molecules from bacterial surfaces that act as adhesins and complementary receptors, which are carbohydrates and proteins respectively. The bacterial aggregative mechanism can occur between bacteria belonging to the same species (autoaggregation) or between bacteria of different genus (co-aggregation). Since the process requires protein-saccharide interactions on the surfaces of both partners, aggregation can be modulated by the regulation of EPSs synthesis. Co-aggregation interactions contribute to the initial development of biofilms through specific recognition and adhesion of single, genetically different bacteria in suspension and the subsequent adhesion of previously co-aggregated cells.

The aim of the present work was to study co-aggregation between *S. meliloti* strains and different rhizospheric bacteria. The co-aggregative phenotype was analyzed in the wild type *S. meliloti* Rm 8530 strain (complete EPS producer) and in their derivative mutants in the EPS synthesis: *exoY* strain (EPS I defective), *expA* strain (EPS II defective) and *exoY-expA* double mutant strain (DM, EPS defective). Each *S. meliloti* strain was tested for co-aggregation with microorganisms that share the same ecological niche, including *Pseudomonas fluorescens*, *Azospirillum brasilense* and *Burkholderia* sp. In general, the strains producing EPS II (wt, *exoY*) were shown to produce more co-aggregation compared to those strains incapable of producing EPS II (*expA*, DM). Co-aggregation assays were also carried out by adding to washed cells different sources of EPSs, i.e. either from supernatants of single cultures or purified EPS. It was always detected that the co-aggregative percentage was increased by providing a source of EPS II. Accordingly, it is concluded that EPS II from *S. meliloti* play an important role in the

co-aggregation process with different rhizospheric bacteria. This exopolysaccharide may be a key factor for microorganisms to start the development of biofilms in nature. Moreover, this molecule could be considered as a possible universal connector with several relevant properties at ecological, biotechnological and agro-productive levels.

Código de Resumen: FM-006

Sección: Fisiología Microbiana

Modalidad: Poster

SIDEROPHORE PRODUCTION BY BIOFILMS AND PLANKTONIC CULTURES OF UROPATHOGENIC *Klebsiella pneumoniae* AND *Escherichia coli* STRAINS

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Uropathogenic strains of *Escherichia coli* and *Klebsiella pneumoniae* form mixed biofilms in urinary catheters. We have recently reported that *K. pneumoniae* partially outcompete *E. coli* in mixed biofilms developed in artificial urine medium (AUM) and that a nutrient limitation is involved in the competition mechanism. A greater ability of *K. pneumoniae* to utilize Fe^{3+} as iron source, compared to *E. coli*, was also evidenced. Both species are capable to produce four iron-scavenging molecules named siderophores: the catechol types enterobactin and salmochelin, the hydroxamate type aerobactin, and the mixed type yersiniabactin. Here, we aim to evaluate siderophore production by clinical *K. pneumoniae* and *E. coli* strains. Bacteria were grown in modified M9 or AUM, supplemented with the Fe^{2+} chelator DIP (200 µM), for 2 d at 37°C. Analysis of siderophore production was performed by using the chrome azurol S (CAS) assays both in solution and on agar plates. Chemical determination of catechol- and hydroxamate-containing molecules was performed in planktonic culture supernatants and supernatants from 5 days-old biofilms, by standard techniques. CAS assays evidenced the expression of siderophores by both species when growing in M9 and AUM. Interestingly, in the CAS agar assay *K. pneumoniae* strains generated yellow halos whereas *E. coli* strains produced pink halos, suggesting a differential ability of these bacterial species to perform Fe^{3+} uptake from the blue CAS-Fe^{3+} complex. When growing in M9, *E. coli* supernatants had both catechol- and hydroxamate-containing compounds [90 and 240 pmol/10^6 cells), whereas *K. pneumoniae* supernatants only displayed catechol-containing molecules (50 pmol/10^6 cells). In AUM, *E. coli* only presented hydroxamate-containing compounds (70 pmol/10^6 cells), whereas *K. pneumoniae* exclusively displayed catechol-containing molecules (7 pmol/10^6 cells). These results suggested a differential siderophore expression depending of the culture media used. Analysis of biofilm supernatants showed similar levels of catechol-containing substances in both single-species *K. pneumoniae* and *E. coli* biofilms and mixed biofilms. Regarding hydroxamate-containing compounds, single-species *E. coli* biofilms expressed higher amounts of these molecules, compared to both single-species *K. pneumoniae* biofilms and mixed biofilms (20-fold and 10-fold higher, respectively). The results observed in mixed biofilms might be a consequence of the *K. pneumoniae*: *E. coli* cell ratio in these biofilms (70:1). Altogether, the results presented show that both bacterial species are able to express siderophores in the conditions assayed. Additionally, even when *E. coli* seems to express higher amounts of siderophores than *K. pneumoniae* when grown as single-species biofilms, in mixed biofilms a differential siderophore expression or utilization might take place, and therefore allowing *K. pneumoniae* to outcompete *E. coli*.

Código de Resumen: FM-007

Sección: Fisiología Microbiana

Modalidad: Poster

PACHYPODANTHINE: AN APORPHINOID ALKALOID THAT INHIBIT PLANKTONIC GROWTH OF *YERSINIA ENTEROCOLITICA*

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Yersinia enterocolitica is a Gram-negative coccobacillus belonging to *Enterobactereaceae* family. It is an important foodborne pathogen causing gastrointestinal disease in humans known as yersiniosis. *Y. enterocolitica* is widespread in the environment and animal populations. The main reservoir of human pathogenic *Y. enterocolitica* strains are pigs. So, contaminated pork and pork products are the most important source of infection. Due to the inappropriate use of antibiotics in the last years has allowed

microorganisms evolve resistance to them, it is important to look for an alternative to battle against bacteria. Alkaloids are abundant secondary metabolites in plants and represent one of the most widespread classes of compounds endowed with multiple and varied pharmacological properties. With the intention of contributing to solve this global problem, in previous studies, we examined a set of aporphinoid alkaloids as possible effective antimicrobial agents on planktonic growth of *Y. enterocolitica* strain. We found that Pachypodanthine inhibited planktonic growth at 100 µM. The aims of this study are determinate the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) and evaluate the growth inhibitory effect at 96 h of culture of Pachypodanthine on *Y. enterocolitica* bio-serotype B1A/O:7,8-8-8,19, isolated from pork sausage in our laboratory. The MIC and MBC was determined by the microdilution test in Moeller Hinton broth (MH) according to Clinical and Laboratory Standards Institute (CLSI), with 100 µM of initial concentration and subsequent serial dilutions in half, at 37 °C for 24 h. A growth curve was made with an starter inoculum at DO_{610} of 0.05 in MH broth using the inhibitor at MIC concentration. Aliquots of 0.1 ml were measured at OD_{655} after 3, 6, 18, 24, 48, 72 and 96 h of culture at 37°C using a microplate reader. Results show that MIC value was 100 µM, as this was the highest tested concentration it was not possible to obtain the MCB value. Its inhibition increased from 62.5 % at 24 h to 76.9 % at 96 h of culture with MIC value. In addition, we assayed MIC and MBC on different biotypes (B) of *Y. enterocolitica* strains, resulting for B1B strain 100 µM; for B2 and B3 strains 50 µM; and for B4 25 µM for both determinations in all cases. These results suggest that Pachypodanthine presents great inhibition of *Y. enterocolitica* B1Aplanktonic growth at 100 µM improving its effect until 96 h of culture, indicating that it does not degrade over time. Furthermore, this compound can inhibit planktonic growth of different *Y. enterocolitica* biotypes showing activity in almost all existing biotypes. We concluded that Pachypodanthine could be used as antimicrobial agent in the future to prevent intestinal diseases.

Código de Resumen: FM-008

Sección: Fisiología Microbiana

Modalidad: Poster

Yersinia enterocolitica IN FOOD: RELATIONSHIP BETWEEN ITS ISOLATION ON CHROMOGENIC MEDIUM AND THE *IN VITRO* AUTOAGGLUTINATION TEST

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Yersinia enterocolitica is a widely distributed enteropathogen, with pigs as major reservoirs. This microorganism is associated to intestinal, extraintestinal and immunological manifestations. Humans are infected by consuming raw or inadequately thermally processed foods, or contaminated water. This species is classified into six biotypes (B):1A, 1B, 2, 3, 4, 5 and more than 60 serotypes (O). The virulence potential of *Y. enterocolitica* strains depends on the presence and expression of both chromosomal and plasmid (pYV)-borne genes. The B1A strains lack pYV but has some chromosomal virulence factors. YadA is a pYV-encoded adhesin that produces autoagglutination (AA) at 37°C, and is strongly linked to adherence to host cells and resistance to the bactericidal effect of serum. On the other hand, CHROMagarTM *Yersinia enterocolitica* allows differentiate pathogenic and nonpathogenic strains of this species by the different color of the colonies. The objective of this work was to evaluate the correlation between results observed on CHROMagarTM and the AA test for a collection of local Y. enterocolitica strains. Seventeen isolates of *Y. enterocolitica* were obtained by standard culture techniques from various foods purchased in retail stores of San Luis city, and identified by classical biochemical tests and Gram stain. Subsequently, the strains were cultured on CHROMagarTM at 30°C for 24 h and discriminated into pathogenic (1B, 2, 3, 4 or 5) and non-pathogenic (1A) isolates; moreover, AA test in trypticase soy broth (TSB) with incubation at 25 and 37°C for 24 h, was performed for each strain. The AA results showed that 35% (6/17) of the isolates were pYV+ carriers and there was a 100% correlation between CHROMagarTM and AA test. Our findings show that the isolation of *Y. enterocolitica* strains on chromogenic medium carried out simultaneously with a simple virulence indicator test as AA, might contribute to the presumptive differentiation of virulence plasmid-bearing and non-bearing *Y. enterocolitica* isolates.

Código de Resumen: FM-009

Sección: Fisiología Microbiana

Modalidad: Poster

SINERGIC EFFECT OF AMPHOTERICIN B AND GOLD NANOPARTICLES COMBINATION ON REGULAR AND PERSISTER CELLS OF *Candida tropicalis* BIOFILM

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Persister cells (PCs) are a small number of cells which were proposed to be dormant and nongrowing phenotypic variants of the isogenic cell population. PCs are distinguished from resistant mutants due to do not exhibit an increased minimal inhibitory concentration (MIC), and represent about 0.1 to 1% of the population. The existence of this small cell subpopulation was described for some fungal species of *Candida*, however, its existence in *Candida tropicalis* biofilms is controversial. The aim of this work was to study the antifungal (ATF) effect of amphotericin B (AmB) and gold nanoparticles (AuNPs) combination over PCs in *C. tropicalis* biofilm.

C. tropicalis NCPF 3111 biofilm formation was assayed by adhesion to 96-well plate and crystal violet (CV) stain (0.1 OD595nm=1BBU). The PCs experimental design proposed allowed comparing supra MIC ATF activity, oxidative stress and antioxidant response between two different biofilms, “Biofilms 1 -B1-” obtained from planktonic cells and exposed to supra MIC AmB treatment; and ”Biofilm 2 -B2-” a second biofilm, derived from PCs that survived to drug treatment. Biofilm was also analyzed by scanning electron microscope (SEM) and PCs fraction was determined by colony forming units counting. Extracellular reactive oxygen species (ROS) were measured by the reduction of the nitro-blue tetrazolium (NBT) reaction, and Reactive nitrogen intermediates (RNI) were measured by Griess assay. Superoxide dismutase (SOD) activity was assayed based on the inhibition of NBT reduction; total reduced glutathione (GSHt) by enzymatic determination and total antioxidant capacity was measured by FRAP assay.

A classic biphasic killing curve, indicative of PCs presence, was obtained and the equal MIC confirmed that they were PCs. We observed that AmB (200 µg / ml)/AuNPs (0.63 mM) combination produced a greater biofilm reduction (95%) than control biofilm. SEM images show a marked decrease in the amount of exopolysaccharides of the biofilm matrix, with predominant yeast form. These images show concordance with the marked decrease in biomass observed by staining with CV. ROS and RNI concentration showed similar increase in B1 and B2, whereas antioxidant defenses (SOD, GSHt and FRAP) were significantly higher in B2 than in B1, indicating that the oxidative misbalance may be important. PCs play an important role in recalcitrance of chronic infections, therefore the finding synergic ATF effect would help to solve the puzzle of biofilm resistance to ATFs.

Código de Resumen: FM-010

Sección: Fisiología Microbiana

Modalidad: Poster

ANTI-CYANOBATERIAL ACTIVITY OF *Bacillus amyloliquefaciens* SL-6 AGAINST *Tolypothrix tenuis*

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The latest trends in agriculture are directed to reducing the use of chemical fertilizers focusing on the search for sustainable alternatives. In this sense, *Bacillus* spp as plant growth promoting bacteria and nitrogen fixing cyanobacteria have been developed as biofertilizers. However, ribosomal (bacteriocins) and non-ribosomal (polyketides, lipopeptides and peptides) metabolites synthesis by *Bacillus* species with antimicrobial activity needs to be considered in order to determine the efficacy by a combined action of both microorganisms. This study aims to determine antibiosis effects of *B. amyloliquefaciens* SL-6 against the filamentous cyanobacterium *T. tenuis;* both organisms used in different agronomic applications.

The SL-6 strain was cultured in Synthetic Mineral Broth with orbital shaking at 200 rpm for 24 h at 30°C. Cell-free supernatant (CFS) was subjected to acid precipitation and methanol extraction (ME10x) for bioactive lipopeptides concentration. *T. tenuis* was grown in Watanabe medium (W), at 30°C under continuous illumination.In a first screening of anti-cyanobacterial activity, CFSs were diluted by serial 10-fold. Minimum Inhibitory Concentration (MIC) was determined by serial two-fold dilutions (up to 1/256) adding 500 µl of W medium containing *T. tenuis* inoculum (1~µg/ml of chlorophyll *a*) and 500 µl of CFS dilutions. Incubation was performed under continuous illumination for 7 d at 30°C. The MIC was defined as the highest dilution that resulted in the absence of photoautotrophic growth and was estimated in arbitrary units per milliliter (AU/ml) using the following formula: (1000/500)×(1/D), where D is the highest dilution that prevented growth of the cyanobacterium during 7 d incubation.

To determine the Minimum Algicidal Concentration (MAC), the biomass of the dilution representing the MIC and two of the more concentrated subsequent CFS dilutions were subcultured in the same conditions and expressed in AU/ml. To evaluate the antagonistic activity in lipopeptides fraction, several volumes (µl) of ME10x in sterile blank disks were impregnated and subsequently dried, due to methanol toxicity. The disks were added to cultures of *T. tenuis* in the conditions mentioned above.

B. amyloliquefaciens SL-6 showed inhibitory activity against *T. tenuis* in the preliminary screening. The MIC and MAC values were 128 AU/ml and 64 AU/ml, respectively. *T. tenuis* grew photoautotrophically in all the concentrations of ME10x tested; however when compared to the control, a partial inhibition in the synthesis of the chlorophyll *a* was observed with significant difference ($P<0.05$).

Antibiosis effects of *B. amyloliquefaciens* SL-6 against *T. tenuis* was evident in assayed conditions, without the lipopeptides extracted in methanol being responsible of such activity. Further studies should be conducted to elucidate the nature of this algicidal capacity of the local bacterial strain.

Código de Resumen: FM-011

Sección: Fisiología Microbiana

Modalidad: Poster

SYNTHESIS, CHARACTERIZATION AND STUDY OF ANTIBACTERIAL PROPERTIES OF MESOPOROUS SILICA NANOPARTICLES FUNCTIONALIZED WITH A COPPER MALEAMATE COMPLEX

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Mesoporous silica nanoparticles (MSN) are an interesting class of nanomaterials with potential application in biomedicine due to their low reactivity and high biocompatibility, high surface area, which allows the functionalization with several agents, and small particle size (below 100 nm) which permit their use in vivo. MSN-based materials have been functionalized with a wide variety of anti-inflammatory, antibiotics, antihypertensive and even anticancer pharmaceuticals, however, the use of MSN in antibacterial studies is still not explored in detail. In this context, bearing in mind the interesting antibacterial properties of copper complexes and the potential applicability of MSN in biomedical uses we have supported a copper(II) complex containing ethoxysilane-based maleamates and bipyridine ligands. The aim of this work was to determine the antibacterial effect of copper complexes against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213. The copper-functionalized nanosystem has been characterized by different methods such as XRD, XRF, SEM, TEM and FTIR observing that the synthesized material is composed of mesoporous silica nanospheres of ca. 80 nm and that the incorporation of the copper complex (with ca. 3.5% wt. Cu in the material) has taken place mainly inside the pores of the MSN. The antibacterial activity of these compounds was tested by the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC of two compounds was determined according to the broth microdilution procedure of Clinical and Laboratory Standards Institute. Serial dilutions of the complex, ranging from 0.02 to 500 mg/mL, were carried out in a microdilution plate (96 wells). The bacterial inoculum was then added to each well. The microplates were incubated at 37 °C for 18 h. MIC was defined as the lowest concentration which resulted in inhibition of visual growth. All these analyses were performed in triplicate. The two compounds assayed evidence antibacterial activity against *E. coli* and *S. aureus*. The data obtained show a better activity against *E. coli*. These results open up new perspectives in the field of biomedicine and in future research through the use of copper complexes as antibacterial agents alone or in combination with other antimicrobial compounds.

Código de Resumen: FM-012

Sección: Fisiología Microbiana

Modalidad: Poster

Candida albicans PLANKTONIC AND SESSILE CELLS TREATED WITH BIOSYNTHESIZED SILVER NANOPARTICLES

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Candida albicans is a normal commensal of the gastrointestinal microbiota in healthy individuals; however, as an opportunistic pathogen, is the most common etiological agent of candidiasis. *C. albicans* has the ability to form biofilms and morphogenetic conversions between yeast and hyphal morphologies contribute to biofilm development. These attached communities of sessile cells are surrounded by a protective exopolymeric matrix that effectively shelters *Candida* against the action of antifungals (ATFs). As fungi are eukaryotic, research and development of new ATFs agents have been difficult due to the limited number of selective targets, also leading to toxicity. Silver nanoparticles (AgNPs) were considered, in recent years, particularly attractive for the production of a new type of antimicrobials. Although the highly antibacterial effect of AgNPs has been described, their mechanism of action is yet to be fully elucidated. This study firstly evaluated the activity of biosynthesized AgNPs in *C. albicans* planktonic cells and then, the effect over biofilms.

The AgNPs were synthesized by an extracellular bioprocess. These were formed from reduction of silver ions by the supernatant of *Pseudomonas aeruginosa,* and were characterized by Ultraviolet-visible spectroscopy (UV-vis), dynamic light scattering (DLS) and transmission electron microscopy (TEM), *as was described* by Quinteros *et al. (*2016). Minimum inhibitory concentration (MIC), minimum fungicidal concentration for planktonic cells (MFC) and minimum inhibitory concentration of biofilm (MBIC) were determined by the AgNPs and amphotericin B (AmB) against *C. albicans* using the plate microdilution technique. Biofilm formation (48 h incubation) was tested by 96-well plate adhesion and crystal violet (CV) staining (0.1 OD595nm = 1BBU). Viable cells were determined by enumerating the colony-forming units per milliliter (CFU/ml) and the results showed a good correlation with the CV assay.

Our results demonstrate that AgNPs had a *stronger inhibition* of *C. albicans* planktonic cells. The MIC results showed that AgNPs were fungicidal against *C. albicans* SC5314 (0.037 pM) and *C. albicans* L20 (0.15 pM) at very low concentrations *compared to* silver standard ($AgNO_3$ 4 x 10^7 pM and 2 x 10^7 pM, respectively) or AmB (2.7 x 10^5 pM). Biofilm reduction of both strains was obtained, however, sessile cells were*not completely removed.* These results are *promising for the future* application, due to the high activity observed at verylow concentrations. *Nanoparticles*are*now consideredaviable alternative*to antibiotics and*seem*to*haveahigh potential*to*solve*the*problem*of the*emergence*of*bacterial multidrug resistance.*

Quinteros MA *et al.*, 2016. Silver nanoparticles: biosynthesis using an ATCC reference strain of *Pseudomonas aeruginosa* and activity as broad spectrum clinical antibacterial agents. Int J Biomater. 2016; 5971047.

Código de Resumen: FM-013

Sección: Fisiología Microbiana

Modalidad: Poster

STUDY OF BIOFOULING IN SURFACES TREATED WITH NANOTECHNOLOGY

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Ship hulls and other structures undergo a process called biofouling, that consists on the colonization of the substrate by microorganisms (i.e. bacteria, algae) that once settled, produce bioactive molecules that aid the invasion of macroorganisms (i.e. barnacles) which in turn can degrade the surface by chemical or physical reactions. Deterioration of ship hulls by biofouling produce an increment in hydrodynamic drag and in fuel consumption with the concomitant economic loss associated. Marine vessels are usually painted with an antifouling coat, that contains metals such as copper and tributyltin that have an adverse effect on the marine environment. Nanotechnology treated surfaces have been proposed as an environmentally friendly alternative to antifouling coats, as the presence of nanoscale roughness has been shown to prevent the colonization of surfaces by microorganisms, through a physical interaction that does not harm the environment. Polydimethylsiloxane (PDMS) is a silicone coating with antifouling properties which may be modified to increase its roughness, in this case with multi-walled carbon nanotubes (MWCNT)

In this project, we studied the growth and biofilm formation of different bacteria on treated surfaces. We selected two marine species, Marinobacter hydrocarbonoclasticus and Cobetia marina, that have a great capacity to form biofilms in hydrophobic and hydrophilic surfaces respectively, and two fresh water bacteria representatives, Pseudomonas fluorescens Pf-5 isolated from soil, and Pseudomonas veronii, isolated from tap water.

First, we tested different culture media and conditions to study growth and biofilm formation of these strains was assessed in 24-well plates. Biofilm formation was quantified via Crystal Violet (CV) Assay in 48 h cultures. M9 minimal medium Glucose as carbon source, was observed to be suitable for biofilm formation of Pseudomonas species, whilemarine bacteria in modify Sea

Salt Peptone (SSP) medium. Preliminary studies with M. hydrocarbonoclasticus were performed with: i) aluminum coated with PDMS, and ii) aluminum coated with PDMS+MWCNT. When biofilm formation on the two silicone coated surfaces were compared, the lowest was present in the bacteria grown on the surfaces containing the carbon nanotubes, indicating that the nanotechnology treated surface inhibited adhesion of bacteria and consequently biofilm formation. Bacterial growth was similar in the presence of both surfaces.

Nanotechnology treated surfaces have a great potential for antifouling applications, as they can lead to a reduction in the colonization by invading species without the use of chemical coating, which is toxic for the environment.

Código de Resumen: FM-014

Sección: Fisiología Microbiana

Modalidad: Poster

THE NOVEL PLASMIDIC TYPE I PROTEIN SECRETION SYSTEM RssDM OF *Rhizobium leguminosarum* bv. *viciae* strain 248 EXPORTS A RTX-PROTEIN.

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Symbiosis between *Rhizobium leguminosarum* (*R.l.*) and the legume host requires a complex interplay between the partners which induce the expression of bacterial genes encoded in the symbiotic megaplasmid (pSym). The identification of type I protein secretion systems (TISS) pSym-associated could contribute to generate genetic tools that could modulate the endosymbiont lifecycle or the symbiosis with legumes. *R.l.*bv.*viciae* 248 strain carries a pSym of about 200 kpb named pRL1JI, which confers the capability to nodulate pea and vicia plants. Our previous results confirmed that a pRL1JI-derivated cosmid, pIJ1552, contained the secretion locus of a novel plasmidic TISS in *R. l.* bv. *viciae* strain 248. This cosmid was able to restore the secretion of an extracellular protein of 49 kDa (EP49) both in a *R.l.* pSym-cured strain and a Tn*5*-pIJ1552 mutant clone that were incapable to export it. Sequence analysis of the Tn*5*-clone corroborated that the transposon was inserted in a putative ABC component of a TISS. In the same orientation and contiguous to the ABC transporter, genes encoding a MFP component and two ORFs of hypothetical target proteins were identified. Both ORFs belong to a family of calcium binding-proteins called RTX (**R**epeated in **t**o**x**in) that form pores in target membranes. The plasmidic TISS was called RssDM for **R**tx **s**ecretion **s**ystem **D** (ABC) and **M** (MFP) components, and the putative target proteins RTX-1 and RTX-2. Based on the amino-terminal sequence of the EP49, we corroborate that RTX-1 corresponds to EP49. Three *R.l.v*. 248 strain deletion mutants affected in *rtx-1*, *rtx-2* and double *rtx-1 rtx-2* genes were generated by homologue recombination. The secretome analysis of extracellular proteins by SDS-PAGE and Western blot using a rabbit antiserum against EP49 confirmed that RTX-1 is absent both in *rtx-1* and *rtx-1 rtx-2* mutants. Coomassie Colloidal staining suggest that a 36 kDa band was absent in the extracellular medium of *rtx-2* and *rtx-1 rtx-2* mutants. Mass Spectrometry (MS) analysis of the triptic peptides obtained from the 36 kDa band showed that it corresponds to RTX-2. To identify possible target proteins of the RssDM system, the secretomes of *R.l.v*. pRL1JI (*wt*) and derivative 248 strains were analyzed by LC-MS. Our results confirmed that although RTX-1 is substrate, RTX-2 secretion is independent of RssDM. In order to dilucidate the role of the TISS RssDM in the bacterial lifecycle, bacteriocin activity assays were performed using *R.l.v*. 248 derivative strains as bacteriocin´s producers against *Mesorhizobium loti* sensitive strains. These results suggest that the observed Mesorhizobium´s inhibition of growth is RssDM-dependent. Finally, we corroborate that RssDM TISS secretes at least a RTX-protein, RTX-1, and although the bacteriocin activity is associated to this system further experiments are required to dilucidate its identity.

Código de Resumen: FM-015

Sección: Fisiología Microbiana

Modalidad: Poster

Salmonella BIOFILMS: PARTICIPATION OF THE RcsCDB REGULATORY SYSTEM

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Bacterial biofilms are complex communities consisting of microorganisms embedded in a self-produced extracellular matrix. *Salmonella* is able to form biofilm on the surface of the gallstones in the gallbladders that produce the persistence of the bacterial colonization in the carrier patients. The RcsCDB phosphorelay system has an important role in the bacterial

physiology, manly in the response to extracytoplasmic stress signaling. It was shown that the factors affecting the cell envelope lead to the activation of the system and consequently the modulation of capsule synthesis, motility behavior and biofilm formation. Previously, in our laboratory we characterize the *rcsC11* mutant as a not virulent strain that can be used as an attenuated vaccine, producing RcsCDB constitutive activation. We here investigated whether the RcsCDB system activation conditions have the ability to produce red dry/rough (RDAR) morphotype and the levels of biofilm formation on polystyrene plates. For this purpose, we used the 14028s wild type strain harboring the p*rcsB* plasmid, or *tolB* and *rcsC11* mutants as RcsCDB system activation conditions. The ability of biofilm formation was also determined on uniform gallstones mainly composed of cholesterol, removed from a single lithiasic patient. To this end, gallstones were incubated in LB medium with and without bile salt, previously inoculated with wild type and *rcsC11 Salmonella* strains. After 7 days, the biofilm formed was evaluated by scanning electron microscopy. Our results demonstrated that the RcsCDB system activation negatively affects the *Salmonella* biofilm development. In addition, our findings on the inability of the *rcsC11* strain to form biofilm highlight once again that this mutant is an excellent candidate for the development of vaccines.

Código de Resumen: FM-016

Sección: Fisiología Microbiana

Modalidad: Poster

EVALUATION OF BIOFILM FORMATION CAPACITY OF *Bordetella pertussis* IN INTERMEDIATE VIRULENCE PHASE

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Bordetella pertussis is a strictly human pathogen that causes whooping cough. The transmission of the bacterium to a susceptible host occurs, in most cases, from a carrier adult where bacteria remain under some form of persistence. One of the mechanisms of colonization and persistence adopted by microorganisms is through biofilm formation. BvgAS signal transduction system regulates biofilm growth in *B. pertussis.* This system represses biofilm development when bacteria are at the avirulent phase (Bvg-), and activates it at the virulent phase (Bvg +) and in the intermediate phase (Bvgi). The transition from one phase to another occurs at temperatures below 30°C and in the presence of high concentrations of $MgSO_4$ or nicotinic acid (NA). It has been proposed that the Bvgi phase would be necessary for transmission of *B. pertussis*. In the nasal cavity the temperature is around 30ºC, so possibly, the bacteria found in this niche could be expressing an intermediate phenotype. Therefore, we decided to analyze the biofilm formation capacity in the intermediate phase, working with the Tohama I reference strain of B. pertussis and a clinical isolate (Bp 2723).

The sessile growth was analyzed using multiwell plates , cultivating the strains in Stainer-Scholte (SS) liquid medium with different concentrations of NA (previously reported as inducers of the Bvgi phase), for 24, 48 and 72 h. Biofilm architectures were examined by fluorescence microscopy. From these assays, we observed that the reference strain produced higher levels of biofilm after 72 h of growth in SS medium with 0.8 mM of NA, while for the clinical isolate the opposite effect was found. Finally, we analyzed the expression of specific genes of the virulence phases, in order to check whether the culture conditions employed induced a modulation to Bvgi phase. Therefore, the quantification of mRNA levels by qPCR of the *bip*A gene (intermediate phase gene) and *cyaA* (virulence phase gene) was performed. We confirmed that the reference strain had indeed changed its phenotype to intermedium, but under the same conditions the clinical isolate did not.

We conclude that *B. pertussis* Tohama I strain in Bvgi phase is able to produce higher levels of biofilm compared to its development in Bvg + phase. This was not the case of the clinical isolate, which rendered an avirulent phenotype at the assayed conditions. Therefore, it would be necessary to study with more detail the modulation conditions that lead to an intermediate phenotype for clinical isolates. These results highlight the differences between a clinical isolate and a reference strain in terms of adaptation to modulation conditions.

Código de Resumen: IN-001

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

DIFFERENTIAL INTERACTION OF TWO STRAINS OF *BIFIDOBACTERIUM* WITH HUMAN DENDRITIC CELLS

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Microorganisms belonging to the genus *Bifidobacterium* have demonstrated health promoting effects (probiotic) in humans. Modulation of host defense response and immunoregulation are some of the proposed mechanisms that involve crosstalk between relevant cell populations such as intestinal epithelial cells (IEC) and dendritic cells (DC). Previous studies showed a strain dependent interaction of bifidobacteria with THP-1 cells demonstrating a higher internalization of *Bifidobacterium bifidum* CIDCA 5310 when compared with *B. adolescentis* CIDCA 5317. Also, bifidobacteria stimulation leads to the expression of HLADR and TLR2 on THP-1 cells. The present study focus on the effect of these strains on the phagocytic activity of human DC and activation in cocultures with IEC.

Bacteria were cultured (24 h; 37°C) in MRS broth in anaerobic conditions. For DC activation studies, HT-29-NF-κB-hrGFP reporter cells, cultured on a transwell filter (3μm pore diameter), and monocyte-derived differentiated DC were cultured in RPMI 1640 + 10% (v/v) foetal bovine serum (48 h, 37°C, 5% CO_2). Inserts were transferred to plates containing DC and the apical surface was stimulated with 30 bacteria per cell (18 h at 37°C, 5% CO_2). Cell response was evaluated by flow cytometry (FC) (MFI: mean fluorescence intensity of HLADR, CD86, CD80 and CD40 markers). For the activation of NF-κB, green fluorescent protein (GFP, %) was assessed in HT-29-NF-κB-hrGFP. Lipopolysaccharide from *E. coli* (LPS, 0.5 μg/ml) was used as positive control. DC phagocytic activity of FITC-labeled bacteria was evaluated by FC. Trypan blue (TB) was used for quenching non-internalized bacteria.

Co-stimulatory molecules expression showed that bifidobacteria modulated HLA-DR and CD86. *B. bifidum* CIDCA 5310 strain increased significantly ($P<0.001$) the expression of HLA-DR (19761.00 ± 373.35) and CD86 (52492.30 ± 515.83) when compared with negative control: 11590.00 ± 251.73 and 14702.80 ± 2072.73 for HLA-DR and CD86, respectively. *B. adolescentis* CIDCA 5317 strain showed the same behavior, with a MFI of 16148.50 ± 173.24 and 42406.30 ± 2323.38 for HLA-DR and CD86, respectively. When bacteria were co-incubated with LPS, *strain* CIDCA 5317 decreased HLA-DR (21371.50 ± 6.36) and CD80 (106.5 ± 0.07) expression in comparison to LPS (24944.30 ± 79.55) and (127.5 ± 3.54) ($P< 0.01$), respectively. Also, this strain modulated CD40 expression (2081.83 ± 296.21) triggered by LPS (2678.50 ± 167.55). No effect on NF-kB signaling was observed for both strains. Uptake of strain CIDCA 5310 by DC (values after TB) was significantly higher ($p<0.05$) (27.8% ± 0.02) as compared with strain CIDCA 5317 (4.18 ± 0.002). Also, values before TB were different: 48.70 % ± 0.024 and 6.57 % ± 0.008, respectively.

Results show that bifidobacteria interact with DC in a strain dependent manner and are able to modulate different activation response

Código de Resumen: IN-002

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

OPTIMIZATION OF NON-COVALENT PEPTIDE IMMOBILIZATION ON SODIUM ALGINATE BEADS FOR PROTEIN PURIFICATION PROCESS

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The alginic acid, a constituent of marine algae, is a copolymer of β-3-D-mannuronic and α-L-guluronic acid. *Yersinia* outer protein P (YopP), a virulence factor of *Yersinia enterocolitica* (Ye), causes suppression of pro-inflammatory cytokines and induces apoptosis both in macrophages and in dendritic cells. Galectin-1 (Gal-1) is a “proto-type” β-galactoside-binding lectin widely distributed in host tissues with an important immunomodulatory role. We previously demonstrated that Ye-induced

apoptosis of macrophages depends on both YopP and Gal-1 and that Gal-1 binds *in vitro* to YopP preventing its auto-degradation. The aim of this study was to establish the optimal parameters for non-covalent peptides immobilization on sodium alginate beads to allow the purification of YopP-Gal-1 complex. In this study, we used alginate beads like solid support for non-covalent adsorption for proteins purification. The beads were prepared beginning with sodium alginate in the presence of calcium ions. Then, we immobilized polyclonal antibodies on alginate beads in water-acetonitrile medium. After sequential binding of YopP and Gal-1 on the bead, we performed enzyme-linked immunosorbent assay (ELISA), in order to evaluate bead capacity of immobilization and the level of non-specific interactions. The time and temperature of incubations and washing-equilibration procedures were optimized. The surface of the beads was blocked to avoid non-specific binding. The presence of Gal-1 and YopP was confirmed by Western blot. We successfully obtained the isolated Gal-1-YopP complex using immobilization of polyclonal antibodies anti-YopP adsorbed on alginate beads surface. We conclude that sodium alginate beads can be used for purification of YopP-Gal-1 complex.

Código de Resumen: IN-003

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

PLEIOTROPIC EFFECTS OF A MUTATION IN *lpsB* GENE OF *Sinorhizobium meliloti* Rm 8530 STRAIN

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Bacterial surface molecules such as exopolysaccharides (EPSs), lipopolysaccharides (LPSs) and capsular polysaccharides (KPSs), are crucial for adherence properties, colonization of surfaces, and as a barrier for defense against stressful environmental factors. For rhizobial bacteria those molecules are also relevant for the development of a successful rhizobia-legume symbiosis. Lipopolysaccharides (LPSs) are the most important structural components of the outer membrane of Gram-negative bacteria contributing to their structural properties and acting as a permeability barrier. Because of their position at the contact zone with the external environment, the LPSs of many bacterial species are the main determinants of interaction with biotic or abiotic surfaces. LPSs contribute to the establishment of the symbiotic relationship through suppressing host defenses and facilitating rhizobial entry into root hairs, infection thread formation, and eventually bacteroid differentiation. The *Medicago* symbiont *Sinorhizobium meliloti* produces a heterogeneous population of LPSs: LPS-1 which includes the O-antigen (S-LPS) and LPS-2 which lacks the O-antigen (R-LPS). The *lpsB* gene codes for a type I glycosyltransferase involved in the synthesis of the LPS *core*.

In the present study, we evaluated the pleiotropic effects of a mutated *lpsB* gene in *S. meliloti* Rm 8530 strain. This mutation was examined alone and combined to deficiency of EPS II (exopolysaccharide II). We studied the mutated LPSB strain in cell-cell and cell-surface interactions, motility and symbiotic parameters with the host plant *Medicago sativa*. The LPSB mutant, which has a defective core portion of LPS, exhibited a reduction in biofilm formation on abiotic surfaces compared to the wild type strain. However, this ability in the in the LPSB mutant was not so reduced when compared to EPS II-defective mutant strains. Cell aggregation studies clearly showed that the LPSB mutant strain formed a greater number of higher cell aggregates compared to wild type strain. Moreover, autoaggregation experiments carried out with LPS and EPS mutant strains showed that both polysaccharides had an impact on the cell-cell adhesive interactions of planktonic bacteria. The *lpsB* mutation had also a marked effect in reducing the motility of strains carrying the mutation. In spite of the effects on several important physiological mechanisms caused by the *lpsB* mutation in this bacterium, the symbiotic process was not altered. In this sense the number and efficiency of nodules as well as the biomass parameters were not reduced because of the *lpsB* mutation. On the other hand, symbiosis was negatively affected in a LPS and EPS II double mutant. Taken into account the results obtained, this work shows that *S. meliloti* interactions with biotic and abiotic surfaces as well as the development of a successful symbiosis could depend on the interplay between LPS and EPS II.

Código de Resumen: IN-004

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

VARIATION OF PREDOMINANT BACTERIA AND ITS ENZYME ACTIVITIES IN *Anticarsia gemmatalis´* GUTS BY DIET MODIFICATION

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Soybean (*Glycine max*) is one of the most important crops in Argentina. Due to climate change, *Anticarsia gemmatalis* is one of the most frequent pests, a Lepidoptera that consumes leaves of soybean plants and/or perforates their pods. The feeding and the physiological conditions of these insects could affect the gut microbial flora. The objectives of this work were to determine the effect of diet variation on the prevalence of isolated bacteria in *Anticarsia´s* guts; isolate, identify and determine the enzymatic proteolytic activities of predominant bacteria.

The used diets were: INTA artificial diet (D_I) and INTA artificial diet with 20% of raw soybeans (soybeans hydrated for 12 hours) (D_{I+S}). The predominant bacteria in caterpillars´ guts were isolated on calcium agar-caseinate medium and were identified with API 20E or API 20NE test systems. The growth was determined as OD_{660}. The proteolytic activities were determined with both, azocasein and casein zymography (8% acrylamide, 1 mg/ml casein); the effect of a serine protease inhibitor, PMSF (phenylmethylsulfonyl fluoride), was studied in this last system. The molecular weight of proteases was determined by electrophoresis PAGE 8%. The amino acids released in different culture media (gelatin, casein and soybean meal) were detected using thin layer chromatography, with ninhydrin as developer.

Colony forming unities with proteolytic activity in insect guts depended on the diet provided (D_I: $1.3.10^4$ and D_{I+S}: $1.0.10^5$ cfu/mg gut); with D_I, the predominant bacterium isolated was *Stenotrophomona maltophilia* (API 20EN), while with D_{I+S} the predominant bacterium was *Burkhordelia cepacia* (API 20E). The proteolytic activity profile of these bacteria was different; in a casein culture the proteolytic activity determined with azocasein was higher for *B. cepacia* (21.9 U/DO_{660}) than for *S. maltophilia* (10.8 U/DO_{660}). The proteases molecular weights determined by gel permeation were: 140, 185 kDa for *S. maltophilia* and 62, 67 for *B. cepacia*. For both bacteria, a high molecular weight protein was observed (greater than 250 kDa). The PMSF inhibited the proteolytic activity of all enzymes detected by zymography, except for the band at molecular weight greater than 250kDa of *B. cepacia*. The released amino acids from different protein media (gelatin, casein and soybean meal) were similar.

The microbiome is a group of microorganisms that inhabit and coexist in the insect's gut throughout its life. The results obtained indicate that the diet affects the predominant bacteria in *Anticarsia´*s gut, this could provide the insect the advantage of using the metabolic activity of the predominant bacteria in its own food digestion.

Código de Resumen: IN-005

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

EFFECTS IN *Mentha piperita* GROWTH PARAMETER'S CULTIVATED UNDER DROUGHT STRESS AND INOCULATED WITH PGPR

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Mentha piperita L. is a popular, commonly-used flavoring agent worldwide. Fresh or dried leaves of Mentha species are used primarily as condiments. Essential oils of these plants are used as food and beverage flavorings, as fragrances, and as fungicides or insecticides in a variety of pharmaceutical and industrial products. Drought is undoubtedly one of the most important stresses having huge impact on growth and productivity of the crops. One alternative for growing plants under dry conditions is the use of plant growth promoting rhizobacteria (PGPR). PGPR can be found in the rhizosphere in association with plant root systems, and can either directly or indirectly facilitate plant growth. Many PGPR have been shown to alleviate drought stress effects in plants by the reduction of ethylene production, increase phytohormone concentration, give protection against reactive oxygen species (ROS), generate compatibles solutes, phosphate solubilization, exopolysaccharide production and phytopathogen control.

To evaluate PGPR effects: *M. piperita* young shoots from Traslasierra Valley (Córdoba province, Argentina) were transferred to test tubes containing sterile distilled water and rooting hormone. *M.piperita* plantlets were transferred to plastic pots containing sterile vermiculite. After 9 days, the plants were inoculated with PGPR strains *B. velezensis* GB03 and *P. simiae* WCS417r. For drought stress determination, two treatments were applied: moderate stress MS (deprivation of water 10 days before harvest) and severe stress SS (deprivation of water 20 days before harvest); and control (C): irrigated 2 times per week, until the end of the assay (36 days). Plants were grown in a growth chamber with controlled conditions of light (16/8 h light/dark cycle), temperature (22 ± 2ºC), and relative humidity (~70%).

Inoculated plants without stress showed significant differences with control (non inoculated)($p<0,05$) increasing leaf area, root dry weight and fresh weight of leaf and stem, the most effective strain was GB03.

Under MS stress conditions, plants inoculated with GB03 showed a 20% increase in fresh weight; leaf number (20%) and area (31%) compared with the respective control (MS) without inoculation.

The strain WCS417r only increased leaf area compared with the control (non inoculated). Under SS stress conditions, plants inoculated with GB03 showed a tendency to improve leaf area, but there was not a statistically significant difference with control (SS) in all parameters evaluated. Plants inoculated with WCS417r did not modify any parameters in relation to controls (SS non inoculated).

The root dry weight in plants inoculated either with GB03 or WCS417, decreased in comparison with controls, in both treatments (MS and SS).

These results stand out the importance of the plant–bacteria interactions in response to drought and open up new possibilities of investigation to improve tolerance to drought stress by aromatic plants.

Código de Resumen: IN-006

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

INCREASE OF SECONDARY METABOLITES CONTENT IN PEPPERMINT IN RESPONSE TO INOCULATION WITH PLANT GROWTH-PROMOTING RHIZOBACTERIA AND FOLIAR FEEDING INSECTS

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Peppermint (*Mentha* × *piperita*) is cultivated worldwide for production of essential oils (EOs) and fresh or dried herbs, and is one of the most important EO crops.Many species of bacteria, most of which are found in the rhizosphere (the narrow region of soil associated with the roots of plants), have beneficial effects on plant growth and on crop yield and quality. Such bacteria, collectively termed *"plant growth-promoting rhizobacteria"* (PGPR), promote plant growth through both direct and indirect mechanisms. *Rachiplusia nu* is a major defoliator commonly found in Argentina. The larval stage of R. nu can cause severe damages to aromatic plants. Plants display a diverse array of inducible changes in secondary metabolites following insect herbivory or PGPR inoculation.

The objective of this work was to determine the effect of inoculation of rhizobacteria and feeding of herbivorous insects on the production of secondary metabolites in *M. piperita.*

M. piperita young shoots were transferred to test tubes containing sterile distilled water and rooting hormone (alpha naphthalene acetic acid). After one week, plantlets were transferred to plastic pots containing sterile vermiculite. After 7 days the plants were inoculated with PGPR strains *Bacillus subtilis* GB03 or *P. putida* SJ04. Thirty days after inoculation, the plants were exposed to 6 larvae of *R.* nu (Lepidoptera, Noctuidae) *for* 5 hs (*enough time to generate considerable damage, approximately 30%).* Levels of the main monoterpenes pulegone, menthone and menthol, EOs yield and phenolic content were assessed 48 h after wounding.

The biosynthesis of the major EO components was increased in inoculated or insect-damaged plants. EO yield and amounts of the main monoterpenes was similar in inoculated plants than in control plants exposed to insects. The content of phenolic compounds was similar in leaves of inoculated and non-inoculated plants damaged by herbivores.

Better understanding of the processes that affect secondary metabolites accumulation will lead to increased yields of these commercially valuable natural products.

Código de Resumen: IN-007

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

FOLATE PRODUCING *Streptococcus thermophilus* CRL808 DECREASED SYMPTOMS OF NEUROLOGICAL DAMAGES IN A MOUSE PARKINSON'S DISEASE MODEL AND EXERTED NEUROPROTECTOR EFFECT IN VITRO

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Parkinson's disease (PD) results from the dysfunction and degeneration of dopaminergic neurons within the substantia nigra pars compacta, and is characterized by persistent tremors, bradykinesia, rigidity and instability in posture. At the cellular level, the pathogenic process involves high levels of oxidative stress, mitochondrial dysfunction and apoptosis. Many studies showed that dietary factors, including B-Group vitamins, may be involved in the etiology of PD. It has been shown that patients with PD present folate deficiencies with increases in homocysteinemia. The aim of this study was to evaluate the effect of *Streptococcus* (*S.*) *thermophilus* CRL808, a folate-producing strain, in an animal model of PD and *in vitro* using neuron cell cultures. For the in vivo study, C56BL/6 mice were injected subcutaneously with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), using 5 doses of 20 mg/kg each during 4 days. Mice were also injected intraperitoneally with probenecid (250 mg/kg) to decrease the renal excretion of MPTP and achieve the chronicity. *S. thermophilus* CRL808 was orally administered ($1x10^8$ CFU/ml) starting 7 days before injection with MPTP until the end of the experiment. Each mouse received 100µl of the bacterial suspension daily. Control animals injected or not with MPTP received 100µl of physiological solution. Each mouse was subjected to behavior tests (vertical and horizontal bar, and the nasal bridge adhesive elimination). For the *in vitro* model, the mouse Neuro-2a (N2a) neuroblastoma cells were cultured in the presence and absence of folic acid. Oxidative stress was induced with MPP+ (toxic metabolite from MPTP). The intracellular extract from *S. thermophilus* CRL808 was added to the cell cultures and the effect was compared with commercial folic acid. Cell viability and proliferation were evaluated using the MTT assay. Results obtained in the *in vivo* model of PD showed that mice that received *S. thermophilus* CRL808 presented milder symptoms of the pathology compared to the PD control group. This effect was observed in the three evaluated behavior tests, in which on average, the time used to complete each test was reduced by half when compared with the PD control, with results close to those of healthy control animals. The *in vitro* assay showed that the exposure to MPP+ significantly increased the death of N2a cells. In contrast, when vitamin B9 or the intracellular extract of *S. thermophilus* CRL808 was added to the culture medium, this effect was reversed or significantly decreased. The results obtained demonstrate the neuroprotective properties of *S. thermophilus* CRL808 against the toxicity of MPTP and MPP+. This bacterial strain has an important potential for future studies in the Parkinson's disease model.

Código de Resumen: IN-008

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

CHEMOTAXIS AND INFECTIVE CAPACITY OF ENDOPHYTIC PHOSPHATE SOLUBILIZING BACTERIA ON PEANUT, MAIZE AND SOYBEAN PLANTS.

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The rhizosphere is a highly dynamic zone for interactions between roots and microorganisms. The roots of plants exude a wide range of compounds and with them the plant shapes the rhizospheric microbiome. In this sense, chemotaxis, the response to chemical agents, is an important phenomenon in these interactions in which bacterial mobility is an essential property involved. This, in addition, is important since it orients the movement of the microorganisms to favor the entrance to the vegetal tissues and thus obtain adaptive and survival advantages. The objectives of this work were to analyze the chemotactic effect of root exudates (RE) of peanuts, corn and soybeans on the native peanut phosphate solubilizing strains *Serratia* sp. S119 and *Enterobacter* sp. J49, and to evaluate its endophytic colonization capacity. The plants used in this study were chosen for their economic importance in Argentina. As a first measure, a physiological study of the strains was performed to know their growth parameters. For this, growth curves in LB medium were performed, and the cell concentration was measured as OD at 550nm and CFU/ml was evaluated by the microdrop method. Kinetic parameters of cell growth such as specific growth rate (µ) and generation time (Tg) were determined for each of the strains. Results obtained indicated µ values of 0.69 and 0.63 h^{-1}, and Tg of 60 and 66 minutes for *Serratia* sp. S119 and *Enterobacter* sp. J49, respectively indicating that both are fast growing strains. The chemotaxis assay was performed following the method of Mazumder *et al*., (1999). For this, an incubation time of 45 minutes was set, to ensure that the quantified cells correspond to the chemotactic movement, and not the duplication of them within the capillary. After that time, the cell concentration in CFU/ml was determined. Two independent assays were performed with n=9 each. The soybean and corn RE showed an attractant effect on *Serratia* sp. S119, while *Enterobacter* sp. J49 was

attracted by soybean and peanut RE. To evaluate endophytic colonization a greenhouse trial was performed by inoculating the two strains in the three plants. At the end of the assay, the genomics fingerprints by ERIC-PCR of colonies isolated from different plant tissues were compared with the rep-fingerprint of the bacteria inoculated. *Enterobacter* sp. J49 strain was isolated from peanut leaves, stems, and roots and also from corn leaves and soybean stems. The strain *Serratia* sp. S119, was only isolated from inside peanut stems. These results supply important information on ecological traits for the colonization of roots, indicating that RE could favor the colonization of the rhizosphere of beneficial endophytic bacteria, inducing a chemotactic response in these strains studied, and allowing in some cases to enter the interior of the plant. This fact would put them at an advantage over other microorganisms colonizing the ecological niche.

Código de Resumen: IN-009

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

IMPROVEMENT OF AGRICULTURAL PRODUCTION SYSTEMS THROUGH THE USE OF REGIONAL BIOFERTILIZERS IN LA PAMPA, ARGENTINA.

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The biological nitrogen fixation for the rhizobium-legume symbiosis is very important in agriculture. This practice is more efficient, less polluting and more economical than mineral fertilization. The native strains of rhizobia are adapted to the edaphoclimatic characteristics of each region. This would give them an ability to nodulate and fix nitrogen effectively with plant symbiont. In the search of strains for the production of biofertilizers, the first step is the selection of those capable of nodulating and establishing an effective symbiosis with the cultivated legume. These microorganisms must reach high cell concentrations in industrial fermentation processes using standard culture media, survive in storage conditions and upon inoculated seed. The objective of this work was to evaluate the symbiotic performance of native strains isolated from the semiarid pampean región, with the purpose of evaluate the use of regional biofertilizers, that is, formulated from strains adapted to each region. In preliminary works, around 200 bacterial isolations were obtained starting from nodules of vicia plants, collected in the areas of General Acha, Victorica and Anguil, province of La Pampa, Argentina. Three bacterial isolates (V39, 270 and 25b) were selected based on their PGPRs properties. The growth kinetics of each isolate was studied in order to determine physiological characteristics and evaluate their development in different culture media. On the other hand, field trials were carried out in two different edaphoclimatic regions: Uriburu (S 36º29,702´, W 63º49,231‘, altitude 161 m) and Chapalcó (S 36° 52’ 51,1", W 64° 47’ 3,9" altitude 233 m). Vicia plants were inoculated with the isolates under study, using the reference strain *R. leguminosarum* D70 as control treatment. Regarding the growth kinetics, the isolates tested correspond to fast growing strains, with a specific growth rate of 0.1 h^{-1}. On the other hand, respect of the selection of culture medium, all isolation used similarly tryptone or mannitol as source of carbon. In the field trials, the 25b isolate showed the best symbiotic performance, based on a higher yield of shoot dry weight. Regarding of infectivity, although the strain 25b originated fewer nodules, these were larger and more effective than the rest, in relation to nitrogen fixation. The results of this work indicate that the use of native strains in the development of biofertilizers, has a greater chance of effectiveness in the field, due to its adaptation to the soil conditions of each region, promoting regional socio-productive development. With climate change and an increasing world population, there is an urgent need to develop the diverse range of nodulated legumes native to dry environments. With regional biofertilizers this goal is more readily achievable.

Código de Resumen: BB-001

Sección: Biorremediación y Biocontrol

Modalidad: Oral

BIOLOGICAL REMOVAL OF ISOXAZOLYL-PENICILLINS BY THE LIGNINOLYTIC NATIVE COLOMBIAN STRAIN *Leptosphaerulina* sp.

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Leptosphaerulina sp. is a Colombian ascomycete strain isolated from lignocellulosic material in the Valle de Aburrá (Antioquia, Colombia), which has efficiently degraded synthetic dyes (Copete et al., 2015; Plácido et al., 2016). However, the capabilities *Leptosphaerulina* sp., for removing other recalcitrant compounds such as antibiotics are still unknown. *Leptosphaerulina* sp. produces ligninolytic enzymes (laccase (Lac), manganese peroxidase (MnP) and versatile peroxidase (VP) with high redox potential, which makes them able to oxidize a large number of organic pollutants. Therefore, *Leptosphaerulina* sp. was considered as a novel alternative to remove Isoxazolyl-Penicillin antibiotics from water. The biotransformation process of three Isoxazolyl-Penicillin antibiotics (40 µmol L^{-1}): oxacillin (OXA), cloxacillin (CLX) and dicloxacillin (DCX) was performed at pH 5.6, 28 °C, and 160 rpm for 15 days. At day 2, 4, 6, 7, 8 and 15 the enzymatic activities, antibiotics degradation and antibacterial activity (AA) were determined. The role of enzymes in Isoxazolyl-Penicillin elimination was evaluated through *in vitro* studies with enzymatic extracts (crude and pre-purified) from *Leptosphaerulina* sp., commercial enzymes (Lac from *Trametes versicolor* and horseradish peroxidase) and enzymatic inhibitors (EDTA, NaCl, sodium acetate, manganese (II) ions). Furthermore, the applicability of *Leptosphaerulina* sp. to a complex matrix (simulated hospital wastewater) was assessed.

It was found that *Leptosphaerulina* sp. significantly (~100%) abated OXA (day 6), CLX (day 7) and DCX (day 8) and theirs AA from the water. Antibiotics removal was related to Lac and VP activities. Tests using commercial enzymes and inhibitors confirmed the important role of enzymatic transformation. Whereas, biomass sorption was insignificant in the Isoxazolyl-Penicillin elimination. Cytotoxicity analyses (using the MTT test) of the final solutions on the HepG2 cell line indicated that they were non-toxic. Finally, *Leptosphaerulina* sp. also was able to eliminate OXA and its AA from synthetic hospital wastewater at 6 days. All these results evidenced the potential of *Leptosphaerulina* sp. to remove Isoxazolyl-Penicillin from wastewater through an environmentally friendly biotechnological process.

Código de Resumen: BB-002

Sección: Biorremediación y Biocontrol

Modalidad: Oral

USE OF BIOLOGICAL CONTROL AGENTS AGAINST *Penicillium expansum*: EVALUATION OF THE MYCELIAL GROWTH AND PATULIN PRODUCTION

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Penicillium expansum is the cause of blue rot and production of patulin in pome fruits. Patulin is a mycotoxin that causes acute and chronic intoxication. Synthetic fungicides are commonly used to control this disease but pose a risk to the environment and the health of both the consumer and the animals. An alternative is the use of biological control agents (BCAs). For this reason, the objective of the work was to evaluate *in vitro* effect of biological control agents on mycelial growth and the production of patulin in two strains of *P. expansum* from INTA, Alto Valle (Rio Negro), INTA-5 and INTA-10, previously selected for their high production of patulin. While the antagonists (BCAs) assayed were *Rhodosporidium fluviale*, *Cryptococcus laurentii*, and *Kosakonia radicincitans*. The mentioned microorganisms were isolated in the Laboratory of Industrial Microbiology (UNSL) from the epiphytic microbiota of apples. The assays were carried out in PDA medium confronting antagonist versus pathogen and incubating at 25 º C for 7, 14 and 21 days. The effect of antagonism of BCAs was evaluated in a semiquantitative manner, by observing the growth of both antagonist and pathogen. The extraction and determination of patulin were carried out following the protocol of the AOAC with modifications, the measure the concentration of patulin was performed by HPLC-UV. The results were expressed in ppm of patulin and percentage of reduction thereof as [(patulin control – patulin treatment) / patulin control].100. The results showed a decrease in mycelial growth in both phytopathogenic strains in the presence of the antagonists, being the most effective *K. radicincitans*. Likewise, the production of patulin decreased in the presence of all

antagonists; *K. radicincitans* and *C. laurentii* showed the most significant reduction. For the INTA-5 strain, the percentage of mycotoxin reduction by *K. radicincitans* was 89% and 61% with *C. laurentii* and for the INTA-10 strain of 94% with *K. radicincitans* and 88% with *C. laurentti* at 21 days. It is concluded that the application *in vitro* of BCAs reduced mycelial growth, but also considerably decrease the production of patulin in strains of *P. expansum* extremely toxicogenics, which allows supposing a reduction of rot and decrease in the toxin in fruits. The application of these BCAs could be healthier and environmentally friendly option to be used in fruit conservation chambers.

Código de Resumen: BB-003

Sección: Biorremediación y Biocontrol

Modalidad: Poster

EVALUATION OF *Kosakonia radicincitans* AS GROWTH-PROMOTING BACTERIA OF LETTUCE UNDER GREENHOUSE CONDITIONS

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Organic agriculture is a greenway to producing healthy food of the highest quality in sufficient quantity and is oriented to provide a clean and balanced environment, without the use of chemical products. Beneficial microorganisms are known to help plants, not only by promoting growth and by accelerating their developmental processes but also with pest biological control. The bacterium *Kosakonia radicincitans* (Kr bSL2 strain), was isolated and identified in the Laboratorio de Microbiología Aplicada (UNSL) from local vegetable products. Molecular confirmation was made by the service of MACROGEN Inc. (Korea) by sequencing the 26S and 16S rRNA. Previous work demonstrated the ability of Kr bSL2 strain, for controlling different phytopathogenic fungi. The objective of this work was to evaluate the capacity of *K. radicincitans* as growth promoting bacterium in lettuce cultured in greenhouse conditions. *K. radicincitans* was cultivated in YGM (yeast extract 5 g L-1 and glucose 10 g L-1), at 30ºC for 24 h. Biomass was obtained by centrifugation in a Sorvall SS-3 centrifuge (DuPont Instruments) 10 min at 10.000 rpm. The bacterial cells were washed with sterile distilled water (15 mL) and then were re-suspended in sterile distilled water. The initial cell concentration was adjusted to 10^8 CFU mL–1 by the standard method of count on plates. This suspension was used for inoculation of young plants of lettuce (*Lactuca sativa* cv. Grand Rapids). Lettuce seeds were disinfected with a 10% sodium hypochlorite solution for 5 min and then rinsed four times with sterile water. Then, they were put into germination trays containing sand wet and were covered with plastic. After three days, young plants were inoculated with the bacterial suspension (10 mL 10^8 CFU mL-1) that was applied by two ways: inundation (a) and spray (b); while the control was sterile water. The experiment was performed in duplicate. After a week, the small plants were translated to hydroponic culture in containers with 4 L of solution nutritive sterile of Hoagland. Plants were grown in no sterile conditions at 20°C (±5ºC), with natural light and adequate humidity conditions. After five weeks, leaves and root of lettuce plants were harvested to determining growth and dry mass. The total length of the plant was significantly influenced by *K. radicincitans* when was applied by inundation way (a) 27%, while with spray way (b) the effect was not significant. The bacterium applied by any of the two ways significantly affected the total length of the lettuce roots: (a) 30%, (b) 13%. The two forms of application significantly increased the dry weight of the leaves (a) 65% (b) 27% and the roots (a) 29%, (b) 30%. The treatment with Kr bSL2 strain improves the growth of lettuce plants, and the way of application could influence its effectiveness.

Código de Resumen: BB-004

Sección: Biorremediación y Biocontrol

Modalidad: Poster

IN VITRO ANTAGONIC ACTIVITY OF A *Fusarium solani* ENDOPHYTIC STRAIN TOWARD PHYTOPATHOGENIC FUNGI

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Chemical interconnection between plants and their endophytic microorganisms is fascinating. It assessment offers, not only the possibility to understand the allelopathic mechanisms that determine the equilibrium of this microcosm, but also to devise biotechnological tools to produce biocompounds. Many of the secondary metabolites that endophytes biosynthesize in order to control their competitors *in vivo* can be produced by *in vitro* cultures (1). These chemical entities, that naturally defend both the

plant and its seeds from predators (2), show several bioactivities, mainly antimicrobial, therefore have promising applications in agriculture (3). In this field, technological developments based on biological control concepts, are appreciated to minimize the use of chemicals of synthetic origin in pest control (4). It is supposed that this kind of bioproducts would be less harmful to humans and environment, since they could act specifically toward particular pathogens; while other chemicals with broader spectrum are often lethal to beneficial insects and microbes, as well as affect the food chain (5).

The goal of this work is the detection of fungal metabolites with antifungal activity toward phytopathogens of agronomic interest species, from *in vitro* cultures of endophytic fungi.

The antagonistic potential of three endophytic fungal isolates was evaluated by facing each other in dual solid cultures. *Fusarium solani* Eb01 (6) was chosen due to its highest inhibition rate. Then, its antagonistic activity was tested toward collection strains of *Aspergillus flavus, Aspergillus niger, Sclerotinia sclerotiorum, Sclerotium rolfsii, Fusariumm oxysporum* and *Penicillium chrysogenum*. Most of the developments were affected by *F. solani* showing radial inhibition rates higher than 40 %. The exception was *S. rolfsii,* which development was not altered by the presence of the endophyte. *A. niger* was chosen for further studies, therefore organic extracts were obtained from *F. solani* liquid cultures with and without elicitation with nonviable *A. niger* mycelium. Elicited broths presented a different metabolic profile, which correlates with its bioactivity toward the other fungi. The present work contributes both to the knowledge of the cross-talk between the two fungal species and to set the bases for a potential biotechnological endeavor.

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Código de Resumen: BB-005

Sección: Biorremediación y Biocontrol

Modalidad: Poster

SELECTION OF HEAVY METAL TOLERANT EXTREMOPHILIC CONSORTIA FROM THE VOLCANIC AREA OF CAVIAHUE-COPAHUE, NEUQUEN

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Biomining and bioremediation are alternatives to the traditional chemical procedures for recovering metals and for the remediation of polluted sites, respectively. Microorganisms useful for such technologies are usually present in very extreme environments with high concentrations of heavy metals under acidic conditions. To survive in such conditions these extreme microorganisms had to develop different tolerance mechanisms. Certain volcanic areas as Caviahue-Copahue (Neuquén) are perfect environments for this kind of polyextremophilic microorganisms. Rio Agrio flows from the crater of Copahue volcano forming several cascades before and after Caviahue lake. A sample from a great cascade after the lake (Salto del Agrio) was taken and several enrichments were performed to select acidophilic iron/sulfur oxidizing microorganisms (using MAC medium), neutrophilic organotrophs (using LB medium), and anaerobic microorganisms (using modified Postgate medium). The maximum tolerant concentration was later assessed for five heavy metals (Cd, Cu, Ni, Zn, and Co) for each consortium obtained from the enrichments. Interestingly, the three consortia were tolerant to relatively high concentrations of each metal being the mean tolerances obtained: cadmium 30 $mg.l^{-1}$, copper 71 $mg.l^{-1}$, nickel 203 $mg.l^{-1}$, zinc 553 $mg.l^{-1}$, and cobalt 173 $mg.l^{-1}$. These results confirm the widely reported observation that extremophilic microbial communities are naturally poly-tolerant even when some of the contaminants are not present at such high concentration. At the same time, tolerance was induced artificially through successive cultures in which the metal concentration was increased until no growth was measured. On this wise, we selected fifteen consortia (five acidophilic, five organotrophic, five anaerobic) tolerant to high concentrations of cadmium, copper, nickel, zinc or cobalt. Remarkably, some of them were tolerant to concentrations higher than the 75 % of those reported in literature for resistant microorganism and consortia suggesting they could be used in different processes for recovering or remediating metals. Acidophilic consortia were the most tolerant ones exhibiting tolerance to approximately 11 $g.l^{-1}$ of cadmium, 0,6 $g.l^{-1}$ of copper, 23 $g.l^{-1}$ of nickel, 26 $g.l^{-1}$ of zinc, and 12 $mg.l^{-1}$ of cobalt. Some preliminary results on the phylogeny of the microorganisms present in the different consortia are also included in this communication.

Código de Resumen: BB-006

Sección: Biorremediación y Biocontrol

Modalidad: Poster

CHARACTERIZATION OF *Bacillus* sp. SFC 500-1E, A BIORREMEDIATION AGENT USED FOR TREATMENT OF TANNERY EFFLUENTS.

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Strain SFC 500-1E was originally isolated from sludge of a tannery industry located in Córdoba province.This isolate belongs to the genus *Bacillus*, which contains Gram-positive aerobic or facultative anaerobic rod-shaped bacteria that form intracelular spores. In a first step, based on biochemical and morpho-physiological tests performed according to Holt and Slepecky and Hemphill keys, this bacterium was identified as *B. cereus*. Supplementary physiological tests were evaluated using API 50CH profile analysis (Biomérieux). Based on this study, the significant taxon to which the SFC 500-1E isolate belongs was *B. cereus* (ID% = 93.5), with a note of possible identification as *B. thuringiensis*. In the last few years systematics of the genus was substantially revised, new species have been described and many others were renamed, even assigned to new genera. Also, the genus *Bacillus* contains several closely related species group, whose delimitation is difficult, especially within *B. cere*us group to which *Bacillus* sp. SFC 500-1E belongs. Analysis of 16S rDNA sequence, using for comparissons the NCBI ribosomal database, indicated a close relationship of the strain with *B. thuringiensis*; however, no parasporal crystal was observed with optical or electron microscopy techniques and no amplification product was obtained when parasporal crystal genes (cryIA, cryIG and cryIIIC) were analyzed. By using a “cured” database (RDB) for 16S rDNA sequences, *Bacillus* sp. SFC 500-1E was 100% similar to *B. toyonensis* type strain (BCT-7112). Further multilocus sequence analysis (MLSA), including *rec*A, *rpo*B and *gyr*B sequences of the isolate, also indicated maximum similarity with *B. toyonensis* strain BCT-7112. In addition, an specific multiplex PCR method that allows discrimination between different *Bacillus* species was carried out. The resultant amplification pattern of SFC 500-1E coincided with that presented by *B. toyonensis* type strain. Despite these similarities, certain peculiarities of the isolate emerged from the taxonomic analysis (with regard to specific profile of carbohydrate utilization, amplification of *gyr*B sequences with *B.thuringiensis* specific primers and fatty acids profile) lead us to consider that it can be described under another denomination. In this sense, the analysis by means of average nucleotide identity (ANI) calculations through the use of data from complete genome sequencing, will allow assigning *Bacillus* sp. 500-1E to a genomospecies with the greatest precision achievable.

Código de Resumen: BB-007

Sección: Biorremediación y Biocontrol

Modalidad: Poster

ASSESSMENT OF ARBUSCULAR MYCORRHIZAL FUNGI ABUNDANCE IN THE RHIZOSPHERE OF NATIVE PLANT GROWING IN HEAVY METAL CONTAMINATED SOILS.

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Arbuscular mycorrhizal fungi (AMF) have the ability to tolerate a wide range of heavy metal (HM) concentrations. AMF can develop different mechanisms to persist in these environments, but high contents of HM could lead to modify spore density. The objective of this work was to evaluate the abundance of AMF spores in the rhizosphere of *Solanum argentinum* and *Zinnia peruviana* growing in soils contaminated with lead (Pb). The study area is located in Bouwer, Córdoba city where a recycling plant of lead batteries leaves high levels of lead in soil. Four sites with different Pb content in the soil were selected (site I: 365 µg g-1, site II: 965 µg g-1, site III: 89 µg g-1, site IV: 544 µg g-1). In each site, 5 individuals of *S. argentinum* and *Z. peruviana* were extracted together with the rhizospheric soil. The last species was only present in sites II and III. Through the decantation technique, wet sieving and sucrose gradient, the AMF spores were extracted, and followed by counting in a stereo microscope the density of spores was calculated (number of spore/100 g of dry soil). In addition, soil moisture, pH, electrical conductivity, content of Cl and N were determined. Although all sites presents high Pb concentrations, the AMF spore density was higher (760.96 ± 67.04 spore/100 g of soil) than the density recorded in other soils with lower Pb content. The AMF spore density differed significantly according to the sites and the host plants. This could be due to the different habits of the species analyzed (*S. argentinum* is a perennial shrub and *Z. peruviana* is an annual herb) that would determine differences in the AMF

community. Respecting to the soil variables, the density of AMF spores was only related with soil moisture, factor know as determinant in the development of these fungal structures. HM are persistent elements in the soil that modify its physico-chemical and biological properties. Phytoremediation is a technology based on the reduction of toxic elements through the use of plants and their associated microorganisms. In this sense, considering the AMF density values in these contaminated soils, it could be inferred that these organisms can be used as a biological source in phytoremediation practices. This preliminary study contributes to the registry of the AMF in the rhizospheric soil of native plants growing in environments contaminated with Pb.

Código de Resumen: BB-008

Sección: Biorremediación y Biocontrol

Modalidad: Poster

ANALYSIS OF BIOSURFACTANTS OBTAINED FROM ENVIRONMENTAL BACTERIAL ISOLATES FOR BIOREMEDIATION APPLICATIONS

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Oil pollution is an environmental problem of increasing importance. Hydrocarbon-degrading microorganisms, adapted to grow and thrive in oil-containing environments, have an important role in bioremediation. One of the limiting factors in this process is the bioavailability of many fractions of the oil. Surfactant Enhanced Bioremediation (SEB) is a biostimulation technique to improve hydrocarbon availability consisting habitually in addition of non-biodegradable surfactants. Due some hydrocarbon-degrading microorganisms produce biosurfactants of diverse chemical nature and molecular size, the aim of this work was to isolate oil-degrading bacteria capable to synthesize biosurfactants and to analyze their tensioactive characteristics.

Samples were isolated from a stagnant stream contaminated with oil from a wastewater treatment plant, located in Moreno, Buenos Aires, Argentina (34º 34' 50.9'' S, 58º 49' 25.6'' W). Oil-degrading bacteria were isolated by supplementing 10 ml of sample water with 10% diesel, a micronutrient solution and different concentrations of yeast extract and incubated at 28°C and 200 rpm until turbidity development. After incubation, culture aliquots were plated on LB agar. Bacteria with different colony morphology were selected; white (W), red (R), green (G), smooth brown (SB) and mucoid brown (MB). To analyze if these five isolates were able to synthesize biosurfactants, cell free supernatants of cultures growing during 5 days on E2 medium supplemented with glucose (g), sunflower oil (so) or diesel (d) as carbon source, were used to determine surface tension reduction by the drop collapsing test and emulsification activity (EI24). All cell free supernatants showed a decrease in the contact angle compared with distilled water, indicating the production of a surface active agent. SB and MB showed the highest superficial tension reduction relative to distilled water in a range of 53% to 59% depending on the carbon source while the other three remaining isolates diminished the superficial tension in a range from 20 to 50%. Regarding to the EI24, SB showed a EI24 of 65% (d), 33%(so) and 50%(g), MB values were 32%(d), 47%(so) and 45%(g) and G showed an EI24 of 47%(d), 47%(so) and 28%(g). W was unable to grow in sunflower oil as sole carbon source, but presented a EI24 of 53%(d) and 44%(g). Finally R showed negligible values in this test.

To analyze the chemical nature of the produced tensioactive, a crude solvent extract from the cell-free supernatants were carried on. TLC analysis showed different retardation factors, most of them were positives for carbohydrates (Molisch reactive) and G and SB presented also spots related to amino-acidic nature (Ninhydrin staining). In conclusion, four environmental isolates produced tensioactive compounds with different chemical nature and surface active proprieties that could be good cantidates to be tested in SEB protocols.

Código de Resumen: BB-009

Sección: Biorremediación y Biocontrol

Modalidad: Poster

MOLECULAR CHARACTERIZATION OF ARSENIC RESISTANT RHIZOSPHERIC BACTERIAL STRAINS ISOLATED FROM AN AGRICULTURAL SOIL

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Contamination of soils with arsenic constitutes a global environmental problem with significant negative impact on human health and agriculture. Rhizospheric microbial populations are known to affect plant growth and metal(loid) mobility and availability through the release of chelating agents, acidification, phosphate solubilization and redox changes. Biological treatments, based on inoculation with appropriate metal(loid)-resistant rhizobacteria, are receiving increasing attention in order to alleviate plant stress and develop sustainable soil management. In this work, we have further characterized six bacterial strains with plant growth promoting (PGP) properties isolated from soybean rhizosphere. The aims of this work were to update bacterial nomenclature and distinguish selected bacterial strains from different rhizospheric strains to improve genetic traceability in co-inoculation microcosms assays. Initially, based on 16S rDNA sequence similarity (NCBI database), these strains were identified as *Enterobacter cloacae AW1, Pseudomonas fluorescens AW2, Rhodococcus erytropolis AW3, P. putida AW4, P. poae AW5 and P. poae AW6*. In the last years, new species have been described, many others have been renamed and databases are constantly being updated. By using a "cured" database (RDB) for 16S rDNA sequences bacterial similarity changed, *P. fluorescens AW2* was 99,2% similar to *P. extremorientalis, P. putida AW4* was 95,3% similar to *P. taiwanensis*, while the other two *Pseudomonas* strains previously identified as *P. poae* showed 99% similarity with *P. extremorientalis* strain BS2774. *Enterobacter* and *Rhodococcus* strains 16S rDNA closest relative also changed. Furthermore, ERIC and BOX-PCR profiles were performed. Also, some morphological, cultural and physiological characteristics were tested (pigment production, hydrolysis of gelatin, growth in differential media, among others) under aerobic conditions. Considering the results, we constructed new phylogenetic trees. Typification of PGP bacteria is necessary, not only to determine their level of kinship with pathogenic strains but also to achieve genetic traceability that allows post-inoculation monitoring of microorganisms.

Código de Resumen: BB-010

Sección: Biorremediación y Biocontrol

Modalidad: Poster

TOXICOLOGICAL EVALUATION OF THE MICROBIOLOGICALLY TREATED SUGARCANE VINASSE USING THE CACO-2 CELL LINE AND WHEAT SEEDS AS BIOINDICATORS

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Microbiological processes fungus-based has been reported as an effective and eco-friendliest for conditioning of sugarcane vinasse. Certainly, we demonstrated the potential of a native fungus identified as *Aspergillus* sp. V1 to neutralize vinasse and remove about 60% of the biodegradable organic matter at 12th days of treatment. However, toxicity studies are usually required in order to determine the effectiveness of the bioprocesses applied to industrial waste materials. To this end, diverse organisms and cell lines can be used as possible bioindicators. Interestingly, the response to toxicity levels of a waste was found to be largely dependent on the organism used. In the present work, it was conducted a toxicological evaluation of a local vinasse sample, both raw as treated with *Aspergillus* sp. V1 for 12 days, using Caco-2 cells and seeds of Triticum aestivum L. (wheat) as bioindicators. Caco-2 cells were incubated for 3 h with 200 µl of vinasse raw or treated. Cells were rinsed and incubated for 3 h with the MTT [(4,5-Dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium bromide], which is converted to the insoluble purple formazan in viable cells. Formazan crystals formed were solubilized with dimethyl sulfide, and the absorbance was measured at 570 nm in order to determine the percent viability. Regarding the wheat seeds, these were placed in Petri dishes containing sterile filter paper (Whatman No. 1) with 30 g of a natural garden soil moistened with 10 mL of vinasse raw or treated. After 7 days of incubation at 25°C under controlled environmental conditions, it was determined the percent germination of seeds. Experimental results demonstrated only a 13% of viability for Caco-2 cells exposed to raw vinasse. This percent increased until 49% when the cells were exposed to the vinasse treated with *Aspergillus* sp. V1, a value increased 3-fold compared to raw effluent. Regarding the *Triticum aestivum L.*, raw vinasse completely inhibited the germination of the seeds. However, application of treated effluent was consistent with an average germination percentage of 60%. It from the current study it was concluded that both bioindicators could be successfully used to predict potential toxic effects of sugarcane vinasse.

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Código de Resumen: MS-001

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

TOWARDS SMART N-BIOFERTILIZERS: CONTROLLING BACTERIAL POPULATION AND FERTILIZING PROPERTIES BY METABOLIC ENGINEERING OF *AZOTOBACTER VINELANDII*

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The growing demand for food to support population increases has generated a strong dependence on fertilizers. Excessive use of nitrogen fertilizers leads to environmental impacts such as eutrophication of fresh water and air pollution. Agricultural promotion of biological N_2-fixation appears as an alternative. However, it could only be exploited in a few symbiotic crops (legumes), displaying a strong specificity of the symbionts. On the other hand, free-living diazotrophs would bypass this specificity constraint, but normally only genetically modified strains display strong N-biofertilizing properties.

To contribute to the development of genetically modified N-biofertilizers that could be safely released to the field in the future, we prepared conditional lethal strains of *Azotobacter vinelandii*. These strains express the glutamine synthetase (GS) gene under an IPTG inducible promoter (*trc$_P$-glnA*). Mutant cells cannot survive long in the absence of the inducer. However, cells can accumulate variable levels of GS as a function of the inducer's accumulation. Most of the GS accumulates as an inactive covalently-modified enzyme. Upon shifting to non-inducing medium, cells gradually activated the GS by reversion of the covalent modification and the life span of the population was somehow proportional to the previous induction intensity. Thus, at low levels of induction cells stopped dividing after a few generations and started to release ammonium as a consequence of failure to assimilate it into amino acids. Conversely, GS overloaded cells, kept producing biomass at the expense of the accumulated GS for an extended life-span. When cell division split the GS pool below a threshold level, cells started to release ammonium, and finally the bacterial population also declined. The bacterial population at which a critical GS level was reached finally determined the overall ammonium that was produced.

When these strains were inoculated into cultures of the alga *Chlorella* sp, we were able to confirm that bacteria with low levels of GS promoted algal growth at the expense of N_2 from the air sooner than bacteria bearing higher levels of GS. However, after some generations, the N-fertilizing property became more prominent for the GS-loaded bacteria. It was also confirmed that after the algal fertilization, the bacterial population tended to decline.

Work is in progress to assess these prototypes of smart-biofertilizers in plants.

Código de Resumen: MS-002

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

MICROBIAL COMMUNITIES IN BORON SOILS FROM SALTA (ARGENTINA)

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Salta Province, in the northwest of Argentina, is the main worldwide producer of hydroboracite and leads in exports of boron mineral and its derivatives in Latin America. This intense industrial development has an important environmental impact on water and soils. In addition to the natural presence of boron in flat-salts and riverbanks, there are others contaminated soils caused by boron mining industry, proximate to urbanized areas.

Here we report the microbial community assembly of soils, natural and anthropogenically contaminated with boron, using next-generation sequencing techniques. We collected soils from three sites: 1) Tincalayu, one of the main deposit extraction flat salts; 2) Animaná, a riverbank with natural boron compounds; 3) Baradero, an urban burden from a former processing factory. Three samples were collected from each site, two exposed to high boron concentration and one control. Soil physicochemical analysis included humidity, organic matter, pH, B_2O_3, and total boron. We used the 16S ribosomal RNA gene, targeting the V4-V5 hypervariable region, and following the QIIME pipeline with SILVA database, we obtained the meta-barcoded microbial

profiling. Multivariate statistical analyses were used to assess the microbial community and its relationships with environmental data.

Microbial community analysis showed variability of richness and α-diversity indices among samples. Taxonomic classification revealed that, at the phylum level, Actinobacteria was the most abundant in the natural boron-soil samples from Tincalayu and Animaná (43.5% ± 2.5 and 27.9% ± 3.4, respectively). Meanwhile, Firmicutes was predominant in Baradero-soils (20.5 to 55.7%) and had very low relative abundance in the flat-salt soils (2.6% ± 1.1). Proteobacteria was the third most abundant phylum (17.0% of total) with higher numbers in Animaná (25.7% ± 8.5) and similar abundance in Tincalayu and Baradero (12.9% ± 9.2 and 11.4% ± 6.4, respectively). The genus level analysis revealed that *Bacillus sp*. was the highest taxonomic group in Baradero and Animana. On the other side, an uncultured bacteria of order Gaiellales (Thermoleophilia class) was the most abundant OTU within Actinobacteria in Tincalayu. DistLM-dbRDA analysis indicated that edaphic properties: pH, humidity, and B_2O_3 concentration shape the microbial assemblies. Microbial community ordination analysis by means of Hierarchical Clustering and nMDS plots showed that exposed-samples from Tincalayu and Baradero formed two distinctive groups (natural vs anthropogenic contaminated soil), and all samples from Animaná grouped together with the control samples from the other two sites.

To the best of our knowledge, this is the first study showing microbial communities of boron associated soils in Argentina. It provides a better understanding of soil-borne microbiome assemblies and valuable information for contaminated sites management.

Código de Resumen: MS-003

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

TRANSGLYCOSYLATION OF TERPENES IN CULTURES OF *Acremonium S*P. DSM 24697

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The fungus *Acremonium* sp. DSM 24697 produces the enzymes α-rhamnosil-β-glucosidase I and α-rhamnosyl-β-glucosidase II. These enzymes catalyze the hydrolysis of diglycosylated flavonoids to the corresponding disaccharide and aglycone, which are presumably further metabolized by the fungus as carbon sources. Moreover, they are able to *in vitro* transfer rutinosyl residues from rutinosylated-flavonoids to various hydroxylated acceptors. We hypothesized that this ("side") transglycosylation activity also plays a role for the survival of the fungus, like the neutralization of toxic compounds through glycosylation. Since many terpenes are synthesized by plants and used as defense against pathogens and herbivory, we decided to expose cultures of *Acremonium* sp. DSM 24697 to sub-lethal concentrations of the terpenes geraniol, 2-phenethyl alcohol and linalool. The presence of transglycosylation products was evaluated in solid cultures of *Acremonium* sp. DSM 24697 with the flavonoids hesperidin or rutin as carbon sources. In the cultures with rutin as carbon source, no transglycosylation products were detected. In the cultures with hesperidin as carbon source, transglycosylation products were found in the presence of geraniol and 2-phenethylalcohol, while no products were detected with linalool. The products rutinosyl-geraniol and rutinosyl-phenethylethanol were identified in the supernatant of *Acremonium* sp. DSM 24697 by TLC and MALDI. These results supported our hypothesis that glycosylation could be a mechanism related to the defense system of the microorganism.

Código de Resumen: MS-004

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

CHARACTERIZATION OF THE EMULSIFYING ACTIVITY OF *Enterococcus mundtii* 278 CULTURE SUPERNATANT: STUDIES OF STABILITY AND TOXICITY

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Microbial emulsifiers or bioemulsifiers (BEs) are amphipathic molecules able to increase the solubility in water of hydrophobic substrates. Therefore, they are usefully used in environmental remediation technologies to remove these contaminants from the environment. Additionally, BEs are widely used in food technology as formulation ingredients since they promote the formation and stabilization of emulsions, improving the texture, consistency and the half-life of food products. Unlike its counterparts obtained by chemical synthesis, BEs are highly stable and non-toxic compounds. In previous studies, was reported the production of BE by the bacterium *Enterococcus mundtii* 278 in a lactic whey-based culture medium. In the current study, BE and two commercial synthetic emulsifying agents (nonionic surfactant Triton X-100 and ionic surfactant SDS) were characterized according to their stability and toxicity. Culture supernatant containing BE was filtered through a cellulose membrane (cut-off = 14,000 Da) and it used as a partially purified product source. Stability studies were conducted by incubating each emulsifying agent at different temperatures (37ºC–100ºC), pH values (2–10) and of NaCl concentration (5%–20%). After the incubation period, residual emulsification index (E_{24}) was determined using kerosene as hydrophobic substrate. Toxicity studies were conducted by determining the percentage of viability of the Caco-2 cell line after being exposed to each emulsifying agent for 3 h at 37°C.

The BE retained 100% of its activity residual for all the temperatures, pH values, and salt concentrations tested. Triton X-100 was also a thermo-stable agent. However, SDS was gradually decreasing its residual E_{24} according to the increase of temperatures. Concerning the effects of the pH, SDS was most stable in the acid pHs (3–6) with residual E_{24}-values among 62%–44%. In opposite, Triton X-100 showed maximum stability at a neutral pH (residual E_{24} = 62%). SDS and Triton X-100 (to a greater extent) were sensible to the salt presence, losing all its residual activity for a 20% NaCl. Finally, the viability of Caco-2 cells was significantly affected by the emulsifying agents tested, with viability percentage of the 61%, 75%, and 94% for SDS, BE, Triton X-100. In fact, ionic agents such as SDS tend to be more toxic to animal cells than non-ionic agents. The results presented in this study demonstrate that BE is a highly stable product, with an intermediate toxicity between SDS and Triton X-100 for Caco-2 cell line.

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Código de Resumen: MS-005

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

EVALUATION OF THE PLANT GROWTH PROMOTING PROPERTIES UNDER SALT STREES OF BACTERIA ISOLATED FROM ANDEAN WETLAND

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Soil salinity is a severe problem affecting agricultural productivity in about 1/3rd of World's irrigated lands. Our country presents 32 million hectares classified as Saline Soils and 53 million hectares as Sodic Soils, which means that we have 85 million hectares with salinity / sodicity problems.

This problem has negative effects on the soil, such as the reduction of biological activity, the reduction in the availability of nutrients, the generation of changes in the structure, degradation and desertification of the land, which is limiting for the development of crops. Salinity changes the agronomically useful lands into unproductive areas, so that desertification of soils is a great concern and if we do not solve this problem we will soon face the situation of the lack of sufficient food in the world.

Plant growth promoting bacteria (PGPB) are a useful alternative strategy for salt tolerance in plants. These bacteria are able to colonize the plant rhizosphere and confer beneficial effects by various direct and indirect mechanisms such as the production of indole-3-acetic acids (IAA), aminocyclopropane-1-carboxylate (ACC) deaminase, phosphate solubilization, among others. The use of halotolerant PGPB is environmental friendly and they are an inexpensive strategy for a better crop production and conservation in salt affected areas.

The aim of this study was to evaluate the effect of saline stress in the promoting properties of PGPB isolated from Andean wetland. For this purpose, the tolerance to different NaCl concentrations (200, 700, 1000,1400,1700 and 2600mM) of thirteen strains was evaluated. Seven of them were able to grow at up to 1700mM of NaCl.

The results of this research showed that the isolates retain its characteristics as promoting plant growth at 200 mM of NaCl. Under this saline stress they can produce IAA, ACC deaminase, N_2-fixation and phosphate solubilization. At higher concentrations of NaCl (1400mM), only three strains maintained the ability to solubilize phosphate. Finally, the results showed that there are strains that are able to produce a greater concentration of AIA as the concentration of salt increases.

These results encourage continued studies for the use of these microorganisms as an alternative to improve the response of crops affected by saline soils.

Código de Resumen: MS-006

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

INFLUENCE OF ACIDOPHILIC MICROORGANISMS IN THE GEOCHEMISTRY OF THE AMARILLO RIVER

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The Amarillo River is located on the eastern slope of the Sierra de Famatina (La Rioja, Argentina) in 'Nevados de Famatina´ mining district where La Mejicana mine operated between 1890 and 1925. This fluvial system drains headwaters through a zone of alteration, an important halo of high-sulfidation epithermal deposits (Au–Cu–Ag–As–Sb–Te) and Cu and Mo porphyries. The river is characterized by sulfated acidic waters (pH ~ 3) of reddish-yellow colorations with high concentrations of dissolved total solids and metals. This high concentration of metals and acidity is generally associated with high rates of oxidation and leaching of pyrite and other sulfide minerals due to natural exposure or mining activity. Along the river a widespread deposition of ochreous sediments associated with the precipitation of Fe(III) compounds can be observed. Jarosite ($XFe_3(SO_4)_2(OH)_6$) is the main precipitated phase in upper riverbed, while schwertmannite ($Fe_8O_8(OH)_6(SO_4).nH_2O$) dominates the middle and lower ones. In this work, we establish the role of the microorganisms present in Amarillo River on the oxidation of Fe(II) and the subsequent precipitation of different hydroxysulfates. Consortia were obtained from the enrichment of samples coming from the upper and middle basins of the Amarillo River using culture medium Mac pH 1.8-Fe(II) 9 $g.L^{-1}$ with yeast extract 2 $g.L^{-1}$ (heterotrophic consortium) and without yeast extract (autotrophic consortium). Biotic oxidation of Fe(II) was confirmed by comparison with abiotic controls. DNA was extracted and bacterial ribosomal RNA genes were amplified, cloned, and sequenced. Iron-oxidizing bacteria and other acidophiles associated to them were detected and isolated. Moreover, yeasts and fungi were also isolated by culture in solid medium from the heterotrophic consortia. To recreate the natural biomineral formation in-vitro laboratory study was carried out: inocula from each consortium was inoculated in 9K medium pH 2.0; abiotic controls replacing the inoculum by fresh medium were also done. After 28 days, the precipitates were harvested, washed, dried at 60 °C, and analyzed by X-ray diffraction (XRD). Two different patterns were obtained: one for biotic precipitates with well-defined and intense peaks suggesting well-crystallized phases belonging to the jarosite group and a second for abiotic controls with broad peaks that indicate low crystalline phases possibly related to schwertmannite precursors. Besides, the morphology of the precipitates was investigated with scanning electron microscopy (SEM) detecting cells and nano-sized precipitates which were covering the cell surface. Mineral aggregates had similarity with synthetic jarosites. These results suggest that the geochemical processes of Amarillo River should be interpreted including the activity of the native microorganisms.

Código de Resumen: MS-007

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

Pseudomonas migulae S1-2 AS PHOSPHATE SOLUBILIZER IN PERIURBAN HORTICULTURE SOILS

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The agricultural activity around big cities (periurban agriculture) is an interesting case study of an environmental concern. Frequently, this type of agriculture uses fertilizers to provide essential nutrients to plants, like nitrogen, phosphorus and potassium. Nevertheless, the indiscriminate use of these agrochemicals causes a negative impact on the environment.

Because of the intensive use of these types of soils, it is very important to manage it in a sustainable way. The stimulation of native microbiota is a strategy to prevent soil deterioration consequence of human activities. Recently, efforts are focused on the development of inoculants with plant growth-promoting bacteria (PGPB) to restore and maintain the agricultural soil quality and optimize crop production without chemical inputs.

The aim of this work was to study the ability of *Pseudomonas migulae* S1-2 to solubilize unavailable phosphate from soils intensively used in periurban horticulture activities. This strain is autochthonous of these soils and exhibited resistance to common pesticides used, such as iprodione, deltamethrin and abamectin.

For that purpose, soils from Cuartel V, Moreno (Buenos Aires) with a total but unavailable phosphorus amount of 0.68 mg/g soil were used. Phosphorus solubilization mediated by *P. migulae* S1-2 was studied in NBRIP broth, supplemented with sterilized soil or calcium phosphate as control. The abiotic solubilization was evaluated in the same conditions in sterile NBRIP broth/soil or $Ca_3(PO_4)_2$ media. The four treatments were incubated 2 weeks at 32 ºC under agitation. Samples were taken at regular intervals, monitoring cell growth by colony-forming unit/ml counts. After centrifugation, soluble phosphate was measured as total phosphorus using a modified protocol of Bray and Kurts method.

P. migulae S1-2 was able to solubilize phosphate from horticultural soils. Total phosphorus in the supernatants was increased almost 18% during the first week of incubation rising to a 22% at the second week, while bacterial counts increased from 10^7 to 10^8UFC/ml. These results are promising since 3% of dry biomass represents phosphorus incorporated as consequence of cell growth.

Pseudomonas migulae S1-2 is a potential candidate to continue studies for the development of bioinoculants for horticultural soil restoration.

Código de Resumen: MS-008

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

THE FECES OF MARA (*Dolichotis patagonum*, Caviidae, Rodentia) AS DISPERSION AGENTS OF ARBUSCULAR MYCORRHIZAL FUNGI AND DARK SEPTATE ENDOPHYTES IN THE NATIONAL PARK SIERRA DE LAS QUIJADAS, SAN LUIS (ARGENTINA)

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In the diet of small mammals, the consumption of fungi is well documented in the Northern Hemisphere, Tropics and Australia. The fungi that are consumed by mammals can be epigeous or hypogeous, and are representatives of Ascomycota, Basidiomycota and arbuscular-mycorrhizal-fungi (AMF). In general, mammals eat the fungal fruiting bodies, digesting and assimilating them, except for the spores that pass through the digestive tract of the animals and are dispersed without being degraded. In the world, the dispersal of spores of fungi by small mammals has been widely studied and has a close and important relationship with the ecology of forests and ecosystems where mycorrhizal associations predominate. Arbuscular-mycorrhizal-fungi are a group of biotrophic fungi that establish arbuscular mycorrhizal symbioses in the roots of most plants. These fungi contribute to the mineral nutrition of their hosts and to the conservation of the soil, among some of their functions of great ecosystemic importance. The dark septate endophytes (DSE) are a heterogeneous group of fungi belonging to Ascomycota, Basidiomycota that also establish endosymbiosis in the roots. There is global and local evidence of the role of rodents in the dispersal of fungal spores. Mara (*Dolichotis patagonum*, Caviidae, Rodentia) is an endemic rodent from Argentina; its diet consists of 70% of monocotyledons and 30% of forbs, with grasses as the herbaceous species with the greatest presence and relative coverage, preferring *Pappophorum*, *Chloris*, and *Trichloris* among the Poaceae, although mara consumes another C_3 and C_4 species. The grasses C_3 and C_4 form symbiosis with the AMF and DSE. The studied mara populations inhabit Sierra de las Quijadas National Park, San Luis (Argentina). The objective of this work was to detect the AMF and DSE in feces of *D. patagonum* and if mara is a potential disperser of AMF and DSE. For this, the feces of mara collected in two areas of the Park ("Jarillal" and "Sierras") were analyzed microscopically to determine the presence of AMF and DSE and trap plants were cultivated with the feces as a source of inoculum for these fungi. In mara feces the presence of AMF and DSE was scarce, and roots were also detected; further, the roots of the trap plants were colonized by AMF and DSE. We concluded that mara contributes to the dispersion of AMF and DSE, although in a meager way. Considering that mara prefers C_4 grasses and that these are obligate mycotrophic host of AMF and are associated also with DSE, the remains of the ingested roots containing these fungi, the spores of the AMF and the anamorphic fructification bodies of the DSE in the feces, are the fungal structures that make up the dispersed inoculum. The presence of sporocarps of AMF and DSE in the diet of mara in the Park is reported for the first time; confirming with their presence the field observations of the consumption of soil and roots by this endemic rodent.

Código de Resumen: MS-009

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN THE CALDENAL, SAN LUIS (ARGENTINA)

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Arbuscular mycorrhizal fungi (AMF) are biotrophic mutualistic symbionts of the 80% of the terrestrial plants; they increase the growth of their hosts through the contribution in the absorption of water and nutrients by means of its extensive network of mycelium in the soil. This hyphal network also contributes to the soil aggregation, prevents erosion and interconnects plants, redistributing resources in the community. Also, these fungi benefit their hosts by increasing the host resistance to drought and pathogens attacks. AMF mycelium and spores are common components of soil microorganisms communities in diverse ecosystems. In worldwide arid areas, mycorrhizal associations have been registered with species of the genus *Prosopis* (Fabaceae). Ecosystem services are the direct benefits to society obtained by the functioning of an ecosystem. Thus, the HMA constitute a key functional group in the soil with an important role as ecosystem services providers through the increase of plant productivity, soil formation and improvement of the soil conditions, the prevention against biotic and abiotic stress. Despite playing a key role in ecosystems, a gap in the knowledge of the diversity of the AMF in Argentina exists. Particularly, in the Caldenal, Fitogeographical Province of the Espinal, there are no records of the AMF diversity. Taking into account that the "Caldén" forests (*Prosopis caldenia* Burkart, Fabaceae) are suffering a constant reduction in their distribution area, driven mainly by the advance of the agricultural-livestock border, the knowledge of the AMF diversity as a basic service ecosystem and as an indicator of the general health of the soil is of vital importance for the preparation of an adequate management plans for the forest and the microorganisms diversity conservation in the soil and for the maintenance and/or conservation of soil quality. The objective of this work was to determine the diversity of the AMF in the Northern limit of the "Caldén" Forest natural distribution. The sampling sites were four forest in Villa Mercedes, San Luis; soil samples extraction and AMF diversity analysis were done with classic technics. The morphospecies were determined by observing the spores and sporocarps in the optical microscope. Ten morphospecies belonging to the genera were found preliminarily: *Acaulospora, Claroideoglomus, Diversispora, Entrophospora, Funneliformis, Gigaspora, Glomus, Rhizophagus, Sclerocystis* and *Scutellospora*; showing a variation in the frequency of appearance of each taxa in the different plots. This work will serve as a starting point for later studies comparing the diversity of AMF between areas with different land uses and for the analysis of the potential ecosystem services of AMF in the Caldenal.

Código de Resumen: MS-010

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

COMPARISON BETWEEN MICROBIAL COMMUNITIES UPSTREAM AND DOWNSTREAM A WATER TREATMENT PLANT IN MORENO DISTRICT, PROVINCE OF BUENOS AIRES

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In Cuartel V, district of Moreno, Province of Buenos Aires, there is an industrial waste treatment plant (WTP) placed near a natural stream. This water course is also surrounded by horticulture farms and urban settles. Local inhabitants have reported recurrent WTP over-flooding that have affected the water course and some adjacent farms, thus water contamination became an important social and economic problem.

The aim of this work was to analyze the microbial community in a reference site located upstream the WTP (R) and a site located downstream the WTP (DS) to find indicators of the biological conditions of the system.

Water samples were collected on 2017 July 19th (winter) and December 12nd (spring) in both DS and R sites. After collection, samples were conserved at room temperature until processing within 24 h. Total DNA was extracted using the DNeasy® PowerWater® kit (QIAGEN) and 16S rRNA gene V1-V3 region was sequenced by Illumina my-seq (Mr DNA-Texas, USA). Community diversity was analyzed using QIIME platform.

Diversity index Shanonn (H) and Simpson (D) showed that in winter samples, DS presented a significant higher diversity compared with R (D: 0.985 and 0.954, H: 7,977 and 6,620 respectively $p<0.04$) but no significant differences were observed for spring samples. On the other hand, R showed significant diversity changes between seasons (p-values of 0.002 for D and 0.004 for H) while DS samples showed no seasonal differences.

Proteobacteria and Bacteroidetes were the dominant phyla despite the season. In spring samples both phyla together represented the 95% of DS reads whereas in R represented only 49%. In this R sample Verrucumicrobia and Actinobacteria become relevant phyla reaching between both of them 38% of the reads. No such pattern was observed in winter samples. Nevertheless, Verrucumicrobia and Actinobacteria were more prevalent in R than DS.

Other interesting changes were that Armatimonadetes, an oligotrophic phylum thrives at 20-40ºC. This phylum was 200 fold represented in R samples in spring compared with winter. This was in accordance with the *in situ* measured temperatures (30°C in spring and 11°C in winter). Among Proteobacteria, class Epsilonproteobacteria, often associated with polluted environments was only found in DS samples.

These results indicate that both, seasonality and the anthropogenic pollution could be responsible for the differences found in microbial community composition.

Código de Resumen: MS-011

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

PGP OF HALOTOLERANT BACTERIA EFFECT IN CHIA (*Salvia hispanica* L.) SEEDS GERMINATION UNDER SALINITY CONDITIONS

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Bad agriculture practices increase salinity in soils. These soils are a serious problem for agriculture since they interfere with the adequate growth of most crops. The high content of soluble salts in the soil exchange complex, produces a negative effect by increasing its osmotic pressure, decreasing available water for roots, and breaking down its structure. In this sense, halophilic microorganisms with plant growth promoting (PGP) properties constitute an alternative for the re-utilization of these soils.

The objective was to evaluate the effect of the inoculation of three halotolerant microorganisms: *Micrococcus sp*, *Bacillus atrophaeus,* and *Halomonas sp*. on the germination of chia in presence of salts.

The assay was carried out on water agar plates 1.5% using saline water with different NaCl concentrations: 15 mM (control-non-saline soil), 50 mM, 100 mM and 150 mM. The experimental strains were: *Micrococcus sp* SA211, *Bacillus atrophaeus* HX11 and *Halomonas sp*. SFsal. Six bacterial inoculums were used: No bacteria (control), SA211, HX11, SFsal, and two consortiums: C1 (SFsal + SA211) and C2 (HX11 + SA211). The selection of these microorganisms and consortiums was carried out from a previous evaluation of their PGP activities, with and without NaCl. Once selected, antagonism tests were carried out to validate the selection. Seeds were sterilized with 70% ethanol, 3% sodium hypochlorite and distilled water, always under stirring at 150 rpm. Microorganisms were grown in nutritive broth with salt (42 mM NaCl) and the OD was adjusted to 0.6. Sterilized seeds were introduced in the bacterial cultures and were agitated for two hours at 150 rpm. Seeding was done immediately, placing 20 seeds per plate and with four replicates for each treatment. After seven days, the number of germinated seeds, the fresh weight of the seedlings, plant and root length, and root dry weight were recorded.

The effect of bacteria at 100 mM and 150 mM was not observed in any of the measured variables for any treatment. Regarding the germination at 15 mM, no differences were observed between the control and the treatments; while at 50 mM an improvement in presence of SA211 and HX11 was observed. Regarding the fresh weight of plants, in 15 mM there was no promoter effect of the bacteria, but in 50 mM there was an increase in the treatments with SA211 and HX11. In the case of root dry weight, a significant increase was observed in SA211 and HX11 compared to the control at 15 mM. Growing at 50 mM of NaCl, the length of the plants increased significantly when the seeds were inoculated with HX11 and to a lesser extent in C2. Inoculation with HX11 produced a positive effect on the elongation of roots under salinity conditions. We were able to observe by fluorescence microscopy, the presence of bacteria in the roots of the treatments at the end of the experiment. The use of halotolerant microorganisms could help chia seeds to germinate in saline soils.

Código de Resumen: BF-001

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

GLOBAL METABOLISM MANIPULATION FOR THE SYNTHESIS OF BIOPRODUCTS IN *Escherichia coli.*

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Transcriptional regulation in *Escherichia coli* comprises a network of specific and global regulators. Some of the latter control operons related to central metabolism affecting carbon flow and reducing power. Among different global regulators that affect central metabolism we studied ArcA and CreC, members of two component systems, Cra and Rob. ArcA is known to be one of the main regulators that affects C metabolism in response to O_2 availability, while CreC is known to respond to both C source and aeration. The majority of Cra targets are genes coding for the enzymes involved in central carbon metabolism. Rob is involved in antibiotic resistance, solvent tolerance and affects some genes of glucose metabolism and TCA cycle. Manipulation of global regulators is one of the strategies used for the construction of bacterial strains suitable for the synthesis of bioproducts. However, the pleiotropic effects of these regulators are not always predictable, since they can vary in different conditions and are often strain dependent.

This study analyzed the metabolic effects of these several major global regulators using deletion mutants of the well characterized and completely sequenced *Escherichia coli* strain BW25113. Production of inherent (organic acids and ethanol) and non-inherent (polyhydroxybutyrate and 1,3-propanediol) compounds was evaluated in different conditions of oxygen availability and different culture media (M9 and LB) supplemented with glucose or glycerol. Additionally, the effects on stress tolerance of each mutation were studied in cultures grown in M9 glucose 0.5% in low and full aeration conditions. Results obtained were utilized to perform multivariate analysis (Principal Component Analysis and Hierarchical Clustering Analysis) in order to identify separation trends and visualize the response of the working strains in the variety of conditions tested.

The simultaneous comparison of the effects of each regulator in different growth conditions, including tolerance assays and the production of several bioproducts, allowed the discrimination of the particular phenotypes that can be attributed to the individual mutants, and singled out Cra and ArcA as the regulators with the most important effects on bacterial metabolism. These two strains also resulted in the most suitable backgrounds for the synthesis of succinate and 1,3-propanediol (1,3-PDO), respectively. The Δ*cra* mutants were further modified to increase succinate production by the addition of carboxylating enzymes, achieving an increase of 80% respect to the wild type. Accumulation of 1,3-PDO in the Δ*arcA* mutant was optimized by overexpression of PhaP, which doubled the accumulation of the diol in a semidefined medium using glycerol, resulting in 23.94 $g.L^{-1}$ of 1, 3-PDO after 48 h, with a volumetric productivity of 0.5 $g.L^{-1}h^{-1}$ in bioreactor cultures.

Código de Resumen: BF-002

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

A CLOSED-LOOP ALGAL BIOMASS PRODUCTION-PLATFORM AND BIOREFINERY FROM RENEWABLE SOURCES OF N AND P AND INTENSIVE RECYCLING OF REAGENTS INTO NUTRIENTS

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Microalgae and cyanobacteria are increasingly considered a promising alternative to conventional crops as feedstock for food and feed and biofuels, mainly because of their much higher photosynthetic productivity. One of the drawbacks of this technology is the very large demand of N and P fertilizers that must be supplied to achieve algal production potential. In this study, we provide proof-of-concept for a closed-loop algal production platform and biomass biorefinery for ethanol at the expense of atmospheric N_2 and P from bone meal. N_2 is assimilated in a N_2-fixing cyanobacterium's biomass that accumulates very high levels of protein (60 % w/w). Water extraction of this biomass produced an organic fertilizer, which as a sole source of nutrients, sustained mixotrophic growth to very high yields of a microalga that accumulated high levels of carbohydrates (60% w/w). The

algal biomass was saccharified in the presence of H_2SO_4 and this acidic condition was secondarily used to release soluble PO_4^{3-} from different P-sources, including bone meal, as a renewable P-source. After increasing the pH with KOH and $Mg(OH)_2$, the resulting preparation was fermented by yeast to quantitatively produced ethanol at about 90 % of its theoretical yield. The resulting fermentation vinasse, supplemented with P, was efficiently recycled as a sole source of macronutrients for the cultivation of the N_2-fixing cyanobacterium's biomass to complete one production cycle. Water recycling and co-production of residual biomass as feed are also shown. This closed loop-algal production platform brings concepts of circular economy into the field of microalgae biomass biorefineries. It basically produces ethanol and feed grade-like biomass at potentially high yields at the expense of atmospheric CO_2 and N_2, and mostly P from food industry waste (bones). Nevertheless, the overall procedure still needs to be subjected to techno-economic and environmental performance analyses for a more realistic comparison to alternative processes.

Código de Resumen: BF-003

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

GENERATION OF *Saccharomyces cerevisiae* HYBRIDS AS A TOOL TO IMPROVE THE OENOLOGICAL PROPERTIES OF A WINE YEASTS COLLECTION

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Saccharomyces cerevisiae. The physiological properties of these yeasts influence the final characteristics of the produced wine. Although several commercial strains are offered in the market, there is a continuous search for novel yeas starters better adapted to different regions, oenological practices or with some special feature. Current winemaking practices favor the harvest of very mature grapes with high sugar concentration, which leads to more alcoholic wines. Thus, it would be convenient to have a yeast strain which combines a resistance to high osmotic conditions (300 g/L of fermentable sugars) and high ethanol concentration (15% $^{v}/_{v}$). From our autochthonous characterized wine yeasts collection, we considered 46 strains which possesses one of these properties and could act as parental strains to generate hybrids capable of tolerating both stress conditions. After a phenotypic re-evaluation, 10 parental strains were selected and induced to sporulation. Considering that native strains are homothallic and no genetic markers are available, the crosses between parental strains were performed with the random spore method. A total of 17 independent crosses were performed, and 207 potentials hybrid were isolated. All generated individuals were phenotypically evaluated for both high osmotic and high ethanol tolerance. So far, 3 hybrid strains and two homozygous cultures from a tetrad dissection have shown improved phenotypes for both characteristics with respect to their parental strains. Furthermore, lab scale fermentations under simulated wine conditions showed promising results for some of these strains as compared to their parental strains. Our results demonstrate that is possible to improve yeast oenological properties of a wine yeasts collection using simple and traditional breeding techniques.

Código de Resumen: BF-004

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

DEGRADATION OF SUGARCANE VINASSE BY AN AUTOCHTHONOUS FUNGUS: MOLECULAR IDENTIFICATION

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Various fungus-based processes can be applied to degrade sugarcane vinasse, an acid effluent (pH=3.5–5.0) from sugar-alcohol industry which contains a high chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Previous studies demonstrated the potential of a native fungus from the provinceof Tucumán(strain V1) to degrade a vinasse sample. In the present study, the molecular identification of this strain was carried out. In addition, it was evaluated the effectiveness of the microbial treatment conducted during 15 d. Mycelium in the exponential growth phase was harvested by centrifugation and total DNA extraction was performed using DNA Kit, MOBIO. Amplification of the rDNA ITS1-5.8S-ITS2 regions was carried out using ITS1 and ITS4 primers and 18S rDNA sequences were compared with partial 18S rDNA sequences published in the GenBank using the BLAST tool from the NationalCenterfor Biotechnology Information (NCBI).

Finally, a phylogenetic tree was constructed using the neighbor-joining method. Regarding the microbiological treatment, 200 mL of vinasse were inoculated with fungus spores at a final concentration of 1×10^6 UFC/mL, and was incubated at 30ºC (150 rpm) for 15 d. Vinasse samples without inoculation were used as abiotic controls (AC). Growth kinetics (measured as biomass production), pH changes, and the removal percentages of COD and BOD was determined each 72 h in the microbiologically treated vinasse (TV) and in AC, by using standard methods for the examination of wastewater. The fungus strain was identified as *Aspergillus* sp. V1 and it was closely related to *Aspergillus terreus* ATCC MYA-4898 (99%). As expected in the AC, no significant growth was detected until the end of the assay. For TV it was observed the maximum growth at 9th d of cultivation (biomass higher than 5 g/l). The TV for 3 d did not show a significant increase in the pH with respect to AC, which remained unchanged throughout the entire experiment (pH = 4.1). However, at 6^{th} d of incubation, pH was significantly increased until a value close to neutral (6.7 ± 0.5). At 12^{th} d of cultivation, it was detected the maximum COD and BOD removal, with percentages of 59% and 89%, respectively. At that point in time, only a 10% and a 30% was removed from AC. Based on these results, a removal of COD and BOD of 49% and 59%, respectively, can be attributed to the metabolism of *Aspergillus* sp. V1. This could involve a significant reduction in the toxicity of the effluent mediated by the action of this strain.

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Código de Resumen: BF-005

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

DEGRADATION OF COMPLEX CARBON SOURCES BY TWO STRAINS OF *Thermoanaerobacterium thermosaccharolyticum.*

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Climate change is a threat to several life forms of our planet. This effect is partially due to the preferential use of fossil fuels to sustain human activities such as house heating, agriculture or transportation. The use of biofuels is one of the most promising alternatives, as it would help reduce the effects of climate change, while avoiding the use of non-renewable sources such as petroleum or gas as sources of energy. Among the most common biofuels are alcohols that can be obtained from microbial fermentation using different kinds of carbon sources. Many different processes have been developed to obtain alcohols from sugars or starch, but the use of these substrates to produce biofuels would compete with food supplies. To avoid this problem, biofuels should be obtained from non-food substrates, such as lignocellulosic biomass.

Several different approaches have been employed to use this substrate, most of which start with the hydrolysis of the biomass to obtain sugars that can be fermented. Although large amounts of energy are required for the hydrolysis steps, and only a fraction of the hydrolysis products can be converted. The use of microorganisms that have the capability to degrade lignocellulosic biomass and synthesize biofuels would help to reduce or eliminate the lignocellulose hydrolysis steps, thereby increasing the sustainability of the processes.

In this work we analyzed differences in the use of xylan and lignocellulosic biomass (sugarcane agricultural residue) by two strains of *Thermoanaerobacterium thermosaccharolyticum. T. thermosaccharolyticum* GSU5 isolated in our laboratory and the collection strain *T. thermosaccharolyticum* DSM 571. These strains are anaerobic thermophilic solventogenic bacteria that can produce butanol and ethanol from these substrates.

Using High Resolution Liquid Chromatograph (HPLC) we observed that both strains were able to degrade corn xylan and sugarcane agricultural residue evidenced by the decrease in the area of peaks corresponding to high molecular weight hydrates and the increase in low molecular weight peaks. Furthermore, using Nuclear Magnetic Resonance (NMR), we observed differences in the degradation of the substrates between the two strains. We were able to determine the composition of Corn xylan by acid hydrolysis and acetylation followed by Gas Chromatography (GC). Analysis of the remaining substrate after bacterial growth allowed us to determine the main type of sugar used.

In conclusion, we could demonstrate that the two strains of *T. thermosaccharolyticum* are able to grow on complex substrates such as xylan and lignocellulosic biomass, and we determined the way each strain processes these substrates. These results are interesting for the development of biofuels, and especially relevant for our country, because sugarcane biomass is an economic substrate that is generated as a contaminating residue during sugarcane harvest.

Código de Resumen: BF-006

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ISOLATION AND CHARACTERIZATION OF DIATOMS FOR LIPIDS OF INTEREST PRODUCTION. COMPARISON OF SAMPLES OBTAINED FROM SITES WITH DIFFERENT GRADES OF POLLUTION.

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Diatoms are a microalgae group that has acquired great biotechnological importance due to their capacity to produce lipids that can be used as food supplements, for the production of biodiesel or in pharmaceuticals. On the other hand, several studies have postulated that lipid production is increased when microorganisms are subjected to stress conditions. However, one of the challenges in the development of some biotechnological processes, is the management of wastes and co-products that are generated. In the case of diatoms, the silica wall (frustule) is obtained as a residue (after solvent extraction of the oils accumulated inside the cells) and the use of these in many industrial and biotechnological processes have been proposed.

The aim of this work is to compare the biodiversity of diatoms isolated from different environments (pristine and polluted); and to compare the amount of lipid accumulation of diatoms from both sites to evaluate their biotechnological potential.

In this study, diatoms from a pristine site were isolated (Reserva Municipal de Rivera Norte), characterized and compared with diatoms isolated from a highly polluted site (Río Luján).

Enrichment of all samples was performed by using modified Diatom medium. After 5 days of growth, samples were analyzed by optical microscope and diatoms were isolated by a Combination of serial and drop dilutions, and transferred to Erlenmeyer flasks. Cultures were kept at 24°C with photoperiods of 16:8 hours (light:darkness).

For the determination of neutral lipids accumulated into diatom cells, Nile Red spectrofluorometric technique was used. Extraction of lipids from diatoms was achieved by using Methanol and Chloroform. The quantification of the extracts was performed by gravimetric analysis. Lipids accumulation was compared between isolated strains.

Frustules, before and after lipids extraction, were studied by SEM in order to analyze their potential biotechnological use in future studies.

The biodiversity found in Rivera Norte was significantly higher than that found in Río Luján. Some of the genus found in Rivera Norte were e.i.: *Fragilaria* sp., *Gomphonema* sp., *Encyonema* sp., *Cyclotella* sp., *Navicula* sp., *Nitzschia* sp. While in Río Luján only a few genus were found.

Results of lipids extraction from the isolated strains (6 from Rivera Norte and 3 from Río Luján) indicated that lipid production was species related and species from Río Luján tended to accumulate lipids at earlier stages of the culture.

SEM images allowed to determine frustule structural analysis for future uses and to determine taxonomic classification.

Results indicated that diatoms could be used for the production of lipids of interest and their frustules could be applied as bio-product for several applications.

This study provides the basis for the development of a cycle of production of a substance of commercial interest while a co-product with potential industrial, agricultural or biotechnological use is being developed.

Código de Resumen: BF-007

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

TECHNOLOGICAL PROPERTIES OF GLUTEN FREE SOURDOUGHS WITH A LACTIC ACID BACTERIA STARTER

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Sourdough is one of the oldest biotechnological processes used in the production of bread. In this system, lactic acid bacteria improve the nutritional and technological properties of the final product, mainly by the acidification and release of volatile components during fermentation. The use of sourdoughs has great potential for the production of gluten-free products (GF) improving their technological and sensory properties. The objective of this work was to evaluate the use of *Lactobacillus plantarum* ATCC 8014 as starter culture in three GF flours: quinoa (Q), buckwheat (B) and rice (R); and compare them with wheat sourdough (W). 24 h (F1) and 10 days backslopping fermentations (F2) at 30 °C were performed. Sourdough with (8014-Q, 8014-B, 8014-R, 8014-W) and without starter (C-Q, C-B, C-R, C-W) were compared considering pH, total titratable acidity (TTA), lactic acid bacteria (LAB) counts, starch gelatinization by differential scanning calorimetry (DSC) and water extractable pentosans. There was a significant decrease of pH for all the 24 h fermentation sourdoughs with starter, reaching 3,43 for 8014-R and 3,46 for 8014-W. The highest TTA was observed with Q with and without starter. 8014-Q y 8014-R showed significant higher LAB counts compared to controls ($p<0,05$). 8014-Q and C-Q presented differences in starch gelatinization with an increase in transition enthalpy (ΔH) and a decrease of To and Tp for 8014-Q. 8014-B showed a decrease of To respect to control ($p<0,05$). Regarding F2, there were no differences among the sourdoughs with starter. There were no differences in pH, TTA, LAB counts, ΔH and water extractable pentosans between F1 and F2 for all the flour types with starters. Similar modifications in the system were obtained with F1 and F2 which allow us to infer that using *L. plantarum* ATCC8014 as starter, it is possible to shorten the fermentation time significantly for gluten free breads.

Código de Resumen: BF-008

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EVALUATION OF PREBIOTIC POTENTIAL OF ARABINOXYLAN FROM HARD AND SOFT WHEAT

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The wholemeal flour is obtained from the milling of whole wheat grain, including the endosperm, the aleurone and the germ. Wholemeal flour is nutritionally richer, with higher dietary fiber content. Arabinoxylans (AX) are part of dietary fiber and have received attention given their emerging prebiotic character. Prebiotics are non-digestible components that stimulate the growth and activity of the beneficial bacteria of the gutl microbiome, mainly *Bifidobacterium* and *Lactobacillus*. The potential prebiotic effect of two extracts of AX from wholeflour flour of hard (bread) and soft wheat was evaluated. Hard wheats are characterized by more protein content and better gluten quality. Both extracts were characterized by protein quantification, water extractable pentosans and xylose/arabinose content. A prebiotic score of each extract was calculated according to the selective growth of *Bifidobacterium* and *Lactobacillus* in a defined medium. Inulin was included as a positive control. The Relative Growth (RG) test was based on the increase in cellular biomass (CFU / mL) after 24 hours of growth of the probiotic strain in a semi-defined medium with 1% of AXs, compared to growth in the same medium supplemented with 1% glucose. In turn, the Prebiotic Activity (AP) was calculated in relation to the change in cellular biomass of *E. coli* cultivated under the same conditions. No significant differences were observed in the ara / xyl composition of the extracts. Both extracts of AX promoted a higher RG of lactic acid bacteria compared to inulin. RG resulted significant higher with AX from hard wheat than from common wheat (1.77 and 1.07, respectively). The PA was higher for *Lactobacillus* and *Bifidobacterium* with the AX extracts than with inulin. PA was significantly higher with AX from hard wheat than from soft wheat. The AX extracts from whole grain flours have prebiotic potential that can be validated *in vivo*. The prebiotic parameters evaluated were significantly higher in AX from hard wheat.

Código de Resumen: BF-009

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

KINETICS OF METHANE PRODUCTION IN A DRY ANAEROBIC DIGESTION OF ALPERUJO

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The alperujo (AL) is a very abundant agro industrial waste in the Cuyo region. It is produced in the two-phase extraction process of the olive oil plants. In previous studies, the possibility of performing a Dry Anaerobic Digestion (DAS) for biogas production has been stated, using a bacterial isolation obtained from an anaerobic digestion of AL and horse manure (preserved at the IBT-UNSJ); relevant variables for biogas production were established by the Plackett-Burman method; and then, optimal values for these variables were calculated using the Box-Behnken method. The aim of present work was to find a model that describes the kinetics of methane production in DAS, using AL as a substrate. For this purpose, DAS were carried out in 60 mL syringes (containing 40 mL of Alperujo supplemented with 0.065 g urea/g AL, 5 mL/L nutrient solution, 0.25 L/L buffer (pH=7) sodium citrate; 2% inoculum), incubated at 32ºC during 25 days (optimal conditions previously obtained). The volume of methane produced was measured after the biogas bubbled in 2N NaOH solution to capture the CO_2, and was reported as mL per gram of initial volatile solids (mL/g $_{IVS}$). Experimental data were adjusted to a Gompertz equation (correlation coefficient R^2 = 0.8904). In this equation, maximum methane production, Pmax, was 94.32 mL/g $_{IVS}$; the time at which the rate of methane production begun to slow, was 254.24 h; and the lag time for methane production, λ, was 47.33 h. This sigmoidal kinetic model is typical for microbial limited behavior, and the Gompetz´s equation has been used for biogas production. This kinetic model of the methane production will be used to scale-up the DAS.

Código de Resumen: BF-010

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

DEVELOPMENT OF POLYHYDROXYBUTYRATE NANOPARTICLES (NanoPHB) FOR ANTIVIRAL DRUG DELIVERY

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Polyhydroxyalkanoates (PHAs) are biodegradable polymers produced by a wide range of microorganisms as intracellular carbon and energy storage compounds. Its thermoplastic properties make them attractive candidates for the replacement of traditional plastics, and their biodegradability and biocompatibility allow them to be exploited in numerous biomedical applications. Poly(3-hydroxybutyrate) (PHB) was the first PHA discovered and it is still the most common and widely researched member of PHA family. PHB has been shown to be fully biocompatible and non-immunogenic, and has been used in drug delivery systems to improve the release of antibiotics and anticancer drugs.

In the present work we aim to develop PHB nanoparticles for antiviral drug delivery. Viral infections pose significant global health challenges, complicated by the limited effectiveness of many antiviral therapies. Among the problems described for antiviral drugs are poor aqueous solubility, short half-life and bioavailability issues. In this context, a PHB-nanoparticle delivery system can help to increase the effectiveness of these drugs by controlling the release and increasing their solubility and stability.

We designed reproducible PHB nanoparticles with precise dimensions using the emulsification method. Particles showed an average size of 180nm, as assessed by dynamic light scattering. Nanoparticles ζ potential values denoted colloidal stability. The size of the nanoparticles allowed us to sterilize the suspension by filtration with 0.22 µm filters. Concentration of the nanoparticles was determined by gas chromatography, after lyophilization and methanolysis of a suspension sample.

Precipitation of the nanoparticles was achieved via ultracentrifugation; after which we were able to resuspend the PHB pellet and retrieve the nanoparticles. This step was fundamental for nanoparticle drug loading, in order to analyze the entrapment efficiency.

Cytotoxicity assays were performed in Vero cells using crystal violet staining and MTT methods in order to evaluate cell viability in the presence of the hydrophobic polymer nanoparticles. Viability of Vero cells did not appear to be affected by our nanoparticles in the range of usage concentration reported in bibliography. We also evaluated the effect of the PHB nanoparticles on Vero cells infected with Zika virus. No differences were observed on infection and virus replication between cells treated with nanoparticles and untreated control cells.

Finally, as proof of concept, we developed PHB nanoparticles loaded with rhodamine B isothiocyanate (RBITC). The RBITC-loaded nanoparticles were observed by fluorescence microscopy to co-localize within two cell types.

Taken together, these results open the road for the application of the PHB nanoparticles as delivery systems for antiviral drugs and other substances of biomedical relevance.

Código de Resumen: BF-011

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

METABOLIC ENGINEERING OF A CYANOBACTERIUM TO INCREASE SUCROSE ACCUMULATION AS AN ALTERNATIVE FEEDSTOCK FOR BIOETHANOL

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Microalgae have great potential as a feedstock for bioethanol and other biofuels. However, the current cost of producing biofuels from microalgae biomass is still high to be able to envision massive and profitable commercialization in the near future. One of the main constraints is the pretreatment and saccharification processes necessary to make the insoluble carbohydrates accessible to microbial fermentation. Hence, we hypothesized that re-routing the carbohydrates' metabolic pathway towards sucrose accumulation would facilitate recovery and fermentation. In order to achieve that, we over-expressed the *spsB* gene, encoding a SPS enzyme and its putative promoter, in *Anabaena sp*. PCC 7120, downstream of the constitutive promoter of pDU1, increasing by 2-fold the SPS activity. Under standard growth conditions, there was no significant difference in doubling time with the *wt* strain. However, the *spsB*$^+$ strain showed an increased tolerance to saline stress, displaying doubling times of 36 ± 11 h in comparison to 53 ± 10 h of the *wt* strain when cultivated in the presence of 80 mM NaCl, and 39 ± 6 h in comparison to 59.4 ± 0.8 hours of the *wt* strain in the presence of 120 mM NaCl. Sucrose content was 10- or 5-fold higher than the *wt* when subjected to 80 or 120 mM NaCl loading, reaching values of 7.65 ± 0.04 % (w/w) or 9 ± 3 % (w/w) of their dry weight in 48 h, respectively. To extract sucrose from the *spsB*$^+$ strain, two different low-energy methods were assessed. In the first one, the collected biomass was air-dried, milled with 15% sand and extracted with water at room temperature, which allowed the recovery of 32 ± 9% of the total sucrose. The second method consisted in an extraction by microwaves at 200 w of power, using 4 pulses of 2 min each, recovering 56 ± 7% of the total sucrose content. Sucrose-rich preparations obtained from both methods were fermented by S*. cerevisiae.* While the preparation obtained by drying and milling allowed an ethanol production of 91% of the maximum theoretical value, the preparation obtained by microwaves exposure was around 50% of the maximum theoretical value. This efficiency was partially improved by adding a nitrogen source to the sucrose-rich preparation. Hence, ethanol productivity was 26 ± 6 mg. L of culture^{-1} for the extract obtained via drying and milling, and 12 ± 1 mg. L of culture^{-1} for the extract obtained via microwave and supplemented with a nitrogen source, a 3-fold and a 2-fold increase compared to the *wt*, respectively. These results present a promising base-line to continue investigating the use of genetically-modified cyanobacteria biomass as an alternative bioethanol feedstock.

Código de Resumen: BF-012

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

DECONSTRUCTING ALGAL BIOMASS WITH FUNGAL ENZYMES AS AN ALTERNATIVE FEEDSTOCK FOR BIOETHANOL

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The most common biofuel is first generation bioethanol, which is produced from agricultural stocks such as corn or sugarcane in the US or Brazil, respectively. Despite the great benefit associated with partial replacement of some fossil fuel, the fact that present and future global food security is still not fully warranted poses a serious concern on the use of these feedstocks for bioenergy purposes. A second generation of bioethanol from plant lignocellulosic feedstocks has been more recently envisioned. Regardless of clear advantages over first-generation biofuels, such as broad availability and low cost of the feedstock, and non-competition with food production, they face hard-to-overcome disadvantages due to the composition and structure of the lignocellulosic biomass, requiring quite intensive mechanical and physicochemical pretreatments, and expensive

saccharifying enzymes for its conversion into ethanol.

Aquatic microalgae and cyanobacteria are increasingly considered a promising alternative to conventional crops as feedstock for food and feed, biofuels, and other higher-value products. This is mainly because of a much higher photosynthetic productivity (a conservative potential of about 50-fold) and more favorable biochemical composition and structural properties than biomass of terrestrial crops, and independence of arable land.

In this study we took advantage of the availability of a cell wall-less mutant strain CW-15 of the microalga *Chlamydomonas reinhardtii*, to advance in the analysis of algal biomass deconstruction as an alternative feedstock for ethanol or other fermentation products. Strain CW-15 was cultivated at different levels of N-deficiency to trigger starch accumulation. We observed that 2.5 to 5.0 mM NH_4Cl in the culture medium resulted in carbohydrates accumulation up to 50% (w/w) of the dry biomass weight.

At the same time we performed preliminary bioprospecting assays to identified fungal strains able to hydrolyze starch and cellulose. Among others, we identified a strain of *Alternaria alternata* which has been isolated as a contaminant of a cyanobacterial culture. Thus, we optimized induction conditions in liquid medium for the production of hydrolytic enzymes, including culture medium, initial amount of spores, and inducers (starch or cellulose). Under these optimized conditions, the fungal spent medium, solubilized starch at 4.0 mg glu . mg de $prot^{-1}$. min^{-1} and released reducing carbohydrates (as a proxy of saccharification) at a rate of 0.4 mg glu . mg de $prot^{-1}$. min^{-1}. Importantly, these enzyme preparations deconstructed *C. reinhardtii* strain CW-15 biomass at a complex-carbohydrates solubilizing and hydrolytic activities of 1.0 and 0.2 mg glu . mg de $prot^{-1}$. min^{-1}. Experiments are in progress to further optimize yields of biomass saccharification and to determine rates of deconstructed biomass conversion into ethanol by fermentation with the yeast *Saccharomyces cerevisiae*.

Código de Resumen: BF-013

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

PROBING BIODIVERSITY OF MICROALGAL PRODUCTIVITY IN ENVIRONMENTAL PHOTOBIOREACTOR SIMULATIONS

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Increasing demand for energy and substantial reduction of unsustainable sources like fossil fuels have led to look towards renewable resources. First generation of biofuels is one of such alternatives and is produced from edible plant crops. The most advanced generation of biofuels (third generation) is proposed to be obtained from photosynthetic microbes, like microalgae, in non-arable lands. However, commercialization of these biofuels is currently very limited mostly due to high production costs. While algal culture in open ponds demands lower capital investments, productivity, especially under winter conditions, normally off-sets profitability. Bioprospecting for native microalgae for desirable traits is a very useful strategy and more broadly accepted than genetic engineering towards strains optimization for increased productivity.

Thus, we started some bioprospecting studies from two contrasting eco-region from Argentina: South eastern Buenos Aires and La Quiaca, Jujuy. The most contrasting weather parameter was maximum irradiation during autumn. La Quiaca's irradiance is among the highest in the planet. We gathered two microalgae strains collections of about 25 entries for each site. Strains were domesticated and identified by morphological and molecular taxonomy. Biochemical analysis of the biomass was performed under N-deficiency to trigger lipid or carbohydrate reserves accumulation. Two pairs of strains, one from each contrasting site were selected as very closely related strains at the rDNA sequence. Biomass productivity and biochemical composition did not change under laboratory culture conditions at low or high irradiance. However, productivity simulations in environmental photobioreactors mimicking cultivation in open ponds under Buenos Aires´s or La Quiaca´s average weather conditions suggested that a *Scenedesmus obliquus* strain (C1S) from Buenos Aires presented a higher productivity under Buenos Aires autumn conditions than the La Quiaca's *S. obliquus* strain (P31). Furthermore, the Buenos Aires's strain was even more productive under La Quiaca's average weather conditions in the same season. However, under simulated weather of La Quiaca of a fully shiny month at 20 cm and 5 cm deep ponds, both strains tended to attain similar and high productivities.

Overall, light appears as one of the main limitations for algal productivity and the case study of these *S. obiquus* strains suggested that strains naturally acclimated to lower irradiances might be broadly more productive in deeper open ponds for an increased areal productivity.

These are pioneering simulations and geographical comparisons of microalgal productivity under environmental conditions in South America and would be very useful for the development of algal biotechnology in the region.

Código de Resumen: BF-014

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

FERMENTATED WHEY WITH A PROBIOTIC BACTERIUM FOR THE FUTURE FORMULATION OF A FOOD ADDITIVE FOR PIG PRODUCTION

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Whey (W) is a byproduct in the dairy industries and retains 55 % of milk nutrients. Disposal of the W is a major pollution problem for the dairy industry because of the high volumes produced and having a high biochemical oxygen demand. To overcome the existing problem, utilization of W in a desired fermented product is highly appreciated. The aim of this work was to fermentate W with a probiotic bacterium (*L. rhamnosus* RC007) and to evaluate the effect of this fermented whey (FW) in a mice model. Previous studies demonstrated beneficial properties of *L. rhamnosus* RC007 such as immune modulation and decrease of intestinal inflammation in mice with TNBS-induced colitis. The W used in the present work was obtained from dairy basin of the south of Córdoba. Their microbiological and chemical composition was characterized. Three different heat treatments were evaluated in order to reduce the microbiological charge without causing coagulated proteins. Heating the W at 65ºC for 30 min was enough to significantly reduce the number of enterobacteria, lactobacilli and total aerobes bacteria. After this pasteurization, cheese whey was evaluated as a base for the development of the probiotic bacterium. Inoculated whey was incubated at 37ºC and bacterial growth was evaluated by taking an aliquot every two hours and plating on MRS agar. For the in vivo assay, 18 BALB/c mice were divided into three groups (n=6): control group: animals received orally 0.1 ml of PBS; FW group: animals received orally 0.1 ml of fermented whey; W group: animals received orally 0.1 ml of whey without fermentation with probiotic bacterium. After 10 days mice were sacrificed by cervical dislocation. Live body weight was measured the day 0 (before W or FW administration) and at the end of the experiment (day 10). Intestinal contents were collected from the small intestines for cytokines determination (IL-10; IL-6 and TNFa) by flow Cytometer. The administration of W or FW did not affect the body weight of the mice. Increases of all the cytokines assayed were observed in mice that received FW compared to control and W group. The ratio between the anti and pro-inflammatory cytokines (IL-10/TNFa) was also evaluated and the results showed that the mean values increased in the group of mice that received FW. The results obtained in the present work showed that FW with *L. rhmanosus* RC007 was able to stimulate and to modulate mouse immune system. This immune stimulation could allow it to respond more quickly to face noxious stimulus. More studies are needed to confirm the beneficial properties of FW, however we could conclude that whey fermentated by this probiotic bacterium is an interesting alternative for development of a new food additive for pig production, taking advantage of the beneficial properties of probiotc bacterium and the nutritional properties of whey, at the same time reducing the environmental impact.

Código de Resumen: BF-015

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SELECTION OF LACTIC ACID BACTERIA FOR THE FORMULATION OF MIXED INOCULANTS INTENDED FOR SILAGE

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Preservation of forage crops for animal feed by ensiling is a well-known method.

The process is based on fermentation of water-soluble carbohydrates by the epiphytic lactic acid bacteria (LAB) present on the crop and the exclusion of air. The fermentation lowers the pH due to lactic acid production which inhibits the growth of many spoilage organisms. Silage quality could be improved by addition of LAB inoculants, alone or in combination with chemical additives.

The aims were to select lactic acid bacteria with potential silage inoculants properties in order to the future development of a mixed inoculant that allows to fulfill different purposes. Ten LABstrains previously isolated from maiz silage without inoculants,were evaluated on their efficacy in reducing the pH of maize extract medium, their bio-control ability against mycotoxicogenic fungi, commonly found in silages, (*Aspergillus fumigatus, A. parasiticus, Penicillium griseofulvum*) and against pathogenic bacteria (*Escherichia coli*, *Salmonella* spp, *Staphylococcus aureus* and *Streptococcus haemolyticus*). With regard to the antifungal properties, studies on lag phase, growth rate and macroscopic characteristics of the fungi were carriedout. *Lactobacillus acidophilus* RC015 was the most efficient in reducing the pH of a maize extract medium, followed by *L. rhamnosus*

RC007; *L. plantarum* RC009 and *Pediococcus acidolactici* RC004, achieving pH 4 or less after 12 h of fermentation. All the LAB strains were able to inhibit the growth of pathogenic bacteria and this fact was more strongly with *L. rhamnosus* RC007. *Aspergillus parasiticus* growth rate and lag phase were inhibited by *L. plantarum* RC009; *L. acidophilus* RC015 and *P. acidolacticci* RC004. *Aspergillus fumigatus* proved to be the most resistant fungi against the LAB tested, only *L. plantarum* RC009 was able to significantly inhibit their growth rate. With regard to *P. griseofulvum*, macroscopic changes were observed with several LAB interactions, such as development of white mycelium and absence of conidiogenesis. *L. rhamnosus* RC007 increased the lag phase of this fungus.

Taking into account all the results obtained, *L. rhamnosus* RC007, *L. plantarum* RC015, *L. plantarum* RC009 and *P. acidolactici* RC004 were selected to formulate a mixed inoculant able to fulfill different purposes. The ‘generally recognized as safe’ (GRAS) status of LAB offers the potential to use these bacteria in commercial applications as biological control agents in foods or feeds. The selected strains could be able to prevent mould growth, to improve quality of fermented silage and to reduce the health hazards associated with mycotoxins in inoculated silages. Future assays will be conducted in laboratory-scale silos in order to provide more knowledge for the future development of a new mixed inoculant intended for silage.

Código de Resumen: BD-001

Sección: Biodiversidad

Modalidad: Oral

WINE YEAST AND THEIR APPLICATIONS IN BIOTECHNOLOGY

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The UNCUYO Facultad de Ciencias Agrarias has a native yeasts´s collection from Mendoza. During the development of different research´s works, it has increased in the number of individuals wich were characterized as Saccharomyces spp. During winemaking, the alcoholic fermentations can be stopped for different reasons, so it is important have microorganisms be able to solving specific problems; such as ferment highly sugary musts, and consequently resist high concentrations of alcohol, or restart stopped fermentations with fructose in a higher concentration than glucose. These microorganisms has continuously being evaluated from a technological and qualitative point of view in order to identify those yeasts so that being used in order to specific winemaking . The aim of this work had been the evaluation of different resistance by yeasts against specific stress situations, such as resistance to 15% ethanol, preference for fructose and growth in breeding ground with 300 g / L of glucose. We worked with 313 strains from five Departments: Luján de Cuyo, Rivadavia, Junín, Maipú and San Martín. First, all the strains of the study were verified in their viability and purity, and then were placed again under maintenance conditions at -20ºC. 16% of the strains were able to grow in alcoholized broths, while all of them grew in fructose broths; their preference for glucose was showed by only 10% of the individuals studied. Finally and with response variability, all grew in strongly osmotic broths. In the yeast´s collection there are strains of interest to be used according to specific winemaking objectives. The maintenance of microbial crops protects biodiversity while offering useful tools to solve everyday problems in the industry.

Código de Resumen: BD-002

Sección: Biodiversidad

Modalidad: Poster

EVALUATION OF AN ALTERNATIVE METHOD FOR THE CONSERVATION OF BACTERIA.

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Microorganisms isolated from their natural environment are preserved in Microbial Culture Collection (MCC) that plays a fundamental role in the *ex-situ* conservation of microbial diversity. The conservation method should minimize the occurrence of genetic events, contamination and ensure cell survival and recovery during the process of long-term preservation. There are different methods such as lyophilization, freezing, repeated sub-culturing. It is common to use vials with "pearls" on which the microorganisms are adhered and preserved by freezing. It has the advantage that when it is necessary to activate the microorganism, a pearl is sown in liquid or solid medium, without defrost the entire vial; however the disadvantage is its cost. The Faculty of Agricultural Sciences, National University of Cuyo, has a MCC with more that 750 bacteria, used in research projects, industrial processes, training courses, quality programs and microbiological diagnosis of food. It is necessary to have a fast, economical and reliable method conservation that at the same time allows the recovery of the microorganism in an accessible form. Therefore, the objective was to standardize an alternative conservation method and evaluate its effectiveness with respect to the internationally recognized method. From a plate with fresh bacterial culture, the description of its morphological characteristics was made by observation under an optical microscope, phenotypic characteristicsl in non-selective and differential media. A pair of colonies was added to the cryopreservation medium with beads (alternative method) or to commercial cryovials tubes. It was homogenized, the supernatant was removed in sterile form and the vials, with the beads coated with bacteria, were brought to -20 ° C. Twelve bacteria were conserved (*E. coli* O157: H7, *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Enterobacter*, 2 strains *Escherichia coli*, 2 *Salmonella* and 3 lactic acid bacteria) in triplicate. Viability, purity and identity were evaluated of each of these strains at the time 0, 24 hours and at 1, 3, 6 and 15 months of conservation. Viable count was checked, by Pour plate method. The morphological and cultural characteristics of these strains remained unchanged. The analysis of the results showed a decrease in the number of microorganisms with respect to the conservation time by both methods and under the conditions analyzed. In strains *E. coli* O157: H7, lactic acid bacteria, *Salmonella*, *Enterobacter*, *Listeria*, *Bacillus* and *Staphylococcus* the viable count did not present significant differences ($\alpha = 0.05$) between both tested methods. These results allow us to conclude that the alternative method can be used to maintain pure, stable and easy to recover this bacterial culture with an accessible cost.

Sección: Biodiversidad

Modalidad: Poster

ARBUSCULAR MYCORRHIZAL FUNGI COMMUNITIES AND INFECTIVITY IN THREE FRAGMENTS OF URBAN FOREST IN CÓRDOBA CITY (ARGENTINA).

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In many countries urban forests and green infrastructures are threatened by the growth of cities, lack of investment and inadequate management to recover vital ecosystem services. Studies that address fungal biodiversity and especially that of arbuscular mycorrhizal fungi (AMF) in these areas are scarce. AMF are fundamental for plant development, especially in degraded soils, where reforestation is necessary. Here, we analyze AMF composition and mycorrhizal infectivity in three sites of urban forest with different land management: a *protected forest* with 10 years of exclosure to the public, a *restored forest with an herbaceous stratum* and a *restored forest with thinned herbaceous stratum* (lawn) removed regularly. In seven soil samples from each situation we evaluated diversity, richness and abundance of morphotypes, edaphic properties, soil compaction and % plant cover (herbaceous, shrub and arboreal strata). Infectivity was evaluated in *Medicago sativa* (alfalfa) plants harvested at 15, 30 and 60 days, at different concentrations of the soil (1:0; 1:4; 1:40) in a greenhouse assay. We evaluated differences by means of analysis of variance (ANOVA or Kruskal-Wallis test). Richness values were different between the three sites, with 14 species in the *restored forest with an herbaceous stratum* and only 11 and 9 species for the *protected forest* and the *restored forest with thinned herbaceous stratum* respectively, which could be due to historical and structural differences such as the different variety and age of plant species. Diversity and abundance did not show significant differences between sites. The most represented families of AMF in the three sites were Glomeraceae, Claroideoglomeraceae and Acaulosporaceae, whose species are considered generalists and resilients to disturbances. Infectivity was different only by the 1:4 treatment in the *protected forest* site (59% at 30 days). There we registered the lowest soil compaction and the dominance of the arboreal stratum, which would benefit the colonization of *M. sativa* roots. Finally, we discuss the need for long-term studies to understand the temporal and spatial dynamics of AMF in urban areas, their study in native flora and their potential role in reforestation strategies of the scarce green areas in the city of Córdoba.

Código de Resumen: MM-001

Sección: Microbiología Molecular

Modalidad: Oral

GENERATION OF DIACYLGLYCEROL IN MYCOBACTERIA: STUDY OF KEY ENZYMES FOR THE SYNTHESIS OF TRIACYLGLYCERIDES

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Mycobacterium tuberculosis, the etiological agent of tuberculosis (TB), infects one-third of world's population, producing the death of 2.4 million people per year. The pulmonary macrophages are the primary host cell of *M. tuberculosis*. During the first week of infection, the bacilli are able to replicate actively. When the host's immune system responses, lymphocytes are recruited at the site of infection and the infected macrophages die. This leads to the formation of the granuloma, a distinctive feature of infection with *M. tuberculosis*. Within the granuloma, *M. tuberculosis* may persist for decades, in a state of dormancy called "latency". The differentiation of macrophages into foamy macrophages (FM) is particularly important since they have been detected in patients who have developed reactivation of a primary TB infection. Within FM, *M. tuberculosis* decreases its multiplication rate and accumulates intracytoplasmic lipid inclusions (ILI) in its own cytoplasm which consist mainly of triacylglycerides (TAG). Furthermore, it is known that the reversion of the foamy phenotype leads to the progressive depletion of the accumulated ILI in bacterial cytoplasm and the resumption of mycobacterial division. However, the mechanisms by which *M. tuberculosis* induces the differentiation of these foamy macrophages and by which ILI accumulates in their cytoplasm within infected cells are not known yet. This is due to the limited knowledge of the regulation network involved in the maintenance of lipid homeostasis in mycobacteria, particularly in the regulation of TAG biosynthesis.

The main biosynthetic pathway for TAG synthesis involves the sequential esterification of glycerol-3-phosphate to produce phosphatidic acid (PA). The PA is a key molecule in the synthesis of membrane glycerophospholipids through the synthesis of CDP-diacylglycerol. In oleaginous bacteria like *M. tuberculosis*, the PA could be dephosphorylated by a phosphatidic acid phosphatase enzyme (PAP) giving as result diacylglycerol (DAG), which is the direct precursor of TAG synthesis. Therefore, DAG synthesis is the first reaction specifically dedicated to the synthesis of TAG, suggesting a key role for the PAP enzyme in the regulation of PA flow towards the synthesis of TAG or membrane phospholipids.

In the present work we demonstrate the phosphatase activity of three putative PAP enzymes of mycobacteria and analysed its physiological role, in order to characterize it at biochemical and genetic level. The main goal of our research project is to elucidate the role of the key enzymatic step that governs the decision of *M. tuberculosis* to synthesize TAG and, therefore, slow its growth and enter dormancy.

Código de Resumen: MM-002

Sección: Microbiología Molecular

Modalidad: Oral

CHARACTERIZATION OF EXOPOLYSACCHARIDES OBTAINED FROM *Exiguobacterium* sp. S17, A POLYEXTREMOPHILE STRAIN ISOLATED FROM LIVING STROMATOLITES.

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The genus *Exiguobacterium* comprises a group of gram positive bacteria with variable morphology ranging from small bacillus to coccus. These bacteria have the capacity to grow under extreme environmental conditions, including cold and hot environments with temperatures ranging from -12 to 55°C and low nutrient concentrations. *Exiguobacterium* sp. S17, is a polyextremophilic strain isolated from a stromatolite found in Laguna Socompa located at 3,570 m.a.s.l., Salta, Argentina. Biofilm formation by S17 is dependent on different stress factors (arsenic concentration and UV-B) and on the surface used for adhesion. It has been observed that the cells which initiated the adhesion were surrounded by Exopolysaccharides (EPS). EPS are complex molecules formed by sugar monomers, attached by glycosidic bonds forming a linear or branched structure made up of thousands of monosaccharide units. These biomolecules have multiple applications in different industrial sectors (food, pharmaceutical, medical and agriculture) as gelling agents, viscosizers, heavy metal adsorption, etc. The aim of our work was to isolate and characterize the EPS produced by S17 in two different media, and to investigate the influence of arsenic on its production. The S17 strain was grown in LB or MME (minimum basal) medium with 3% glucose supplemented or not with 1 mM arsenic (As) for 48 h. The cultures were centrifuged and the supernatant was precipitated with cold ethanol for 48 h. The

obtained EPS were deproteinized with 10% TCA, dialyzed and lyophilized. Samples were weight and diluted in distillated water. The EPS MW and monomeric constituents were determined by chromatography with a HPLC System, equipped with a Waters Ultrahydrogel column using 0.1 M of $NaNO_3$ as eluent at a flow rate of 0.6 mL/min. Different EPS concentrations were observed depending on the growth media used, being approximately 1.9 times higher for LB than for MME. Although EPS concentrations when adding As were similar to those obtained in the control media, in the latter two peaks were detected while only one EPS was observed in the presence of As. The MW observed for the EPS isolated from LB were 188.5 and 44.5 KDa while when As was added a peak corresponding to 44.6 KDa was observed. On the other hand, when S17 was grown in MME two peaks corresponding to MW of 224.0 and 12.8 KDa were detected, while in presence of As only one peak of 246.0 KDa was observed. In all samples, the EPS monomeric structure was composed of glucose and fructose. The ability of *Exiguobacterium* sp. S17 to synthesize EPS and produce biofilm could be in part responsible for the high adaptation capacity of this strain to the adverse environmental factors present in the ANDEAN PUNA. *Exiguobacterium* sp. S17 EPS could be used for biotechnological applications and especially for arsenic bioremediation processes.

Código de Resumen: MM-003

Sección: Microbiología Molecular

Modalidad: Oral

CHARACTERIZATION OF THE PHYSIOLOGICAL ROLE OF THE FABH ENZYME IN *M. smegmatis*: IMPACT ON LIPID BIOSYNTHESIS AND VIABILITY.

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Mycobacterium tuberculosis has a very complex life style. The flexibility in its metabolism allows it to adapt and survive within the infected host. During this process, one of the most affected pathways is lipid metabolism, both in the host and in the pathogen. Despite there is a lot of information about the biosynthesis, structure and biological function of the main lipids present in *M. tuberculosis* envelope, little is known about the mechanisms that allow the bacteria modulate and adapt the biosynthesis of the components of the cell wall in response to changes in environment. Thus, the study of the processes involved in the regulation of the biosynthesis of lipids in *M. tuberculosis* represents a crucial step in the comprehension of the physiology and physiopathology of this pathogen, as well as to understand the interaction between the mycobacteria and its environment.

The biosynthesis of fatty acids in *M. tuberculosis* involves two different systems of fatty acid synthases (FAS I and FAS II). Both synthases are involved in the biosynthesis of membrane fatty acids and lipid components of the cell wall, like mycolic acids which are essential for viability and pathogenesis, and have to work in a coordinate way to keep lipid homeostasis. These two systems are linked by a beta-ketoacyl-acyl carrier protein synthase III, named FabH, that catalyzes a condensing reaction combining acyl-CoAs produced by FAS I with malonyl-ACP to form beta-ketoacyl-ACP. This product is the substrate of the FAS II system which is elongated to produce the precursors of the mycolic acids. Although FabH has been studied at the biochemical level, there are no genetic analysis that could help to unequivocally establish the physiological role of this enzyme. In this work, using a double homologous recombination event strategy we constructed a knockout mutant strain in the putative gene for FabH in *Mycobacterium smegmatis* and carried out a physiological characterization. We determine that the gene is not essential for growth in the different growth conditions studied. However, the mutant strain presents a longer lag phase in 7H9 medium. When grown on 7H10 agar plates, the mutant strain colonies are very smooth and round, a phenotype in stark contrast to the dry, rough, and rugose morphology of the wt parent strain, suggesting altered lipid composition of the envelope. Lipid analysis are being carried out to determine the basis of these phenotypes. Our results will help to better understand lipid metabolism and regulation of this organism.

Código de Resumen: MM-004

Sección: Microbiología Molecular

Modalidad: Oral

SCREENING AND CHARACTERIZATION OF INHIBITOR COMPOUNDS OF THE *Salmonella enterica* PhoP/PhoQ REGULATORY SYSTEM

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Salmonella is an enteropathogen that causes a wide range of diseases in humans and animals. In *S. typhimurium*, the PhoP/PhoQ two-component system (TCS), composed by PhoQ, the histidine kinase sensor, and PhoP the cognate transcriptional regulator, controls key virulence phenotypes such as the adaptation to limited Mg^{2+} conditions, the invasion and proliferation within host cells or the resistance to antimicrobial peptides. As signal transduction in mammals does not involve TCS, histidine kinases are attractive targets to develop new antimicrobial agents to control bacterial diseases. We performed a high-throughput screen for compounds that would modulate PhoP/PhoQ activity, using an open-source set of small molecule kinase inhibitors spanning over 30 chemotypes. To accomplish this task, we measured β-*galactosidase* activity in a 96-multiwell plate to quantitatively asses the action of PhoP-dependent reporters. Hit compounds were progressed through a variety of triage assays to eliminate false positives, exclude cytotoxic compounds, and to determine the signal transduction pathway mode of inhibition. Of 686 molecules, we initially selected 16 that showed a repressing effect towards PhoP-regulated reporter genes and not towards CpxR or OmpR-regulated genes, used as controls, to continue the characterization. To further understand the specificity of the inhibition, we performed a fluorescence-based thermal shift assay (FTS) to examine whether the selected molecules were ligands that could bind to the purified periplasmic sensor domain of PhoQ (PhoQp). We used long chain unsaturated fatty acids (LCUFAs), that we have previously determined that repress the transcription of PhoP regulated genes by inhibiting the PhoQ autokinase activity, as control of the assay. In parallel, and to further understand LCUFAs-PhoQ interaction dynamics, we collected a series of (1H, 15N)-HSQC NMR spectra of PhoQp as a function of LCUFAs concentration. Assignments for the chemical shift perturbations of backbone amide allowed us to identify residues in the PhoQp structure that experience LCUFAs-dependent changes. Taken together, we successfully set up a protocol to test a large library of samples in a short time in order to establish a high throughput screening strategy to test and identify bioactive compounds with modulatory effect on the *Salmonella* enterica PhoP/PhoQ regulatory system and we also provide the structural basis for LCUFAs as PhoP/PhoQ input signals.

Código de Resumen: MM-005

Sección: Microbiología Molecular

Modalidad: Oral

PRTA METALLOPROTEASE EXPRESSION CONTRIBUTES TO *SERRATIA MARCESCENS* BIOFILM FORMATION

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Serratia marcescens (Sma) is an environmentally ubiquitous bacterium also acting as an opportunistic pathogen. Moreover, *Sma* displays biofilm formation capacity that has been shown to be related to its ability to colonize, persist, and proliferate on either biological or inert surfaces. This capacity to adapt and survive in either hostile or changing environments can be related to the expression of numerous secreted hydrolytic enzymes, including proteases. Genomic analysis of *Sma* clinical strain RM66262 identified four zinc-metalloprotease-encoding genes. Amongst them, we previously showed that PrtA is the most abundant in the secretome and its expression depends on the bacterial growth temperature, being transcriptionally upregulated at 30°C in comparison with 37°C. Considering this, we examined whether PrtA could influence *Sma* biofilm formation capacity. To that aim, we performed *in vitro* biofilm assays in polystyrene microwell plates, followed by biofilm quantitation using crystal violet staining. When the strains were grown at 30°C or 37°C in SLB medium the lack of PrtA expression in the *prtA* strain reduced the capacity of the bacteria to form biofilm compared with that of the wild-type strain, being more attenuated at 37°C. Results of confocal microscopy also showed biofilm formation deficiency in the *prtA* strain. This defect could be complemented to wild-type levels by adding catalytically active purified PrtA. To further understand PrtA influence on biofilm formation, we built a single-aminoacid-mutant protein that annuls the protease hydrolytic capacity and performed biofilm assays. This resulted in a defective biofilm phenotype that could also be rescued by the addition of catalytically active PrtA. In sum, our results demonstrate that PrtA expression and activity contribute to the ability of *Sma* to structure a biofilm community.

Código de Resumen: MM-006

Sección: Microbiología Molecular

Modalidad: Oral

ROLE OF RapD IN THE DEVELOPMENT OF THE BIOFILM MATRIX IN *Rhizobium leguminosarum* bv. *viciae* 3841

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The ability to form biofilms confers bacteria several advantages to survive in unfavourable environments or colonize new niches. Understanding the process of developing a proper biofilm structure in *Rhizobium leguminosarum* is crucial to further expand the knowledge of this symbiont's interaction with the host legumes and the soil particles

Capsular (CPS) and extracellular polysaccharides (EPS) are key components of the biofilm developed by *R. leguminosarum*. Besides, extracellular proteins secreted by the Type I secretion system PrsDE participate in the formation of a mature biofilm structure, processing the chains of the polysaccharides or affecting the adhesive properties. Previous studies have shown that all members of the Rap proteins secreted by PrsDE share at least one EPS/CPS-binding domain called Ra (*Rhizobium* adhering) and could harbour another specific domain. In particular, RapA2 is an EPS-lectin that consists only in two Ra domains and is involved in the binding to the EPS/CPS and affects both the competition to infect the legume host and the biofilm matrix. Other members of the Rap proteins are the EPS-glycanases PlyA and B, which modify the length of the chains of the EPS and the recently identified RapD. RapD harbours one Ra domain with high similarity to Ra2 of RapA2 and a specific domain of unknown function. The aim of this study is to understand the role of RapD during biofilm formation.

Using *Rhizobium l. bv. viciae* 3841 as a model, we generated isogenic strains harbouring a deletion in *rapD* gene as well as RapD overexpressing variants. We quantified extracellular RapD on the culture supernatant from different growth conditions and strains. Presence of the protein on the cell surface was also quantified in a wild type context and on an overexpressing context. Our findings suggest that almost all RapD secreted by the bacteria in a wild type context is sent to the extracellular media. We tested different aspects of biofilm formation like adhesion to abiotic surfaces, the possible impact on EPS-glycanases, surface characteristics and auto aggregative properties.

The absence of RapD promotes bacterial autoaggregation and alters biofilm structures in Y minimal medium whereas swimming type motility was reduced in a RapD overexpressing context both in rich and minimal media. Our studies on the purified recombinant RapD suggest that, unlike RapA2, RapD might be able to form homo multimeric structures.

Ongoing studies are focused on determining the polysaccharide binding capabilities as well as the affinity and the conditions under which multimeric structures are formed.

Código de Resumen: MM-007

Sección: Microbiología Molecular

Modalidad: Poster

IDENTIFICATION OF GENES RESPONSIBLE FOR THE POTENT ANTIFUNGAL ACTIVITY OF *Burkholderia ambifaria* T16

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Burkholderia ambifaria T16 is a bacterial strain isolated from the rhizosphere of barley which showed the ability to inhibit mycelial growth and conidial germination of several phytopathogenic fungi, including different *Fusarium* spp. With the aim to identify the genes responsible for this potent antifungal activity, an insertional mini-Tn*5* library was constructed in *B. ambifaria* T16 by triparental mating. A total of 8,950 mutants were screened for the ability to inhibit growth of *F. oxysporum* by using an overlay assay with homogenized mycelium. The insertion sites of the mini-Tn*5* were mapped in 20 mutants, which showed reduced or null antifungal activity, by an arbitrary primed polymerase chain reaction (AP-PCR) method. In half of these mutants, the mini-Tn*5* was inserted in a modular non ribosomal peptide synthetase (NRPS) gene cluster, encoding proteins involved in the biosynthesis of cyclic antifungal lipopeptides (CLPs) known as occidiofungins/burkholdines (bks). In these mutants, the mini-Tn*5* insertion abolished completely the ability of *Burkholderia ambifaria* T16 to inhibit mycelial growth of *F. oxysporum*. When these mutants were tested against other important pathogenic fungi, such as *F. graminearum*, *Macrophomina phaseolina* and *Candida albicans*, the antifungal activity was significantly reduced compared to the wild type strain, but not completely abolished. These results suggest that besides burkholdines, other compounds produced by *B. ambifaria* T16 are capable to inhibit growth of *F. graminearum*, *M. phaseolina* and *C. albicans*. Nevertheless, for all fungi analyzed, burkholdines were responsible for most of the antifungal activity. The identification and characterization of NRPS gene clusters responsible of strong antifungal activity against phytopathogenic fungi would provide key information for engineered biosynthesis of innovative antifungal compounds.

Código de Resumen: MM-008

Sección: Microbiología Molecular

Modalidad: Poster

EFFECT OF DIFFERENT PH ON *Pseudomonas extremaustralis* GROWTH AND POLYHYDROXYALKANOATES ACCUMULATION

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P. extremaustralis is a versatile Antarctic bacterium able to grow under aerobic and microaerobic conditions and is related to several non-pathogenic *Pseudomonads.* An interesting characteristic of *P. extremaustralis* is its capability to accumulate polyhydroxyalkanoates (PHAs) as polyhydroxybutyrate (PHB) and medium chain length PHA under unbalanced nutritional conditions. PHAs accumulation was shown to be related with *P. extremaustralis* high resistance to environmental stress, having also biotechnological interest by its properties similar to petroleum derived plastics. Due that changes in the medium pH could be considered as a stress factor the aim of this work was to analyze the effect of different pH in *P. extremaustralis* growth and how pH affects PHAs accumulation.

To study the effect of pH in *P. extremaustralis* growth, overnight precultures performed in LB medium supplemented with 0.25% sodium octanoate, to favor PHA accumulation, were used to inoculate fresh culture medium in which pH was adjusted to different values (5.5, 6, 7, 8, 9, 9.5 and 10). Initial OD600nm was 0.05 (about $1x10^7$ CFU/ml). Cultures were incubated at 30°C and 200 rpm for 24h. After incubation time, growth was measured by both OD and viable (CFU/ml) counts. Qualitative PHA accumulation was analyzed by Nile blue staining and microscopy observation. Quantitative PHA content was determined by gas chromatography. Experiments were performed using 3 independent cultures.

Our results showed strong differences in the growth of the cells at different pH. Marked loss of viability at pH 5.5 was observed. The initial bacterial number decreased immediately 3 orders of magnitude (from about 10^7 CFU/ml to 10^3 CFU/ml) and no viable cells were detected after 24h incubation. On the other hand, *P. extremaustralis* was able to grow when pH ranging between 6 to 9, showing a high increase in viable cell number and PHA content. For cultures grown at alkaline pH between 9 to 10, a decrease in viability of around two orders of magnitude in comparison with initial counts was observed and an increasing cell size associated to a greater PHAs synthesis was observed. Results showed that both acidic and alkaline pH affected growth of *P. extremaustralis*, however alkaline suboptimal growth conditions resulted in higher PHA accumulation.

These results suggest that PHA conferred to *P. extremaustralis* an adaptive advantage, enabling it to survive under suboptimal conditions for growth. In addition, information regarding conditions leading to higher PHA accumulation can be useful to improve the design of strategies for bioplastics production.

Código de Resumen: MM-009

Sección: Microbiología Molecular

Modalidad: Poster

FUNCTIONAL PANGENOME ANALYSIS AND GENOME CHARACTERIZATION OF A COLLECTION OF *Staphylococcus aureus* ISOLATES FROM PATIENTS WITH OSTEOMYELITIS IN ARGENTINA.

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Staphylococcus aureus is a highly prevalent human pathogen that causes osteomyelitis. The aim of this study was the analysis of the pangenome and genome characterization of *S. aureus* isolates from patients of Argentina with osteomyelitis. *S. aureus* isolates from 27 patients with chronic and acute osteomyelitis from 5 different hospitals were sequenced with Illumina MiSeq. Reads were assembled de novo with SPAdes and filtered. The genomes were annotated with Prokka and pangenome analysis was performed using Roary. Abricate was used to screen virulence factors and antimicrobial resistance genes. Functional analysis of pangenome was performed using the EggNOG server, using COG annotation. Scoary was used to perform the

pangenome wide association study.

The *S. aureus* genome mean size was 2,81 Mbp, with an N50 of 1,04 Mbp, an average of 25 contigs per isolate and a 32,82% GC content. An average of 2.812 CDS per genome and 59 tRNA were predicted. A total of 4.094 different genes were detected and 2.032 of these genes were shared by all isolates (core genome, CG), 486 genes by only one isolate (unique genome, UG) and 1575 genes were shared from 2 to 26 isolates (shell genome, SF). Metabolism related genes were more frequent in the core genome (38,6%) when compared with shell (7%) and unique genome (8%). In the metabolic category, the CG represented group was mostly aminoacid transport and metabolism (23%), in SF the most represented group was inorganic (26%) and aminoacid (22%) transport and in UG was inorganic ion transport and metabolism with 50% of the total metabolic genes. In genes related with cellular process and signaling, 14% were in CG, 9% in SG and 5% in UG. In the category assigned to information storage and processing in CG was 17%, 15% in SF and 25% in UG. In this category, in the CG, the most represented group was translation and transciption related proteins (39% and 35%) but in SG and UG the most represented group was replication and recombination related proteins (67 % and 74%), most of them related with transposons and phages. The uncharacterized proteins or unassigned proteins were 29% in CG, 67% in SG and 60% in UG.

Every isolate contained from 63 to 69 characterized virulence genes. 17/27 isolates carried genes coding for resistance to aminoglycosides, 27/27 to fluoroquinolones, 4/27 to erythromycin, 27/27 to chloramphenicol (one including the gene *fexa*) and 15/27 were MRSA (*mecA* gene). Pangenome association analysis of virulence and resistance genes information was performed to find genes associated with acute or chronic infection or with a given hospital. No gene was associated to any hospital and no gene was associated with chronic or acute infection. In conclusion, the sequence analysis of *S. aureus* isolates from Argentina revealed a conserved core genome, with predominance of metabolic genes. Functional pangenome analysis is a suitable tool to identify conserved target genes for vaccine and drug development.

Código de Resumen: MM-010

Sección: Microbiología Molecular

Modalidad: Poster

TRANSFORMATION AND CHARACTERIZACION OF *YopP* DEFICIENT *Yersinia enterocolitica* MUTANT STRAIN WITH A PLASMID ENCODING THE GREEN FLUORESCENT PROTEIN

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Yersinia enterocolitica (Ye) is a Gram-negative bacterium that causes gastrointestinal and genitourinary infection. Ye translocates, through a type III secretion system, bacterial effector proteins into the host cells, interfering with different cellular functions. The machinery of secretion and a set of six effector *Yersinia* outer proteins (Yops) (YopE, YopH, YopM, YopO, YopP, YopT) are encoded in a 70-kb virulence plasmid (pYV). YopP induces apoptosis in macrophages and dendritic cells by inhibition of NF-kB and MAPK pathways. The green fluorescent protein (GFP), isolated from the jellyfish *Aequorea victoria,* is frequently used as a reporter for the *in vivo* tracking of GFP-transformed-pathogens. The purpose was to transform a YopP-deficient Ye mutant strain (Ye Δ*yopP*) with a GFP-containing plasmid. Moreover, we evaluated the effect of GFP- transformation on the bacterial growth at 27ºC, and on the virulence and invasiveness of this transformed-mutant strain after *in vivo* infection of mice. Therefore, the pACYC_EGFP$_{1000}$ plasmid (pGFP) of 4569 bps, carrying a cassette of Clorhanphenicol (Cam^r) resistance was electroporated into competent Kanamicin-resistant Ye Δ*yopP* (Ye Δ*yopP* Kan^r) in 0.2 mm cuvette (2,5KV, 25µF, LR200ohm, HR 600ohm), and recovered in SOC medium. Transformed bacteria were selected by plating on Kan (50 µg/ml) and Cam (35µg/ml) supplemented Luria Bertani (LB) agar, and green fluorescence was evaluated by UV illumination. Then, several fluorescent clones were picked at random and stored at -80ºC in 10% glycerol-LB broth. Final cell density and specific growth rate (h^{-1}) of GFP-transformed Ye Δ*yopP* (GFP-Ye Δ*yopP)* was compared with its corresponding parental strain. Therefore, both Ye Δ*yopP* and GFP-Ye Δ*yopP* were cultured overnight at 27 °C in LB broth in presence of Kan or Kan and Cam, respectively. After 1:1000 dilution in fresh media, bacterial growth was measured hourly for 9 h in a spectrophotometer at 655nm. Moreover, C57BL/6 mice were oragastrically infected with 1-5 x 10^8 Ye Δ*yopP* or GFP-Ye Δ*yopP*. After 1 and 5 days, Peyer's patches (PP), mesenteric lymph node (MLN) and spleen (Sp) were obtained and colony forming units (CFU) were recorded. Successful pGFP transformation of Ye Δ*yopP* was obtained since green fluorescence was observed in the colonies under UV exposure. GFP-Ye Δ*yopP* exhibited similar growth rate to Ye Δ*yopP* strain. We did not find significantly differences in the number of CFU/organ in PPs, MLNs and Sp between mice infected with Ye Δ*yopP* or GFP-Ye Δ*yopP* at 1 or 5 days after infection (Mann Whitney test). Although further studies are necessary, our results indicate reliable expression of GFP-reporter plasmid in Ye Δ*yopP*, which does not impact on the bacterial growth nor *in vivo* virulence and invasiveness.

Código de Resumen: MM-011

Sección: Microbiología Molecular

Modalidad: Poster

INVASIVE CAPABILITY OF *Streptococcus uberis* FIELD ISOLATES WITH DIFFERENT BIOFILM FORMING PROFILES.

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Bovine mastitis is a cattle disease that causes large annual economic losses around the world. Different pathogens, classified as environmental and contagious, are associated with the disease as their ability to form biofilm leads to the advance of the infection. *Streptococcus uberis* is one of the most important environmental pathogen. The aim of this study was to evaluate the ability to adhere and internalize of eight *S. uberis* field isolates with different biofilm forming capacity. Invasion assays were performed using a bovine mammary epithelial (MAC-T) cell line. Previously, we characterized the *S. uberis* according to the biofilm formation ability. We selected *S. uberis* strains according to the biofilm profile: one weak strain, three moderate strains and four strong strains. Adherence and internalization ability of the strains was determined with $5.0x10^4$ MAC-T cells/well cultured in 96-well plates for 24 h. The cells were washed and co-cultured with 200 µl of the bacterial suspensions (approximately 1×10^6 CFU/ml) during 2 h. Then, cells were lysed with Triton 0,025% and MAC-T lysates were 10-fold serially diluted and plated on Tripticase Soya Agar. A parallel assay was performed to evaluate the internalization. After 2 h of co-culture with bacterial suspensions, cells were incubated for 1 h in DMEM culture medium supplemented with gentamicin (100 µg/ml) to eliminate adhered extracellular bacteria. Then, cells were washed and lysed. An aliquot of the lysates was collected and serial 10 fold-dilutions were made to determine the internalized CFUs per well. Our results showed that all the *S. uberis* assayed were able to adhere MAC-T cells. No statistical differences were found among the strains suggesting that the ability to adhere depended on the strain. In addition, all *S. uberis* strains were able to internalize MAC-T cells, although no differences in invasion capacity among strains were observed. Furthermore, the ability to internalize was not associated with the biofilm profile. The results provide a better understanding of virulence factors of this important environmental pathogen associated with mastitis, and may contribute to help the design of new therapeutic approaches.

Código de Resumen: MM-012

Sección: Microbiología Molecular

Modalidad: Poster

HEAVY METAL RESISTANCE IN EUKARYOTIC MICROORGANISMS ISOLATED FROM AN ABANDONED GOLD MINE SEDIMENTS IN SAN LUIS, ARGENTINA

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In San Luis, Argentina, the acid mine drainage of an abandoned gold mine is released into La Carolina stream. In previous studies we demonstrated that the drainage influences on the physicochemical parameters of the sediments of the stream and on microbial community structure. The aim of this work was to isolate and characterize microorganisms heavy metal tolerant from sediments of this acid mine drainage-affected environment and to investigate the ability of those isolates to remove heavy metal from culture medium.

A total of 28 sediment samples were taken from inside the mine and from La Carolina stream bed. Sediments were used for a sequential isolation process in the presence of Cu(II), Fe(II) and Cr(VI) as selection pressure, using EG* medium (g L^{-1}): glucose 10.0; yeast extract 1.0; K_2HPO_4 0.5 and $KH_2PO_4$0.5. Isolated microorganisms were then subjected to qualitative tolerance tests on solid medium. The resistant microorganisms were identified by PCR amplification with universal primer and 18S rRNA gene sequencing. The Minimum Inhibitory Concentration (MIC) was determined in liquid culture medium for each metal. Growth performance and heavy metal toxicity by the isolates were evaluated. Heavy metal concentrations were performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in cell-free supernatants prior dilutions during 120h and intracellular metal concentration, cells prior acids digestion in microwave heating.

After the isolation processes, three microorganisms were isolated and identified as: *Fusarium* sp. M6 (NCBI Accession number KY596033); *Pochonia* sp. M8 (NCBI Accession number KY596046); *Apiotrichumloubieri* M12 (NCBI Accession number KY596699).

MIC values obtained were (µg L^{-1}): 125 (Fe), 250 (Cr) and 90 (Cu) for *Fusarium* sp. M6; 250 (Fe), 45 (Cr) and 90 (Cu) for *Pochonia* sp. M8; and 375 (Fe), 60 (Cr) and 90 (Cu) for *A. loubieri* M12. None of the three microorganisms showed removal capability of Fe(II) or Cr(VI) from the culture media. Consequently, the growth and removal capability of microorganisms were evaluated in the presence and absence of 35 µg L^{-1} Cu(II). *Fusarium* sp. M6 and *A. loubieri* M12 showed a similar behavior, they showed a growth inhibition around 60% in the presence of the metal. Both microorganisms showed a Cu(II) removal capability between 30 and 35%. Pochonia sp. M8 was the most affected by the heavy metal presence.

These results were compared with *Saccharomyces cereviciae* ATCC 32051. This strain showed MIC values of 60 µg L-1 of Cu and their growth was affected by around 70% and the metal removal capacity was around 15% showing a similiar behavoir with *Pochonia* sp. M8

The selected microorganisms were obtained from sediments with low pH values and the highest heavy metal concentrations. These extreme environments allow us to isolate microorganisms with heavy metal resistance phenotype for their subsequent study of metalloproteomics and compare their metalloprotein profile with *S.cereviciae*.

Código de Resumen: MM-013

Sección: Microbiología Molecular

Modalidad: Poster

LONG-TERM EVOLUTION OF β-LACTAMASE AMPC ISOLATED FROM *Pseudomonas aeruginosa* CYSTIC FIBROSIS CHRONIC AIRWAY INFECTIONS

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Antibiotic resistance has emerged as a global health concern with serious economic, social and political implications. Among high-risk pathogens, *Pseudomonas aeruginosa* is one of the most feared cause of nosocomial infections and is responsible for about 10-20% of hospital-acquired infections. As an opportunistic pathogen, *P. aeruginosa* causes acute and chronic infections, and represents the main cause of morbidity and mortality in immunocompromised patients suffering from cystic fibrosis (CF).

P. aeruginosa from chronic CF infections provide unique opportunities to get insights into long-term bacterial evolution, and an extraordinary natural scenario to explore evolution of antibiotic resistance. By whole-genome sequencing, we previously evaluated the genetic changes undergone by mutator populations of *P. aeruginosa* during long-term chronic infections. Remarkably, *P. aeruginosa* isolates from one patient (CFD), who was intensively treated with β-lactam antibiotics, showed accumulation of mutations within the *ampC* gene with evidence of at least 4 different allelic variants coexisting in the same *P. aeruginosa* population, suggesting that this gene underwent a high evolutionary pressure in the CF lung. Moreover, *ampC* showed convergent evolution across the different sub-lineages, suggesting a role of this mutagenic process in the pathogenic fitness of *P. aeruginosa*.

In this work, we aimed to characterize the evolution of the spontaneous mutations acquired in the *ampC* gene during the long-term adaptation of *P. aeruginosa* to the CF airways. To explore the genetic diversity within *ampC* as well as the dynamics of their allelic variants in the population, we used a sequential collection of isolates obtained from single sputum samples from the CFD patient, spanning 26 years of chronic infection history. Likewise, in order to understand the plasticity of the enzymes for β-lactam hydrolysis and to identify potential therapeutic implications of mutations, we explored the impact of *ampC* mutations from the different allelic variants on β-lactam MICs. Finally, we performed Amplicon Sequencing to study the genetic diversity of *ampC* and common tendencies across different CF patients.

Our results show that evolution is still occurring and driven by antibiotic treatment, and that the *ampC* sequence is highly diverse across populations. Furthermore, some dominant *ampC* allelic variants are associated to high resistance towards cephalosporins and monobactams. Remarkably, we show that some positions in the *ampC* sequence are frequently hit by mutations across different CF patients suggesting a key role of these mutations in antibiotic resistance.

Código de Resumen: MM-014

Sección: Microbiología Molecular

Modalidad: Poster

SEQUENCE ANALYSIS AND ACTIVITY DETERMINATION OF *narU* AND *narZ* PROMOTER GENES OF *Salmonella* Typhimurium

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Salmonella Typhimurium and *Escherichia coli* cells have three nitrate reductases (Nap, NR-A and NR-Z) involved in adaptation to growth on anaerobic and/or on oxidized carbon environment. Previously, we demonstrated that RcsB and RstA regulators control the *narZ* gene expression, involved in the synthesis of NR-Z, in a carbon source-dependent pathway. Since the regulatory region that controls the *narZ* expression is not well defined and the data is controversial, here we investigate which is the promoter whose activity allows the transcription of *narZ*. In *Salmonella*, some reports postulated that *narU* gene is upstream of *narZ* and it is the first gene of the operon, while others argue that they are independently transcribed. We localized the transcription start site, the -10 and -35 boxes in both promoters, by bioinformatics analysis. Then, these regulatory region were cloned into the plasmid pFU62, harboring the β-galactosidase encoding gene as reporter of the activity of each promoter. The effect of the RcsB overproduction was also analyzed and compared with data obtained from chromosomal *narZ*::*lacZ* transcriptional fusion to define it *narZ* promoter sequence and to determine the impact of this regulator in the gene expression.

Código de Resumen: MM-015

Sección: Microbiología Molecular

Modalidad: Poster

GENETIC AND BIOCHEMICAL STUDY OF A NOVEL MICROCIN PRODUCED BY A CLINICAL ISOLATE OF *Shigella flexneri 2*

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Enterobacteria have the ability to produce antimicrobial peptides called bacteriocins, which allowed them to thrive host gut. *Shigella* is the most common causative agent of diarrhea, an important worldwide health problem. Previously, we have reported a new low molecular-weight bacteriocin (*Sh*pCI172) produced by a *Shigella flexneri* 2 CI172 strain, which was isolated from a fecal sample of a child suffering acute diarrhea. This was the first report of a microcin-producer *Shigella flexneri*, suggesting a rare occurrence. The plasmidic profile of this strain suggested that the genetic determinants for the production of *Sh*pCI172 could be encoded in a plasmid. In order to eliminate the harbored plasmids, strain CI172 was successively sub-culture in LB medium containing increasing concentrations of ethidium bromide. After 3 days of treatment, the cultures were plated onto LB medium, for further analysis of plasmidic profiles. We also performed cross-streaking tests to analyze whether the CI172 strain display immunity to other microcin groups. Finally, the *Sh*pCI172 cell-free supernatant was used to perform a preliminary purification step by ammonium sulfate and/or acetone precipitation, and then subjected to HPLC fractionation. Taking together our results suggest that *Sh*pCI172 would be a novel plasmid-encoded antimicrobial peptide belonging to the microcin family. *Sh*pCI172 is produced and released into the culture medium, facilitating large scale purification for its potential use as an antimicrobial agent.

Código de Resumen: MM-016

Sección: Microbiología Molecular

Modalidad: Poster

EFFECT OF SHEAR STRESS ON *Bordetella pertussis* BIOFILM FORMATION

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Whooping cough is a highly contagious respiratory tract infection, caused by *Bordetella pertussis*. Numerous studies have reported the ability of *B. pertussis* to grow in microbial communities known as biofilm. In the case of another respiratory tract pathogen, such as *Pseudomonas aeruginosa,* it was reported that a shear stress, similar to the one present in lungs, regulates many cellular functions promoting bacterial attachment and biofilm formation. Nevertheless, for *B. pertussis*, the hydrodynamic shear stress effects on adhesion and biofilm formation have not been studied, so far. Our aim was therefore, to investigate the attachment and biofilm formation of *B. pertussis* reference strains and clinical isolates under shear stress conditions.

For this purpose, *B. pertussis* Tohama I reference strain, the clinical isolate *B. pertussis* 2723 -in both cases in virulent (Vir+) and avirulent (Vir-) phases-, and *B. pertussis* 537 strain -an avirulent phase-locked mutant-, were used in this study. Cells attachment and biofilm formation were analyzed using Stainer-Scholte liquid medium in flow chambers (IBIDI, Germany) (17 mm in length, 3.8 mm in width and 0.4 mm in height) under both static and continuous flow conditions. Fluid flow was adjusted at a shear stress similar to the one reported for respiratory tract (0.45 dynes/cm^2). Adhesion images were acquired by epifluorescence microscopy and analyzed by IMAGE J software; and mature biofilm structure was evaluated by CLSM and COMSTAT2 software.

We observed that in all strains tested shear stress reduced the attachment level. However, the clinical isolate in virulent phase showed lower reduction of its capacity of adhesion under hidromechanical stress than reference strain. Interestingly, *B. pertussis* Tohama I (Vir+) under shear stress presented an adhesion value similar to that recorded for both the clinical isolate and reference strain in Vir- phases growing under static conditions. In addition, the clinical isolate in Vir+ phase showed a higher biomass formation, after 48 h of incubation under shear stress, than the reference strain (Vir+). Moreover, the biofilm thickness recorded for this clinical isolate in virulent phase was higher when it was grown under fluid flow than in static conditions. Therefore, although for the clinical isolate a direct impact of mechanical forces was observed on the biofilms thickness, the biofilms biomass was not affected. In contrast, the hydrodynamic forces impacted crucially and negatively on both the biomass and the structure of the biofilm formed by the reference strain. Taking these results into account, we could assume that the clinical isolate shows an adaptive advantage over the reference strain to grow as biofilm under physiological shear stress. Our results shed light on *B. pertussis* biofilm response to hydrodynamics forces which has not been described so far.

Código de Resumen: EM-001

Sección: Educación en Microbiología

Modalidad: Poster

IMPROVING THE UNIVERSITY TEACHING PROCESS: LEARNING BASED ON PROBLEMS.

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Food Microbiology is a signature belonging to the careers of Bromatology and Licenciature in Bromatology, dictated in the Faculty of Agrarian Sciences, National University of Cuyo. Within the scope of the professional is to establish, monitor and direct measures to obtain safe food. For that is necessary to possess the theoretical and practical disciplinary knowledge as well as interpersonal and cognitive skills such as oral and written expression, leadership, teamwork, decision making, critical thinking, daily reasoning and creativity. Therefore, with the aim of improving the teaching-learning process in university teaching, problem-based learning was incorporated as part of the curricular activities to be carried out in this space. At the beginning of the course was exposed the problem which consisted in present a training workshop for food handlers in establishments that presented vulnerable conditions. The students formed work teams, accompanied by a tutor teacher. They searched for an entity where food is prepared and / or consumed for children and / or elderly people in the area. They contacted those responsible and offered the training. They made the diagnosis of the place (habits, activities carried out, level of training of the manipulators, age group that receives the food service, hygiene conditions, etc.) They prepared the training according to the establishment, in the form of workshop / discussions. They attended meetings established with the tutor teacher. At the end of the course, they presented to their classmates and teachers, using various resources created by the group. Finally they repeated this presentation at the establishment. This activity has been carried out since 2016. All the students of the 3 cohorts actively participated and committed to the activity. They presented a receptive attitude towards the exchange of ideas and suggestions from their classmates and teachers. They acquired and applied knowledge, vocabulary, skills and attitudes according to a real scene of their professional future. They valued being accompanied by the tutor in one of the first steps that relates them to the environment. Likewise, a positive impact was generated in the establishments visited. Therefore, these scientifically based educational practices allow training competent professionals committed to their environment.

Código de Resumen: EM-002

Sección: Educación en Microbiología

Modalidad: Poster

SCREENING OF *Staphylococcus aureus* COLONIZATION BY SECOND YEAR MEDICINE STUDENTS OF SAN LUIS CATHOLIC OF CUYO UNIVERSITY

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Staphylococcus aureus is the biggest human pathogen causing skin and tissue infections, pneumonia, septicemia and associated infections to devises. The outcome of strains resistant to antibacterial agents has come to be a critical concern especially in hospital environments; this is because of the high mortality due to the systematic infections caused by methicillin resistant *Staphylococcus aureus* (MRSA).
Naturally, an understanding of the dissemination dynamics and transmission identification are of interest not only to the public health epidemiologists, but also to clinical microbiologists involved in the daily patients' treatment. In addition, since 2009 the USA health insurance for elder and disabled Americans stopped covering the costs related to hospital stay, being this practice followed by insurance private companies. One of the fundamental steps to prevent the possibility of new intra-hospital infections consists on checking the nasal and pharynx colonization by *Staphylococcus aureus* from all the sanitary staff involved in surgical practicing or constant contact with patients. Our principal objective was to identify *S. aureus* colonization in medicine students which allows preventive actions to avoid new transmissions. During this process our students were able to work with cultures of *Staphylococcus* in blood agar plates and perform the catalase and coagulase reactions, which are the main reactions for the appropriate identification of this microorganism. As a secondary objective our students could work in a microbiology lab, acquiring new abilities and supporting the theoretical knowledge with practice.

As a result of our study we found that 28% of our students are colonized by *Staphylococcus aureus*, allowing them to quickly start the treatment consisting in the elimination of this pathogen. Even more, 30% of our students claimed to be the first time they worked in a laboratory doing the work themselves, and 85% obtained a better performance in the evaluation of these concepts after practice.