Sección 1 - Biodiversidad

ANTIFUNGAL ACTIVITY OF ANACARDIACEAE SPECIES NATIVE FROM NORTHWESTERN ARGENTINA

Pasa a la sección Biorremediación y Biocontrol

[POSTER]

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Anacardiaceae family has an important participation in the dendrologic flora of northwestern Argentina and are mostly represented by species of the genus *Schinopsis* and *Schinus*. Some of them cause severe contact dermatitis, which is mainly attributed to the content of phenolics compounds, biflavonoids and phenolic lipids (alkylcatechols and resorcinols). These substances are defense metabolites against plant pests and diseases, and become Anacardiaceae species as sources of antimicrobial compounds. In this work, we check the antifungal activity and phenolic contents of leaf extracts of five Anacardiaceae species native from northwestern Argentina. Leaves of *Schinus gracilipes*, *Schinus fasciculatus*, *Schinus molle*, *Schinopsis haenkeana* and *Schinopsis lorentzii* were sequentially extracted with dichloromethane, ethyl acetate and methanol. The organic extracts were evaporated to dryness, solubilized in methanol and filtrated. Percentage of participation of total phenolic compounds, flavonoids and phenolic lipids were estimated in the dry residue of the filtrates by microcolorimetric methods based on Folin Ciocalteu reagent, aluminum chloride and Fast Blue B salt, respectively. Minimum antifungal dose (MAD) was determined by the disk diffusion method (10-2000 µg dry extracted material/disk) on the cereal pathogens. Dichloromethane and ethyl acetate extracts of *S. lorentzii* and *S. haenkeana*, and the methanolic extract of *S. gracilipes* had a MAD of 125 µg (dry material/disk) on *F. graminearum*. Dichloromethane and methanol extracts of S. fasciculatus had no an inhibitory effect on this fungal species and the remaining organic extracts had MADs at 500-1000 µg. Dichloromethane extracts of *S. lorentzii*, *S. haenkeana*, and *S. molle*, and ethyl acetate extracts of *S. molle* and *S. haenkeana* had MADs of 500-1000 µg on F. verticillioides while the remaining extracts had no a fungitoxic effect on this fungus. Quantitative participation of phenolic compounds, flavonoids and phenolic lipids in the extracted dry materials were not directly associated to the antifungal effects, suggesting that qualitative differences among extracts determine the observed biological activity. Our results indicate that *F. graminearum* was more susceptible than *F. verticillioides* to the leaf extracts suggesting that Anacardiaceae species has a selective antifungal effect on the *Fusarium* species assayed.

Characterization and technological properties of *O. oeni* and *L. plantarum* isolates from Patagonian Pinot noir wine: an approach for selection of new starter cultures.

Pasa a la sección Biotecnología y Fermentaciones

[POSTER]

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The malolactic fermentation (MLF), i.e. the conversion of L-malic to L-lactic acid and carbon dioxide, is the biochemical process by which lactic acid bacteria (LAB) contribute to the wine deacidification, and improve the wine flavour and microbiological stability. The MLF becomes especially relevant in red wines from cold regions and from early *Vitis vinifera* L varieties, i.e. Pinot noir, where L-malic acid can reach high concentrations. Spontaneous MLF is not always guaranteed and, when it occurs, its evolution is difficult to predict. Many studies have focused on the stimulation of MLF by selected bacterial strains and recent winery practices consist in using malolactic starters for direct inoculation in wines.
The aim of this work was to study the technological properties of five *O. oeni* and fifty one *L. plantarum* isolates obtained from Pinot noir wines from Nor Occidental Patagonia. The isolates were identified by *rpo*B fragment analysis and 16S rRNA sequences. Genotypic diversity was assessed by RAPD analysis with COC primer. Forty one different profiles were founded for *L. plantarum* isolates and five for *O. oeni* isolates. MRS-ethanol supplemented growth screening was carried out for selecting some *L. plantarum* isolates for further characterization. Eight *L. plantarum* selected isolates and the five *O. oeni* isolates were analyzed for tannase and B-glucosidase activities and citrate fermentation obtaining positive results for all isolates studied. Finally, wine media tolerance and malolactic activity were evaluated in sterile red wine Pinot noir. Although all *L. plantarum* isolates were able to grow in wine containing 14% ethanol, none *O. oeni* isolate was able to grow in this condition. When malolactic activity was studied both, *O. oeni* and *L. plantarum* isolates analyzed, were able to consume L-malic acid. Interestingly, *L. plantarum* isolates take four times less time to consume the same amount of L-malic acid than *O.oeni* isolates.
This study is the first report of *O. oeni* and *L. plantarum* species isolated from Pinot noir wine in Argentinean Patagonian region. The isolates obtained were well-characterized showing phenotypic and genotypic differences; they also evidenced adequate technological properties that suggest these isolates might be used as malolactic starters in winemaking. Further studies, including simulated microvinifications, are necessary to accomplish a more integrated approach.

Expression of human intestinal trefoil factor in *Escherichia coli*

Pasa a la sección Biotecnología y Fermentaciones

[POSTER]

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Lactic acid bacteria (LAB) are used as a delivery system for a range of molecules that have different applications, including therapies for allergic and gastrointestinal diseases. Intestinal trefoil factor (ITF), a small, compact protease-resistant peptide, is abundantly expressed in goblet cells of large and small intestine. Several biological activities of ITF have been identified, including promotion of wound healing, stimulation of epithelial cell migration, and protection of intestinal epithelial barrier. The therapeutic use of recombinant ITF has been suggested for the treatment of patients with chronic inflammatory bowel diseases, peptic ulcer, necrotizing enterocolitis and gastrointestinal surgery. However, one of the factors that limit its use is the high cost associated to their production and purification. Furthermore, when the ITF is administered orally, it adheres to the mucus of the small intestine being absorbed in the cecum. On the contrary, intragastric administration of ITF by the action of recombinant LAB would lead

to the release of active peptides in the mucosa of colon. The aim of this study was the expression of human ITF in *Escherichia coli* for its subsequent expression in LAB for the prevention and treatment of intestine damage. Human ITF gene encoding mature peptide was obtained by RT-PCR using the plasmid pSport (Clontech) as template. The amplified fragment (approximately 200 bp) was cloned into pBlueScript SKII (Stratagene) resulting the plasmid pSal. By using specific primers with cut sites for *Sal*I and *Nco*I the ITF was amplified and inserted into the expression vector pET28b to construct the recombinant pET28b-ITF. After confirmation by gene sequencing, pET28b-ITF was transformed into *E. coli* BL21(DE3), and the fusion protein was expressed by isopropyl-β-d-thiogalactopyranoside induction in shake flask and analyzed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The subsequent expression of ITF fusion in LAB is ongoing, constituting the recombinant LAB strain a promising tool for the treatment of intestinal damage.

Pyrosequencing analysis of nitrogen fixing communities in soils with different agricultural management.

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Despite the key role of bacteria in soil processes, there is still limited information about the effect of crop management on the diversity of soil microbial community. In this study, we examined the diversity and abundance of the free-living diazotrophic microorganims in soils under different agricultural practices (crop rotation and nutrient amendment, monocropping without nutrient reposition, and non-cultivated soil), using nifH gene as a molecular marker. Soil samples used in this study were collected at two sites, Monte Buey (Córdoba) and Viale (Entre Ríos) as part of the PAE project called “BIOSPAS” (Soil Biology and Sustainable Agricultural Production).
The relative abundance of the nitrogen fixers in soil samples was quantified by real-time PCR. The copy number of nifH varied according to the soil type and agricultural management. Viale samples had a higher number of copies than Monte Buey. Moreover, Viale did not show differences related to agricultural practices, whereas Monte Buey soils under monocropping had a higher level of nifH.
The nifH sequence diversity found in samples was analyzed by pyrosequencing of nifH, using the GS FLX Titanium platform (Roche). 183,000 reads were obtained, aligned and grouped in Operational Taxonomic Units (OTUs) using the Dotur program. Diversity among samples was analyzed comparatively with different statistic indexes (rarefaction, Chao1 and Ace), considering different degree of sequence dissimilarity (0-10%). In Monte Buey samples, OTU abundance was higher in soils under monocropping practices, followed by those under rotation and finally by non cultivated soil, independently from the taxonomic level used to group them. In contrast, variation in the number of OTUs was found in Viale analysis according to the level of sequence dissimilarity. The monocropping soil samples presented the highest diversity with low levels of dissimilarity (0 to 3%) and the lowest with high level of dissimilarity (5 to 10%). Rarefaction analysis suggests that our sequencing approach has covered approximately 99% of nifH diversity in soils under rotation and in the non-disturbed soils from Monte Buey, while the other soil samples ranged between 65 and 77% revealing the high variability in these samples. In conclusion, these results show that the pattern of diversity and abundance of nifH sequences varies among soils from different locations, as well as in soils with different agricultural management.

CULTURABLE ORGANOTROFIC BACTERIA FROM HIGH-ALTITUDE ANDEAN LAKES

[POSTER]

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The Andean Altiplano is a sedimentary volcanic plateau at about 4.000 m. Several lakes in the Altiplano are formed over evaporitic endorheic basins. These aquatic ecosystems present extreme conditions (high solar irradiance, arid conditions, low nutrient concentrations, heavy metals, large temperature fluctuations) and are a source of microorganisms with potential biotechnological applications.
Lately, many studies have reported that the assessment of bacterial diversity by cultivation-independent methods is the most appropriate for studying diversity, owing to the existence of a host of unculturable. However, cultivation remains the preferred method for the acquisition of an accurate picture of the physiology and complex ecological interactions in which microorganisms engage.
In this study, we attempted to determine the culturable bacterial diversity and extend the culture collections of Andean lakes in Argentina. Bacteria were isolated from Socompa and Diamante lakes in the Argentinean Puna. They were grown in different broth: LB, MGM, PY and 6WS. The pure and cellular morphology were determined by phase contrast microscopy and Gram stain. Bacterial diversity among the isolated was evaluated by PCR-amplified 16S rDNAs followed by phylogenetic analysis of 16S rDNA sequences.
Twenty six strains were found. A neighbor-joining tree of the partial 16S rDNA sequences resulted in the division of the 18 strains of Socompa into two major groups, 14 strains of (γ)*Proteobacteria* (77.7%) and 4 strains of *Firmicutes* (22.3%). Eigth strains of Diamante resulted in the division into 6 strains of *Firmicutes* (75%) and 2 strains *Actinobacteria* (25%). The (γ)*Proteobacteria* was group more diversity, including several genus: *Salinivibrio, Shewanella, Pseudomona, Halomona, Aeromona, Idiomarina* and *Chromohalobacter*. Additionally, seven new species candidates were found in Socompa, based on similarities of the 16S rDNA sequences to those of previously reported species.

Isolation, partial identification and evaluation of the beneficial properties of *Bacillus* strains isolated from *Piaractus mesopotamicus*

Se sugiere presentación de POSTER, pasa a la sección Biorremediación y Biocontrol

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The genus *Bacillus* constitutes a wide group of rod-shaped Gram-positive bacteria, and is characterized by the ability of producing endospores. Although the genus is not commonly found in marine and fresh water environments, it has been isolated from the guts of several marine and fresh water animals and sediments. Bacteria belonging to this genus have been widely used as probiotics due to the ability to compete for nutrients with other bacteria and to produce antimicrobial substances such as antibiotics and enzymes. In this work the isolation, partial characterization and beneficial properties of eight *Bacillus* strains from the intestinal tract

of *Piaractus mesopotamicus*, the most frequently cultivated fish species in the north Argentinean region, was evaluated. 70 of the 140 strains isolated from 10 healthy fishes were rod-shaped bacteria, 8 of which were resistant to the temperature treatment indicating its ability to produce endospores. The results of the Shaeffer and Fulton staining, catalase, Voges-Proskauer reaction, NO_3^- reduction, and growth at 50°C, 65°C and 7% NaCl allowed us to indentify this strains as *Bacillus* spp. None of the 8 strains were able to produce hydrogen peroxide, and all of them showed an autoagregation index lower than 50% and a hidrophobicity between 0% and 5%. One strain was able to inhibit the growth of *Salmonella enteritidis* and *Klebsiella* spp.; another strain inhibited both pathogens and also *Stapylococcus aureus* and another strain inhibited only the growth of *Staphylococcus aureus*. All this inhibitions were produced by a metabolite different to organic acids and did not affect the growth of *Aeromonas salmonicida*, *Yersinia rukeri*, *Streptococcus agalactiae*, *Streptococcus dysagalactiae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Proteus vulgaris*. The inhibition halo was evident only with the in situ growth of the producing strain. The compatibility assays showed that any of the strains inhibited the others. Further studies are being performed to determine the inclusion of these strains in a product to be applied in aquaculture.

Copper accumulation by Eichhornia crassipes and role of rhizospheric bacteria

Se sugiere presentación de POSTER, pasa a la sección Biorremediación y Biocontrol

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The high levels of heavy metals in lower basin of Matanza-Riachuelo river would affect soil and water quality which consequently hampers plant growth. The recent management government plan includes revegetation with native plants. Water hyacinth (Eichhornia crassipes (Mart.)) grows in heavily polluted water bodies like Matanza-Riachuelo river and accumulates metal ions. Rhizospheric microbes can increase the tolerance of their host plants against heavy metal stress. The aims of this research are a) to assess the removal efficiency and copper tolerance of E. crassipes harvested in Riachuelo b) to evaluate copper tolerance of its rhizospheric bacteria.
Harvested water hyacinths were grown outdoors in Hoagland nutrient solution for 50 days. Three treatments with this solution and different added copper concentration were performed: Control (0.1mg/l Cu), T1 (15 mg/l Cu) and T2 (25 mg/l Cu). Water hyacinths of uniform size, previously rinsed with de-ionized water were placed in plastic containers of 2.8 cm^2, were grown in a greenhouse, at room temperature between 19-23 °C, 11:13 photoperiod and pH: 4.12-5.14. Harvested at 7 days, total chlorophyll was determined in leaves. Final dry weight was determined in above and belowground parts and copper concentrations in water and plant tissues. Roots were separated in sterile vials for bacterial count. Bacterial count was performed by spreading on the surface of minimal medium supplemented with 100 mg/l of copper. Morphologically different colonies were selected to study the copper tolerance. The level of tolerance was evaluated by streaking on the surface of minimal medium supplemented with 200 mg/l, 500 mg/l, 1000 mg/l and 2000 mg/l of copper. Plants absorbed copper mainly in the roots according to supply (Control: 0.082; T1: 13.5 and T2: 23.8 mg Cu/g dry weight). Translocation rate was low and copper concentrations in the belowground parts were: Control: 0.016, T1: 0.056 and T2: 0.133 mg Cu/g dry weight. Productivity declines corresponding to the increase in copper concentration (Control: 0.57 g/pot. day and T2: 0.25 g/pot. day). Total chlorophyll concentration was: Control: 0.037 mg/cm^2 and T2: 0.004 mg/cm^2
The number of bacteria cell resistant to 100 mg l-1 of copper ranged between 9.2 x 10^5- 1.4 x 10^6 CFU g-1 in the control and 6.4 x 10^6 - 1.1 x 10^7 CFU g-1 in T1, while T2 values were

between 6.6 x 10^6 and 1.6 x 10^7 CFU g.-1 The 81.8% of the selected bacteria tolerated up to 2000 mg/l of copper.
E. crassipes was capable of removing copper during incubation period with some symptoms of toxicity (chlorophyll and productivity decreasing). In T1 and T2, the numbers of bacteria resistant to 100 mg/l Cu were higher than control and were highly tolerant to copper. However, rhizospheric microorganisms did not seem to improve the uptake and plant toxicity symptoms.

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GENOTYPIC DIFFERENCES BETWEEN PEANUT RHIZOBIA POPULATIONS OBTAINED FROM DIFFERENT CROPPING AREAS OF CÓRDOBA PROVINCE IN ARGENTINA

[POSTER]

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Peanut (*Arachis hypogaea*) is one of the most important crop of Córdoba province in central region of Argentina. Peanut establish a symbiotic relationship with rhizobial strains which constitute stable populations in soils of typical peanut cropping area. Degree of genetic diversity of peanut nodulating populations has not been fully addressed. Aim of present work was to carry out a genetic characterization of rhizobia isolated from peanut nodules growing on soils with previous peanut cropping history (Río Cuarto and Cabrera) and soils with no previous peanut cropping history (La Aguada and Chaján) and compare them. Ten different *16S rRNA* RFLP genotypes were obtained as result of combination of restriction patterns obtained with four endonucleases. Cluster analysis of genotypes showed at 80 % similarity the formation of two main groups. Group Ib clustered majority of strains from unrelated origins Río Cuarto and Chaján, whereas group IIc clustered majority of strains from unrelated origins Cabrera and La Aguada, suggesting genetic relationship between peanut nodulating populations isolated from different peanut cropping system. Diversity indexes showed that populations obtained from soils with strong previous history were less diverse compared to soils with no previous history, suggesting that the presence of the legume selects particular taxa of rhizobia. Sequence analysis of *16S rRNA* gene demonstrated the identity of isolates with strains of *Bradyrhizobium* sp., *Bradyrhizobium japonicum* or *Bradyrhizobium elkanii* genus and the phylogenetic association between rhizobia populations from sites with different peanut cropping history. Higher polymorphism and diversity was reached in the analysis of ERIC-PCR. Peanut strains clustered at very low levels of similarity (55 %). Populations of different origins, such as Cabrera and La Aguada, clustered together although at elevated genetic distance. ERIC results indicate the presence of very different rhizobia populations in soils of Córdoba province. In spite of the presence of certain linkage between non related rhizobial populations according their origin, global analysis of genotypic properties showed that peanut nodulating populations have high diversity independently of their geographical origin and that the presence of a legume crop affects soil biology through influencing rhizobia nodulating populations. We speculate that knowledge of such properties can contribute to improve the global development of peanut crop.

PURIFICATION AND PROPERTIES OF A THERMOSTABLE α-GALACTOSIDASE FROM *Lenzites elegans*

Pasa a la sección Fisiología y Metabolismo

[POSTER]

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White rot fungi are important in the forest ecosystems because of their unique capacity to degrade wood components (i.e.,cellulose,hemicelluloses and lignin).The major constituents of hemicelluloses are the hetero-1,4-β-D-xylans,hetero-1,4-β-D-mannans, galacto-glucomannans and glucomannans.The biotechnological importance in the hydrolysis of hemicelluloses for paper and feedstock industries has revived interest in the enzymology of hemicellulose degradation.Galacto-glucomannans enzymatic hydrolysis requires, among other enzymes, the action of α-galactosidases. These enzymes have other industrial uses like hydrolysis of raffinose in beet sugar molasses and the raffinose family of oligosaccharides in soybean milk. α-Galactosidases (α-D-galactoside galactohydrolase, EC 3.2.1.22) catalyze the hydrolysis of α-D-galactopyranosyl linkages from alkyl, aryl or glucosyl (mono or oligo) residues or groups.
An α-galactosidase was isolated from a culture filtrate of Lenzites elegans grown on pectin as carbon source.It was purified to electrophoretic homogeneity by ammonium sulfate precipitation, gel filtration chromatography and ion-exchange chromatography.The molecular mass of the native purified enzyme was 158 kDa by gel filtration.It is a homodimer with subunits of 61 kDa.The optimal temperature for enzyme activity was 80°C.This α-galactosidase showed a high thermostability, retaining 94% of its activity after preincubation at 60°C for 1 h.The optimal pH for the enzyme was 4.5 and it was stable from pH 3 to 7.5 at 0°C and from 3.9 to 6.5 at 50°C.It was active against several α-galactosides like p-nitrophenyl-α-D-galactopyranoside, α-D-melibiose, rafinose and stachyose.The α-galactosidase is a glycoprotein with 26 % of structural sugars. Galactose is a non-competitive inhibitor with a Ki = 22 mM vs. p-nitrophenyl-α-D-galactoside and 12 mM vs. α-D-melibiose as substrates.Cations as Hg2+, Ag1+ and p-chloromercuribenzoate were also inhibitors of this activity,suggesting the presence of –SH groups in the active site of the enzyme. The sequence of the N-terminal end of the α-galactosidase from L. elegans was SPDTIVLDGTNFALNN. This sequence data was aligned and compared using Basic Local Aligment Research Tool (BLAST) and CLUSTALW Programmes.No similarities were found in the protein sequence using protein-protein BLAST from the National Center for Biotechnology Information, USA(http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).The alignments of sequences with enzymes belonging to GH 36 were carried out with CAZy database.Consequently, the studied α-galactosidase isolated from Lenzites elegans was classified as member of glycosyl hydrolase family 36(GH 36).Given the high optimum temperature and heat stability of L. elegans α-galactosidase, this fungus may become an alternative source of α-galactosidase production for industrial use.

PCR-DGGE analysis of native LAB population associated with malolactic fermentation of Merlot wine from Argentinean North Western Patagonia.

Pasa a la sección Biotecnología y Fermentaciones

[POSTER]

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Winemaking is a complex microbial process in which yeast and bacteria play key roles. During alcoholic fermentation (AF) yeasts consume sugars to produce ethanol. Subsequently, lactic acid bacteria (LAB), mainly *Oenococcus oeni* and *Lactobacillus plantarum*, are responsible for the sensorial wine properties converting malic acid into lactic acid and carbone dioxide during malolactic fermentation (MLF). The MLF has important influences on acidity, flavour and biological stability of wine.
Traditional microbiological methods to study LAB in wine are not efficient enough, because exist viable but non cultivable bacteria. The use of PCR-DGGE is a very sensitive tool for species separation and identification.
The aim of this work was the identification of LAB isolates and the study of the bacterial population associated to Merlot wine produced in the area of the Argentinean North Western Patagonia by using PCR-RFLP and PCR-DGGE techniques.
A collection of 30 LAB obtained by enrichment cultures from three different stages of natural and yeast inoculated MLF were identify by PCR-RFLP of a 294 bp *rpo*B gene region after two digestions with *Aci*I and *Hinf*I. The restriction products allowed the identification of *Lactobacillus plantarum*, *Lb. paracasei* and *Lb. fermentum* and *Leuconostoc mesenteroides* species in the natural MLF and *Lb. plantarum*, *Lb. paracasei* and *Lb. collinoides* in the inoculated MLF. Greater diversity and most of the isolates were obtained from natural MLF. On the other hand *Oenococcus oeni* was not found in the MLF isolates. At the present does not exist studies by direct PCR-DGGE analyses of LAB biological diversity from Patagonian wines. With this aim, genomic DNA was directly obtained from wine and identification of LAB was performed using primers rpoB1, rpoB1o, and rpoB2 able to amplify a region of 336 bp of the rpoB gene. PCR fragments were separated in a 30–65% DGGE gradient, and the DGGE profile analysis showed greater diversity in the initial and medium stage of the natural MLF. At the end of both MLF processes a reduction of the diversity were observed, nevertheless the presence of *Oenococcus oeni* was detected in all fermentation stages. Monitoring microbial populations by PCR-DGGE revealed the presence of conventional wine species: *O. oeni* and *L. plantarum*. PCR-DGGE offer an approach to wine species involved in different stages of MLF winemaking processes. In order to develop a malolactic starter it is necessary to deep the LAB diversity analysis.

Sección 2 - Biorremediación y Biocontrol

2.1 METAL BIOSORPTION BY S-LAYER PROTEINS FROM *Bacillus* SPECIES

[ORAL]

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Bacillus species have been involved in metal association as biosorbents, but there is not a clear understanding of this chelating property. In order to evaluate this metal chelating capacity, cultures and spores from Gram-positive bacteria of species either able or unable to produce surface layer proteins (S-layers) were analyzed for their capacity of copper biosorption. Only those endowed of S-layers, like *Bacillus sphaericus* and *B. thuringiensis*, showed a significant biosorption capacity. This capacity (nearly 50%) was retained after heating of cultures, thus supporting that structural elements of the envelopes are responsible for such activity. Purified S-layers from two *Bacillus sphaericus* strains had the ability to biosorb copper. Copper biosorption parameters were determined for strain *B. sphaericus* 2362, and after analyses by means of the Langmuir model, the affinity and capacity were shown to be comparable to other bacterial biosorbents. A competitive effect of Ca^{2+} and Zn^{2+}, but not of Cd^{2+}, was also observed, thus indicating that other cations may be biosorbed by this protein. Spores that have been shown to be proficient for copper biosorption were further analyzed for the presence of S-layer content. The retention of S-layers by these spores was clearly observed, and after extensive treatment to eliminate the S-layers, the biosorption capacity of these spores was significantly reduced. For

the first time, a direct correlation between S-layer protein content and metal biosorption capacity is shown. This capacity is linked to the retention of S-layer proteins attached to *Bacillus* spores and cells.

2.2. Cr(VI) bioremediation by *Streptomyces* sp. MC1: Effect on *Zea mays*

[POSTER]

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In natural water and subsurface soils, chromium (Cr) occurs in two major oxidation states: III and VI. Cr(VI) is the major chromium species used in industry and is the common pollutant in soil and waste water, while Cr(III) is a relatively insoluble and non-toxic. Cr(VI) produce toxicity acute and chronic, neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity and immunotoxicity. Cr(VI) compounds are 1,000 fold more toxics than Cr(III) compounds. Biological transformation of Cr(VI) to Cr(III) by enzymatic reduction is a means of decontamination. This biological reduction may provide a less costly and environmentally friendly approach to remediation. On the other hand, vegetables can be used as contamination level markers because they can accumulate these compounds.
A Cr(VI) resistant actinobacteria strain, *Streptomyces* sp. MC1, previously isolated, in our lab, from sugar cane, has the ability of reducing Cr(VI) in sterile soil samples.
The aim of this work was to evaluate the ability of this strain to bioremediate non sterile soil samples contaminated with Cr(VI), using *Zea mays* as bioindicator.
Streptomyces sp. MC1 was grown in Tryptic Soy Broth (g L^{-1}: Tryptone 15, Soy Peptone 3, NaCl 5, K_2HPO_4 2.5 and glucose 2.5) during 3 days at 30 ºC. Glass pots were filled with 200 g of soil and kept at 20% humidity with distilled water. $K_2Cr_2O_7$ solution was added at final Cr(VI) concentration of 200 mg kg^{-1} of soil. Later these soil samples were inoculated with pre-cultured *Streptomyces* sp. MC1.
Zea mays seeds were sterilized and sown on plates with agar. Maize young plants were potted at the same time of *Streptomyces* inoculation (t0), 14 days (t14) and 28 days (t28) after the inoculation, and cultivated during 14 days. Chromium bioavailability was measured by atomic absorption spectrophotometry (AAS) after centrifugation 1 g of soil at 5540 g. Maize biomass was estimated as dry weight. Chromium accumulate by plants was measured by AAS after plant treatment with concentrated H_2SO_4.
Streptomyces sp. MC1 was able to reduce Cr(VI) bioavailability up to 73% after 42 days. Similarly, *Zea mays* reduced up to 70% of chromium bioavailability, in absence of *Streptomyces* sp. MC1.
On the other hand, *Zea mays* biomass decreased up to 88% with Cr(VI) and without *Streptomyces* sp. MC1. However, *Zea mays* biomass decreased only 32% in presence of Cr(VI) and *Streptomyces* sp. MC1, when the sow was made after 28 days of inoculation, and Cr(VI) bioavailability was reduced up to 97%.
This is the first report where *Streptomyces* sp. MC1 and *Zea mays* show a synergic effect that could be useful to bioremediate Cr in soil samples.

2.3. Biosurfactant production by autochthonous bacteria from Reconquista River basin: screening and extraction.

[POSTER]

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Biosurfactans are natural surface-active compounds with a hydrophilic head and a hydrophobic tail. They are involved in the emulsification of non-aqueous phase liquids, allowing the degradation of several organic compounds. Moreover, biosurfactans can act as metal ligands decreasing their bioavailability in the environment. Biosurfactants can be produced by a large group of microorganisms and are mostly glycolipids, phospholipids, polysaccharide-lipid complexes, lipoproteins-lipopeptides, hydroxylated and cross-linked fatty acids. The aim of our work is to make a screening for surfactant production of the isolates: *Pseudomonas veronii* 2E, *Delftia acidovorans* AR, *Ralstonia taiwanensis* M2, *Klebsiella ornithinolytica* 1P and *Klebsiella oxytoca* P2 to study complexing capacity with Cu(II), Cd(II) and Zn(II) for their application in future metal bioremediation techniques. Surfactant production was evaluated by three methods: a) Blue Agar (BA) plates (g/L: $(NH_4)_2HPO_4$ 1.5, KH_2PO_4 4, yeast extract 0.4, CTAB 0.2, glucose 20, methylene blue 0.015, $MgSO_4.7H_2O$ 1.97, agar 3) were inoculated and surfactant production was evidenced by the development of a dark blue halo after 24-48 hs at 32°C; b) Blood Agar (BLA) plates were inoculated to test for haemolytic activity, which is related to biosurfactant production and c) Surface Tension (ST) of bacterial cultures´ supernatants was measured using a stalagmometer. In addition, haemolytic activity was tested in cultures´ supernatants in BLA plates. Surfactant production was examined in the presence of two different carbon sources in a minimal broth: sunflower (SF) oil (5%) and glucose (20 g/L). Our results indicated that SF oil was a better carbon source for surfactant production stimulation. Supernatants of 2E and M2 cultures presented a decrease in ST values (68.03 and 60.59 dyn/cm respectively), compared to medium (71.73 dyn/cm) or water (72.75 dyn/cm) and haemolytic activity was registered when SF oil was the carbon source. Persistent emulsification of SF oil in aqueous phases was observed in M2 cultures. Although P2 presented low ST value (68.61 dyn/cm) when glucose was the carbon source (78.55 dyn/cm in control medium), no haemolytic activity was observed in its culture supernatant. However, culture supernatants obtained with SF oil developed haemolysis in BLA. Neither AR nor 1P cultures decreased ST comparing to control. In accordance, no haemolytic activity was detected in culture supernatants of AR, eventhough haemolytic activity was observed by growing in BLA plates. These evidences indicate that 2E and M2 are biosurfactant producers, and extraction techniques from cultures had to be developed for a proper chemical identification of the compounds involved. These molecules with emulsificating properties are potential ligands for further metal complexing capacity experiments.

2.4. Aerobic removal of methoxychlor contaminated soil by *Streptomyces* sp. A14

[POSTER]

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Organochlorine pesticides (OP) have aroused global concern due to their long persistence, low biodegradability, wide range distribution in the environment and chronic adverse effect on wildlife and humans. Methoxychlor (MTX) is a toxic OP that was used in industrial and agricultural activities and for the malaria control. Although the use of MTX has been internationally banned it can still be detected in the environment like in the northwest of Argentina.
Bioremediation offers the possibility to environment cleanup of pollutants, such as OP, using natural biological activity. However, there is little information available about microbial MTX degradation.

Actinobacteria have a great potential for bioremediation of toxic compounds, in addition strains of Streptomyces genus may be well suited for soil inoculation as a consequence of their mycelial growth habit, relatively rapid rates of growth and colonization of semi-selective substrates.
In our laboratory, we isolated an actinobacteria strain from OP-contaminated soil in Santiago del Estero, Argentina, identified as *Streptomyces* sp. A14, able to remove and degrade MTX from liquid minimal medium. Thus, the purpose of this work is to contribute to the study of bioremediation of MTX by actinobacteria in contaminated soils.
Glass pots were filled with 80 g of soil at 20% moisture. The soil samples were sterilized and the soil humidity was adjusted with sterile water and a MTX solution for different final concentrations (8.30 and 16.60 mg kg^{-1} wet weight (ww) soil). For soil samples inoculation, the microorganism was precultured in Tripticase Soya Broth with MTX (1.66 mg L^{-1}). Soil samples pots with MTX and without MTX as control, were inoculated with *Streptomyces* sp. A14 (2 g kg^{-1} ww soil). Soil pots were incubated at 30 ºC for 28 days. Samples were taken each 7 days. The growth was measured as CFU kg^{-1} and residual MTX from soil was determined by gas chromatography.
Our results indicate no significant differences in the growth at the different MTX concentrations added and in the control without MTX. The cell concentration increased up to 2 log units and the maximum growth of *Streptomyces* sp. A14 was 1.78 x 10^7 CFU g^{-1} ww soil, at 14 days of incubation. In both concentration assayed the microorganism was able to remove the soil pesticide, reaching the maximum removal percentages (36.14 and 76.02%) at 28 days of incubation. Finally, we suggest that *Streptomyces* sp. A14 has a big potential for bioremediation of soils contaminated with high MTX concentrations.

2.5. Characterization of *Pseudomomas veronii* 2E biofilm establishment for fixed-bed bioreactors to be applied in metal loaded wastewater treatments.

[ORAL]

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Biofilms are commonly referred as populations of surface-attached microorganisms comprising either single or multiple species. The environmental conditions promote changes in microbial enzymatic activities that can disrupt the structural organization of biofilms. Over the course of the past decades, biofilm-mediated wastewater treatments became a proficient and safer alternative, especially when metal retention is involved. *Pseudomonas veronii* 2E is an indigenous strain, isolated from polluted sediments of Reconquista River (Buenos Aires Metropolitan Area), which showed the ability to biosorb Cd(II) and Zn(II) on fixed-bed reactors. The aim of our work is to improve the biofilm development of *P. veronii* 2E by changing environmental conditions to be applied on fixed-bed bioreactors for metal loaded wastewater treatments. For that purpose, biofilm establishment and maintenance were explored over glass coverslips under different culture broth composition (M9, minimal medium, and PY, peptone and yeast extract), carbon sources (glucose, glutamate, succinate or citrate), nutrient concentration, presence of Fe(II) and incubation times at 32ºC. For the structural analysis of biofilms, samples, previously stained with crystal violet (CV), were microscopically observed and scanned for image analysis by MATLAB program. Finally, for a semi-quantitative estimation of attached cells the CV was extracted with ethanol and Absorbance 590nm was measured. With M9, biofilm structure appeared to be a monolayer with small clusters scattered over the glass surface, while in PY-Glucose numerous cell clusters and channels were appreciated by microscopic observation. Best immobilization was achieved with PY-0.05%Glucose at 55hs, but the presence of 3µM Fe(II) negatively influenced on biofilm development. M9-0.5%Glucose-3µMFe(II) was the best combination for *P. veronii* 2E biofilm establishment. The attached cells´ density correlated highly with spectrophotometric determinations of CV ethanol solutions and

satisfactorily described biofilm evolution with time. MATLAB program allowed an approximate simulation of the surface profile of the obtained biofilms from scanned 2D-images. For *P. veronii* 2E, the final biofilm structure was defined by medium composition and nutrient quality. These parameters directly influenced on cellular differentiation, necessary for a proper biofilm establishment to acquire an efficient bioreactor performance.

2.6. Characterization of *Pseudomonas* strains able to hydrocarbon degradation and polyhydroxyalkanoates production

[POSTER]

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Bioremediation is an attractive and environmental friendly approach for the cleanup of contaminated sites. It exploits the potential of naturally occurring microbial populations, or in some cases, introduces microorganisms with a known ability to degrade the contaminants. This technique is named bioaugmentation. Desirable characteristics in strains used in bioremediation are the high stress resistance and increased fitness. We propose that those characteristics could be conferred by the polyhydroxyalkanoates (PHA) accumulation capability. Under this context, our approach consisted in to isolate and characterize bacterial strains able to accumulate PHA from hydrocarbon contaminated environments. Two strains - *Pseudomonas* sp. KA-08 and *Pseudomonas* sp. KB-08 - were selected by their ability to grow in high amount of kerosene and diesel and to accumulate PHA from sodium octanoate as well. Comparison of the sequences and phylogenetic analysis of 16S rRNA gene allowed determine that both strains are related to *P. putida*. PHA was determined by gas chromatography (GC) analysis showing medium-chain length PHA, especially C8. In minimal medium with sodium octanoate as carbon source, KA-08 accumulated 15.3 ± 1.5 % of dry weight and KB-08 7.5 ± 0.8% of dry weight. We studied the type of PHA synthase present on each strain, obtaining positive results for class II *phaC2* and *phaC1* synthases. The comparison of nucleotide and amino acid sequences of class II synthases in both isolates showed more than 90% of similarity with sequences of Class II polyhydroxyalkanoate synthases from various *Pseudomonas* strains. In KB-08, a nonsense mutation was observed in *phaC2* gene. Both strains were able to grow in crude oil, kerosene, diesel and xylenes as sole carbon source in high agitation conditions (300 rpm). Diesel and kerosene degradation were determined by GC. Kerosene degradation was higher than diesel's reaching 39% and 35% for KB-08 and KA-08, respectively, after 25 days of culture. No growth in hexane or octane was observed in liquid medium under high agitation condition. However, both strains were able to grow in solid media supplemented with hexane but when octane was used as sole carbon source, only KA-08 was able to grow. PCR analysis of the *alkB* gene showed differences between the amplicons obtained from KA-08 and KB-08 that could explain these differences.In addittion, both strains showed different results regarding xylenes mix. While KB-08 showed a good growth at 0.5% v/v, KA-08 needed at least 1% v/v to growth, perhaps because a different usage of the xylene isomers. These capabilities of these isolates regarding PHA accumulation and growth in different hydrocarbons and its degradation, will allow us to construct a rational design of bacterial consortia.

2.7. En revision

2.8. En revision

2.9. INFLUENCE OF SUBSTRATE CONCENTRATION, ADAPTATION AND INOCULUM SIZE ON 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) BIODEGRADATION BY AN INDIGENOUS *DELFTIA* SP. STRAIN

[POSTER]

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2,4-dichlorophenoxyacetic acid (2,4-D) is a persistent and toxic compound widely used as an herbicide in agricultural practices for the control of broad-leaved weeds in cereal croplands. Because of its high solubility and low tendency to accumulate in organic matter this compound can enter as contaminant into streams, rivers or lakes directly from drainage of agricultural lands. Moreover, contamination of the aqueous environment could be the result of pesticides discharges from manufacturing plants, storage sites and accidental spills. In Argentina, the National Act on Hazardous Wastes (Act 24051/92) recommends restricting 2,4-D concentrations in surface waters to 4 µg l^{-1} in order to protect the aquatic wildlife. Biodegradation is a feasible alternative for the remediation of polluted waters. In a previous work we isolated an indigenous *Delftia* sp. strain able to use 2,4-D as the sole carbon source. The aim of this investigation was to study the influence of substrate concentration, adaptation and inoculum size on 2,4-D biodegradation by *Delftia* sp. Biodegradation assays were performed in a 2 l aerobic microfermentor, at 28 ºC, with agitation (200 rpm). Initial 2,4-D concentrations of 100, 200, 300 and 400 mg l^{-1} were tested. To assess the influence of adaptation the system was inoculated with 2,4-D pre-exposed bacterial cultures. The assay was also performed with non adapted cells. In order to evaluate the influence of inoculum size cell concentration was adjusted to 1 x 10^5 or 1 x 10^6 CFU ml^{-1}, according to the assay. 2,4-D biodegradation was determined by UV-spectrophotometry and chemical oxygen demand (COD), and bacterial growth by the plate count method. The indigenous strain was able to degrade 100, 200, 300 and 400 mg l^{-1} of 2,4-D in 24, 28, 32 and 40 h respectively. Process efficiency was higher than 95% and 90% at all the concentrations tested, expressed in terms of 2,4-D and COD removal respectively. Absence of adaptation produced an increase in the biodegradation time from 24 to 44 h. The reduction in the inoculum size from 1 x 10^6 to 1 x 10^5 CFU ml^{-1} increased in 8 h the biodegradation time. *Delftia* sp. was able to degrade high concentrations of 2,4-D. The factors studied in this investigation did not affect the efficiency of the biodegradation process. We hope these results will help in the optimizing of 2,4-D biodegradation processess for the remediation of polluted waters. *Supported by University of Buenos Aires (UBACyT Program-Project B022)*.

2.10. DEGRADATION AND DETOXIFICATION OF 2,4-DICHLOROFENOXYACETIC ACID (2,4-D) AND 4-CHLORO-2-METHYLPHENOXYACETIC ACID (MCPA) MIXTURES BY AN INDIGENOUS BACTERIAL STRAIN

[POSTER]

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The structurally related compounds 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) are among the most widely used herbicides to control dicotyledoneous weeds in cereal crops and pastures throughout the world. In addition to their usage in agriculture, relevant sources of environmental pollution with these herbicides are wastewater treatment processes from manufacturing plants. Because of their water solubility and persistence convencional wastewater treatment plants do not remove these compounds efficiently, therefore they are discharged into surface waters, thus presenting a potencial risk for drinking water supplies. The isolation of native microorganisms able to degrade these herbicides and their use in bioaumentation processes allow the optimization of treatment processes. In a previous work we isolated an indigenous bacterial strain able to degrade 2,4-D and MCPA. The aims of the present investigation were: a) to study degradation kinetics of MCPA by the bacterial strain; b) to assess the ability of the strain to degrade a mixture of 2,4-D and MCPA and c) to evaluate detoxification after biodegradation processes. Biodegradation assays were performed in a 2 L aerobic microfermentor, at 28 ºC, with agitation (200 rpm). To assess biodegradation of MCPA the compound was added as the sole carbon source to the culture medium at a concentration of 50 mg L^{-1}. A biodegradation assay was also performed with a mixture of 2,4-D (50 mg L^{-1}) and MCPA (50 mg L^{-1}). 2,4-D and MCPA degradation was determined by UV-spectrophotometry and gas chromatography, and bacterial growth by the plate count method. Detoxification was evaluated by the use of toxicity tests. Root elongation test was performed by using *Lactuca sativa* seeds as test organisms, according to EPA 600/3-88/029 protocol. The indigenous bacterial strain was able to degrade MCPA (50 mg L^{-1}) as the sole carbon source, and a mixture of 2,4-D (50 mg L^{-1}) and MCPA (50 mg L^{-1}) within 72 hours. The efficiency of MCPA biodegradation process was higher than 99% expressed as compound removal. 2,4-D and MCPA removals in the mixture were higher than 99%. Toxicity was not detected after biodegradation processes. The use of this indigenous bacterial strain in 2,4-D and MCPA degradation processes is a promising strategy for the purification and detoxification of liquid effluents that contain these herbicides. *Supported by University of Buenos Aires (UBACyT Program-Project B022)*.

2.11. Combining culture dependent and independent approaches to characterize bioremediation potential at Córdova Cove, a chronically polluted site of Northern Patagonia

[POSTER]

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Polycyclic aromatic hydrocarbon (PAH)-bioremediation potential was assessed in intertidal sediment samples collected from Córdova Cove, a chronically polluted site located in Northern Patagonia, using culture dependent and independent methods.
We estimated the relative abundance of various genes encoding PAH dioxygenases by qPCR, in DNA extracted directly from the sediment samples. We quantified the following genes: *phnA1* (*Cycloclasticus* spp.), *nahAc* (*Pseudomonas* spp.), *phnAc* (&- beta*Proteobacteria*) as well as A, B, C and D dioxygenase genes, which are novel gene groups that we determined to be abundant in sediments from Southern Patagonia and for which no cultured representative is known. Additionally, we quantified bacterial 16S rRNA genes using a degenerate primer set. *Cycloclasticus phnA1* genes were abundant in the most polluted sediment sample (8.10^{4} ± 3.10^{4} copies/&g sediment mu DNA, vs. ca. 10^{8} copies for the 16S rRNA gene). Their abundance was found to be two orders of magnitude higher in sediments with twice the concentration of 3-ring PAHs. The other analyzed genes were also detected, although below quantification limit for this technique. These results suggest that *Cycloclasticus*, a cosmopolitan marine obligate hydrocarbonoclastic bacterium, plays an important role in the biodegradation of low molecular

weight PAHs in coastal sediments of Northern Patagonia too.
On the other hand, culture-dependent methods consisted in the isolation of bacterial strains by enrichment on ONR7 synthetic medium using crystals or vapors of naphthalene, phenanthrene or pyrene as carbon and energy source. After two months of incubation, colonies were purified by multiple streaking or by transferring the hydrocarbon crystal on ONR7 plates with PAH vapor. This resulted in the isolation of twelve gram-negative bacteria, four of them based on their ability to form biofilms on the crystal surface. All twelve isolates presented unique RFLP and ITS amplification patterns. These isolates are being identified by 16S rRNA gene sequencing. The isolates were tested for their ability to produce indigo from indole, a functional assay suitable for some oxygenase enzymes. Only one of the 12 isolates (naph1) produced blue color on solid media in the presence of indole. PCR amplification of oxygenase genes involved in PAH metabolic pathways (*nahAc* naphthalene dioxygenase gene and *c12* and *c23* catechol dioxygenase genes from *Pseudomonas* spp.), were used to assess the presence of archetypical naphthalene degradation genes. The expected amplicons were found only in naph1 strain. None of the isolates showed the presence of *phnA1* genes, suggesting that although very abundant in the sediments, *Cycloclasticus* could not be obtained in culture by this method.

2.12. Use of *Escherichia coli* strains as bioremediators of soil and water polluted with copper

[POSTER]

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Copper is an essential element for all living organisms, acting as a cofactor of several cellular enzymes. However, in excess, this metal becomes potentially toxic and because it is not degradable, their remediation turns complicated. Contamination of soils, rivers and deep waters with copper increased dangerously, especially from industrial effluents. On the other hand, when applied in the field as a fungicide ($CuSO_4$, Bordeaux mixture, copper oxychloride, etc.) it accumulates in the soil, and when crops take it, copper may become toxic for animals and humans if it reaches certain level in those foods. The microorganisms have been studied as a tool for bioremediation of polluted environments. In our laboratory it was demonstrated that certain strains of *Escherichia coli* could detoxify liquid media with high copper levels. Additionally, we standardized a sequential oxidative treatment (SOT), which is able to eliminate postharvest citrus pathogens. In one step of SOT, $CuSO_4$ is used combined with H_2O_2 for catalyze the formation of reactive oxygen species. In this process, H_2O_2 is transformed into a harmless compound, but the concentration of $CuSO_4$ practically remains unchanged at the end of the processing. Because we are interested in protecting the environment, we explored ways to capture of copper from the SOT effluent.
Here, we proposed: a) finding conditions suitable for extracting copper from soil by solubilising it to a liquid medium, b) finding appropriate conditions for copper capture by *E. coli* cells, both from liquid medium after the soil treatment and from SOT effluent. To perform this investigation, soil samples were contaminated with different amounts of copper and then we tried to solubilize it by using several types of acids and different extraction conditions, to obtain the most effective combination of them. Afterwards, supernatants of the extractions were treated with the *E. coli* strains studied. The SOT effluent was subjected to several procedures to degrade the residual H_2O_2 and subsequently treated with *E. coli* as well. Results shown that: 1) HCl, was the acid with which the better extraction was obtained; 2) three extractions of 30 min were capable of solubilize around 90% of the added copper; 3) when the solutions were treated with bacteria, the highest copper capture was at pH upper than 5, and with two bacterial extractions in a sequential manner, 90% of the metal was recovered; 4) the best condition to remove the

residual H_2O_2 from the SOT effluent was irradiating with UV-B for 30 min; 5) the condition for the highest capture of copper from the SOT effluent was similar to that of the liquid medium from the soil treatment. These results suggest that the *E. coli* strains used in this study have the potential capability to detoxify soils and waters polluted with copper.

2.13. BIOREMEDIATION OF PHENOL BY A BACTERIAL STRAIN ISOLATED FROM TANNERY SEDIMENTS

[POSTER]

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Many industrial activities produce a large volume of pollutant wastes, generating a deleterious effect on the environment. In particular, wastewater derived from leather production may contain phenols, which are highly toxic and their degradation could be possible through bioremediation technologies. The aims of this work were: a) isolate bacterial strains, tolerant to phenol, from tannery effluents and sediments, b) select a strain with high phenol tolerance and evaluate its tolerance to other phenolic compounds, c) identify and characterize this isolate by biochemical and molecular assays, d) determinate the effect of pH, culture media composition, temperature and phenol initial concentration for its degradation, e) evaluate the ability of the strain to grow and degrade phenol in tannery effluents.
Effluent and sediment samples were obtained from a tannery located in Elena (Cba. province). Some physicochemical parameters of these samples were evaluated as well as total phenol concentration, which was 17.5 mg/L. A bacterial strain, namely as CS1, showing fast growth in TY medium supplemented with 100 mg/L of phenol was selected. CS1 was characterized as a Gram positive, oxidase-negative and catalase-positive bacterium. Physiological and biochemical tests were performed in order to characterize the strain. It was identified as *Rhodococcus* sp. by 16S rDNA gene PCR amplification. The strain was able to grow in 1000 mg/L of phenol and guaiacol, and 500 mg/L of 2,4-dichlorophenol, whereas it was not able to tolerate pentachlorophenol, in mineral medium with these contaminants as sole carbon source. Phenol biodegradation studies were carried out in Erlenmeyer flasks with different culture media (MM1, MM2, MM3, MM9), at different pH (5-11), temperature (25-37 °C) and initial phenol concentrations (200-1000 mg/L). Bacterial biomass and phenol consumption were determined in these experiments. CS1 strain was able to remove completely 1000 mg/L of phenol in MM1 medium at 30 ± 2 °C and pH 7, as optimal conditions. Furthermore, growth and phenol degradation of CS1 strain in Erlenmeyers flask containing tannery effluents was evaluated. After 9 h of incubation, the bacterium showed tolerance to this effluent and ability to completely degrade phenols.
In conclusion, *Rhodococcus* sp. CS1 could be an appropriate microorganism for bioremediation of tannery effluents or other environments contaminated with phenols.

2.14. BIOREMEDIATION OF WASTEWATER CONTAINING CHROMIUM (VI)

[POSTER]

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Chromium is present in the industrial wastes as chromate and dichromate primarily. Conventional methods require large amounts of chemicals and energy input, which is not economically feasible. Low cost methods for the remediation of hexavalent chromium from the polluted environment are therefore of considerable interest. Reduction alters the valence and may therefore alter the toxicity and environmental mobility of a metal. One potential method is the use of bacteria that can reduce toxic hexavalent chromium to non-toxic trivalent form.
The aim of this work was to study the use of *Escherichia coli* in wastewater detoxifying Cr (VI) belonging to a metalworking plant at laboratory and pilot scale.
Effluent bioreduction process was simulated in laboratory conditions (discontinuous batch reactor) and pilot scale reactor (fluidized bed). In both cases it was worked nutrient broth, 5% sand as support material, E. coli strain (initial concentration of 10^6 UFC.ml^{-1}), constant agitation, mechanical aeration and room temperature (25 (±)3 ºC) during a period of 24 hours. Once obtained the biofilm, the effluent was added obtaining an initial concentration of 25 mg. L^{-1} of Cr (VI). Samples were taken at various time intervals, which were centrifuged at 3,500 rpm (20 minutes) and the Cr (VI) content in supernatant was colorimetrically determined. Significant Cr (VI) concentration reductions were attained at laboratory scale during the test period. The total elimination was achieved after 72 hours, turning most of the Cr (VI) into Cr (III). Favorable results were also obtained working with a pilot scale reactor, with a total reduction of Cr (VI) at 45 hours. A second addition of effluent was made, but, in this case, the removal was not as important as in the first aggregate. This could be due to bacterial activity reduction. The results prove the possibility of using E. coli in the bioremediation of industrial effluent containing Cr (VI). Further research will be conducted in order to accomplish optimum conditions for a total removal of hexavalent chromium from the effluent in less time.

2.15. KINETICS OF BIOFILM FORMATION OF Pseudomonas plecoglossicida IN THE PRESENCE OF AN ANNONACEOUS ACETOGENIN, SQUAMOCIN, AND ITS RELATION TO BIODEGRADATION OF NAPHTHALENE

[ORAL]

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Squamocin, an annonaceous acetogenin extracted from *Annona cherimolia* (“chirimoya”), has been shown to increase the biofilm production of *Pseudomonas plecoglossicida*, a polycyclic aromatic hydrocarbons degrading bacterium, by induction of autoinducers production. Biofilm-mediated bioremediation presents a proficient and safer alternative to bioremediation. Due to its carcinogenic and toxic properties, naphthalene is considered a priority pollutant for bioremediation. Natural products, as squamocin could enhance the efficiency of bioremediation processes.
In this work we study the kinetics of biofilms formation of *P. plecoglossicida* in the presence and absence of squamocin and its relation to the rate of degradation of naphthalene.
The kinetics of biofilm formation was carried out by measuring absorbance at 560 nm in a microplate spectrophotometer (Biotek-Power Wave XS2 with GEN5 data analysis software). Measurements were taken every hour for a 12 hours period.
Naphthalene degradation was assessed by RP-HPLC with an UV/visible detector (wavelength 276 nm). The inicial naphthalene concentration was 1mM.
Treatment with squamocin at 2.5 µg.ml^{-1} produced 47% stimulation in the production of biofilms of the strain *P. plecoglossicida* at 6 h of incubation ($p \leq 0.05$). At the same time, the most significant difference in naphthalene consumption between control (18%) and treated (43%) assays was registered.
The rate of consumption of naphthalene at 6 h was determined by mathematical treatment of

experimental data. At that time of incubation, the calculated speed of degradation in the control assay was 21.5 $\mu g.h^{-1}$, against 38.1 $\mu g.h^{-1}$ in squamocin treated assay. Data analysis also showed that at this time, the biofilms formation was almost reaching stationary phase or, which is the same, reaching its maximum value.
The results of the kinetics of biofilms and quantification of residual naphthalene, enabled us determine that the naphthalene consuming was increased with the biofilm production.
This led us to conclude that the increase in the naphthalene degradation is due perhaps to an increase in the production of biofilms since the biofilm phenotype is more suitable for bioremediation.

2.16. Influence of sulphate and phosphate ions on Cr(VI) removal by *Streptomyces* sp. MC1

[POSTER]

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Chromium (Cr) is a natural element which is found in rocks, soil, plants, animals, volcanic dust and gases. It can exist in several oxidation states, but the most stable and common forms in the environment are trivalent [Cr(III)] and hexavalent [Cr(VI)] chromium. Cr(III) is an essential micronutrient required for the growth of many organisms but toxic at high concentrations. However Cr(VI) is toxic, mutagenic, carcinogenic, teratogenic at all concentrations and it is the most frequently used in industrial processes like leather tanning.
While, Cr(VI) is highly soluble in water, mobile and biologically available in the ecosystems, Cr(III) shows poor solubility and is easily adsorbed on mineral surfaces.
Due to the problems that high Cr(VI) concentrations produce in the environment, the treatment strategy could include the reduction of Cr(VI) to Cr(III).
Reduction ability has been found in a large range of eukaryotic and prokaryotic microorganisms. *Streptomyces* sp. MC1, isolated from sugar cane, was selected because it showed significant growth and capacity to remove Cr(VI) in liquid minimal medium, abilities that might be useful for bioremediation.
It is believed that Cr(VI) uses sulfate and phosphate transport routes to penetrate the cellular membrane due to its structural similarity to these anions.
The aim of this work was to study the influence of sulfate and phosphate ions on the resistance and Cr(VI) removal.
Cells of *Streptomyces* sp. MC1 were grown in liquid minimal medium supplemented with glucose and with or without 20 mg/L of $K_2Cr_2O_7$ as Cr(VI) source and 5mM of Na_2SO_4 or K_2HPO_4 for 120 h at 30 ºC in an orbital shaker (170 rpm).
Biomass was determined by dry weight, Cr(VI) concentration in the medium by the colorimetric method of 1,5-diphenylcarbazide, total chromium by flame atomic absorption spectroscopy and residual glucose by the method of dinitrosalicylic.
The presence of sulfate or phosphate ions induced the growth of *Streptomyces* sp. MC1. However, only sulfate ion enhanced the Cr(VI) removal significantly. On the other hand, total chromium concentration in the supernatant was constant in all assayed conditions. It is known that Cr(III) is a stable and soluble component formed by Cr(VI) reduction. Therefore, it could be inferred that chromium remaining in the supernatant will be as Cr(III). This finding may indicate that the Cr(VI) removal by *Streptomyces* sp. MC1 was due to reduction to trivalent form but not to chromium bioaccumulation under these conditions.
Cr(VI) reduction to Cr(III) by microorganisms is an interesting strategy of bioremediation. These results may have significant implications in the biological treatment of Cr(VI) in environmental polluted. Further more studies are required for future applications in bioremediations processes.

2.17. Lindane removal by *Streptomyces* sp. strains immobilized in agar and PVA-alginate

[POSTER]

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γ-Hexachlorocyclohexane (γ-HCH) or lindane is a chlorinated pesticide, which initially played important roles in the control of pests and disease vectors. Nowadays it is well established that it is a toxic, carcinogenic and persistent compound which not only accumulates in animals and plants tissues, but also persist in the environment for long periods.
There has been much work on γ-HCH biodegradation. Bacteria and consortia of bacteria capable of degrading lindane under aerobic and anaerobic conditions have been described. Immobilization techniques are gaining importance in bioremediation because of their advantages such as protection of the cells against the pesticide toxicity, reuse of the cells and the facility to recover them from the system.
The aim of this work was to evaluate the ability of streptomycetes strains immobilized in agar cubes and polyvinyl alcohol-alginate beads to remove lindane in a liquid system.
For this purpose, four streptomycetes strains (*Streptomyces* sp. A2, A5, A11 and M7) previously selected because of their ability to degrade γ-HCH in pure and mixed cultures, were pre-cultivated in TSB medium for 72 h. Biomass pellets were individually entrapped using: a) 3% agar cubes and b) PVA-alginate beads. 5 g of cubes or beads were put into an Erlenmeyer containing 100 mL liquid minimal medium (MM) supplemented with lindane (1.66 mg L^{-1}) as carbon source. After 96 h of incubation, the cells were collected to determine microbial growth by estimating the colony forming units (CFU mL^{-1}) and supernatant samples were taken to determine residual lindane concentration by gas chromatography.
The four studied strains were able to grow in MM supplemented with lindane as sole carbon source. All of them showed lower growth values when they were immobilized in PVA-alginate beads than in agar cubes. Maximal growth (1.35×10^8 CFU mL^{-1}) was obtained by *Streptomyces* sp. A11 immobilized in agar cubes. However γ-HCH removal was more efficient when actinobacteria were immobilized in PVA-alginate beads, showing *Streptomyces* sp. M7 the greatest lindane removal ability, while no lindane removal was observed with the agar-entrapped bacterial strain, *Steptomyces* sp. A11.
The results showed that PVA-alginate can be used as potential actinobacteria immobilization matrix for lindane bioremediation.

2.18. ANTIBACTERIAL ACTIVITY OF COMPOUNDS ISOLATED OF A MEDICINAL EXTRACT FROM *Caesalpinia paraguariensis*

[ORAL]

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Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Substantial investment and research in the field of anti-infectives are now desperately needed if a public health crisis is to be averted. Phytochemical research based on ethnopharmacological data is an effective approach in the discovery of new bioactive molecules. Moreover, the bioguided purification and analysis of plant medicinal extracts is a good method for isolating bioactive molecules.

Infusion prepared from stem bark of *C. paraguariensis* (D. Parodi) Burk. (CPBI), is traditionally used because of their vulnerary properties; these properties suggested possible antimicrobial activity. We investigated the antibacterial activity of a medicinal extract and compounds isolated from this by bioguided purification. Collection microorganisms were used: *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). The microbial susceptibility tests were microdilution in liquid culture medium, and sub-cultured in solid medium, in order to obtain minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), respectively (according to CLSI, 2007). During bioguided purification bioautographic technique was useful. Amoxicillin and Ciprofloxacin were used as positive controls. Lyophilized infusion was fractioned by sequential extraction with organic solvents. Methanolic fraction (MF, bioactive) was fractioned by Sephadex LH-20 column chromatography, and XII fractions were pooled. Five different compounds were isolated from III and XI fractions (ABF, bioactive) by RP-HPLC.They were identified by spectrometric techniques (MS(ESI/ Q-TOF), UV-VIS, H^1-NMR, C^{13}-NMR, etc.). According to the values of MIC/MBC obtained, expressed as µg/ml, CPBI as well as their purified fractions were active against the tested bacterial strains (CPBI: 200-3.000/800- > 3.000; MF: 75-100/> 3.000; ABF: 50-75/> 3.000), and bioactive concentration values were lowering through purification. The isolated compounds were: gallic acid (A), ellagic acid (B), 3-*O*-methyl ellagic acid (C), 3,3'-*O*-dimethyl ellagic acid (D) and 3,3'-*O*-dimethyl ellagic-4-β-D-xylopyranoside (E). These were tested up to 128 µg/ml, in accordance with CLSI for pure substances. B compound was active against both bacterial strains (MIC/CBM: 8-16/8-16 µg/ml). C and D showed activity at higher concentration (32/64 and 64/128, respectively), A was active only against *S. aureus*, and E showed not activity at the assayed concentrations. The MIC/CBM values show that isolated compounds are promising antimicrobial agents, and we raise the possibility of carry out chemical change in bioactive molecules and to assay synergisms, in order to low those values. Our research results validated the medicinal property of this plant.

2.19. *Lactobacillus paracasei* subsp *tolerans* ISOLATED FROM INTESTINAL MICROBIOTA OF FISH OF BAHÍA BLANCA ESTUARY FOR USE AS BIOLOGICAL CONTROL AGENT IN AQUACULTURE

[ORAL]

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The aim of the current study was to evaluate the probiotic properties of a lactic acid bacteria strain for rainbow trout (*Oncorhyncus mykiss*) culture. The strain F2 was isolated from the fish saraquita (*Ramnogaster arcuata*). According to 16S rDNA phylogenetic analysis, the isolate was affiliated as *Lactobacillus paracasei* subsp *tolerans* (GenBank accession FJ892732), and was selected for this study by presenting growth inhibitory effects against the salmonid pathogens: *Aeromonas salmonicida* subsp. *salmonicida*, *Yersinia ruckeri* and two strains of *Lactococcus garvieae*.
The characteristic evaluated were: a) tolerance to rainbow trout gastric pH, b) tolerance to rainbow trout bile, c) *in vitro* adhesion to rainbow trout mucus, e) competitive exclusion of salmonid pathogens: *Y. ruckeri* and *A. salmonicida*, and f) assessment in vivo of possible pathogenicity in rainbow trout.
When the strain F2 was exposed at pH 3.0 for 1.5 h, no changes in viable count were observed. Although the strain can survive at pH 2.0, a reduction of 4 logarithmic units with respect to control (pH 6.5) was detected. F2 tolerated a fish bile concentration of 10% for 1.5 h of incubation and no significant changes in viable counts were observed. The strain was able to attach to rainbow trout skin mucus, between 10^4-10^5 cells/cm^2. F2 inhibited the attachment of salmonid pathogens, *Y. ruckeri* and *A. salmonicida*, to mucus at different assay conditions (competition, exclusion and displacement). Under conditions of competition, F2 showed a high

percentage of inhibition of *Y. ruckeri* (92.3% ± 2.9) and *A. salmonicida* (97.0 % ± 3.0). Futhermore, F2 was able to exclude *Y. ruckeri* and to displace *A. salmonicida* adhered to mucus (75.5 ± 2.9 % and 91.1 ± 5.2 % of inhibition, respectively).
To evaluate the pathogenicity of the potential probiotic against rainbow trout, the strain F2 was added daily to the water of a close recirculating-water system for intensive culture of rainbow trout in a final density of 10^3 UFC/mL. Under probiotic supplement, among the experimental fish normal behaviour and no pathology were observed and no differences in the survival were detected, compared to the control tanks (without probiotic supplement) during 45 days of treatment. F2 showed the capacity of colonizing the rainbow trout mucus, ability to compete with pathogens or remove them, stability against fish bile and the usual gastric pH (2.5-3.5). On other hand, the strain has not been harmful to rainbow trout under culture conditions. These results suggest a probiotic potential of *Lactobacillus paracasei* subsp *tolerans*, isolated from Bahía Blanca Estuary, for application in rainbow trout culture and encourage further studies.

2.20. Diversity of lepidopteran specific *Bacillus thuringiensis* strains from Argentina

[POSTER]

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Several lepidopteran species cause significant damage to many agricultural important crops in Argentina. Among them *Anticarsia gemmatalis* and *Spodoptera frugiperda* are important insect pests of soybean, corn, wheat, and forage grasses. Currently, the use of entomopathogenic bacteria *Bacillus thuringiensis* as biolarvicide offers a viable alternative for insect control. This bacterium produces proteinaceous inclusions during sporulation that are toxic towards insect larvae upon ingestion. The parasporal body of *B. thuringiensis* consists of one or more insecticidal crystal proteins (ICP). Most ICP-coding genes are located in megaplasmids. Typically, *B. thuringiensis* harbours a set of native plasmids, which vary in number from 1 to 17 and in size from 2 kb to 600 kb. Plasmid patterns and different PCR approaches have frequently been used to characterize *B. thuringiensis* strains around the world.
In this work, we described the characterization of nine *B. thuringiensis* strains isolated from soil samples of Argentina. In preliminary tests, we performed mortality assays to determine the toxicity spectrum of these strains. They were highly toxic against *A. gemmatalis* and moderately toxic against *S. frugiperda*. Also, protein patterns of spore-crystal complexes analyzed by SDS-PAGE showed similar patterns with major polypeptides of about 70 and 130 kDa. Molecular characterization of these strains was made by determining plasmid patterns, nested-PCR and, denaturing gradient gel electrophoresis (DGGE). According their plasmidic pattern, the isolates were classified in three groups containing four, eight or no plasmids. On the other hand, characterization of *cry* gene content in these strains was performed by a nested-PCR and DGGE approaches both with degenerate primers. Using DGGE methodology we got identical profiles in eight strains except in strain FCC25. Additionally, these methods allowed us to identify partial *cry* sequences that are currently under analysis and will be further used to recognize and characterize new *cry* sequences.

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Sección 3 - Fisiología y Metabolismo de Microorganismos

3.1 SYNTHESIS OF ESTERS BY PROPIONIBACTERIA AND LACTOBACILLI

[POSTER]

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Esters are important contributors to the flavor of many cheese-types, conferring fruity notes. Among several esters detected in cheese, ethyl butanoate is a common flavor-active compound. Propionibacteria (PAB) and lactobacilli used as starter/adjunct cultures in the manufacture of Swiss-type cheeses play a major role in ester synthesis. Esters are synthesized by esterases from free fatty acids and alcohols (esterification) or triglycerides and alcohols (alcoholysis). The availability of alcohols is the limiting factor of ester synthesis in Swiss and Cheddar cheese. Our aim was to evaluate the ester-synthesizing activity of two PAB strains (*Propionibacterium freudenreichii* CIRM1 and CIRM508) and two lactobacilli strains (*Lactobacillus fermentum* CRL1446 and *L. casei* CRL1430) isolated from cheeses. PAB were cultured in YEL broth at 30°C, and lactobacilli in MRS broth at 37°C. Cells were harvested at stationary phase, and disrupted using glass beads. Cell-free extracts were then incubated in reaction mixtures containing either butanoic acid + ethanol or tributyrin + ethanol, to investigate ester synthesis via esterification and alcoholysis, respectively. After 24 h incubation at 30°C (PAB) or 37°C (lactobacilli), esters and alcohols were determined using dynamic head space-gas chromatography-mass spectrometry (DHS-GC-MS). Both PAB strains showed ethyl butanoate-synthesizing activity via esterification, *P. freudenreichii* CIRM508 activity being 2-fold higher than *P. freudenreichii* CIRM1. Ethyl butanoate-synthesizing activities via alcoholysis were also detected, but were significantly lower than via esterification, *P. freudenreichii* CIRM1 activity being 2-fold higher than *P. freudenreichii* CIRM508. Regarding lactobacilli, both strains showed ethyl butanoate-synthesizing activity. In *L. fermentum* CRL1446 higher activity via alcoholysis was observed, whereas in *L. casei* CRL1430 higher activity via esterification was detected. Significant pentyl butanoate and butyl butanoate-synthesizing activites via alcoholysis were also observed in these strains. No direct relationship between the presence of alcohols and the corresponding esters was observed. Our results demonstrate that the evaluated strains have the enzymatic potential to synthesize esters that are crucial for flavor development in Swiss-type cheeses. *Supported by ECOS-MINCYT A08B02*.

3.2 En revisión

3.3 ISOLATION AND SELECTION OF POTENTIALLY PROBIOTIC STRAINS FROM CHICKEN INTESTINAL CONTENT

[ORAL]

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There is a worldwide attempt to reduce antibiotic use in animal production because increased microbial resistance to antibiotics and residues in animal products can be harmful to consumers. Therefore, feed companies and researchers are looking for alternative products and strategies that can help to maintain animal gut health in order to prevent or reduce the prevalence of pathogens in the food chain. An alternative and effective approach to antibiotic

administration is the use of probiotics, which can help to improve gut microbial balance and therefore the natural defence of the animal against pathogenic bacteria. In the poultry industry, probiotic supplementation has been shown to improve daily weight gain, feed conversion ratio and mortality rate in broiler chickens. Probiotics used in broilers include *Lactobacillus*, *Bifidobacterium* and *Enterococcus* among other lactic acid and related microorganims. The aim of this paper was to isolate and select lactic acid bacteria (LAB) strains from chicken intestinal content, with potential use as chicken probiotics. The media used were HHD, LBS and KF, selective for *Bifidobacterium*, *Lactobacillus* and *Enterococcus* respectively. The LAB strains were isolated from chicken gastrointestinal tract; same niche previously isolated *Propionibacterium* strains were also studied. In order to analyse their capacity to grow at 42 °C, growth kinetics of the strains isolated in HHD, LBS and KF media were studied in LAPTg + 1% lactose, MRS and LAPTg broths, respectively. Then, the strains were subjected to a simulated gastrointestinal digestion, performed at 42 °C. Finally, their capacity to grow in co-culture was studied towards the selection of a combination of 3 strains. Out of 104 isolated strains 81 were considered as possible LAB (Gram and catalase negative). Fifty eight of these strains grew at 42 °C; the viable cells of the 14 possible LAB strains and 4 of the 9 propionibacteria strains previously isolated decreased less than a unit log after the simulated gastrointestinal digestion. Finally, all strain combinations grew in co-culture and showed a lower biomass than the addition of those of the corresponding mono-cultures. Thus, the combination that showed the least biomass decrease was selected for further studies as a potential probiotic supplement for chickens. In conclusion out of 104 strains isolated from chicken intestinal content, 13 were Gram and catalase negative, grew at 42 °C and survived a simulated gastrointestinal digestion. A combination of 3 strains showed compatibility and thus was selected as a potential probiotic supplement for chickens.*Supported by CONICET PIP 0996, ANPCyT PICT 2168 and CIUNT D429*.

3.4 Conjugated linoleic acid synthesis by lactic acid bacteria cultured in skim milk

[POSTER]

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Conjugated linoleic acid (CLA) is a biolipid with beneficial properties for humans, such as anti-carcinogenic effect. Several authors have hypothesized that CLA production is a detoxification mechanism by which bacteria reduce the toxicity of free fatty acids. The aim of this study was to evaluate the CLA-synthesizing ability of lactic acid bacteria (LAB), using skim milk as culture medium and vegetal oils as substrate. *Lactococcus lactis* CRL 1434 and *Lactobacillus paracasei* CRL 575 were previously selected due to their high linoleic acid (LA) conversion rate observed in LAPTg and MRS medium, respectively. Strains were inoculated at 2 % (v/v) in 10 % (w/v) reconstituted skim milk. At regular intervals (0; 4; 8; 12; 18; 24 h), samples were taken to measure pH and growth (viable cell count). Lipid profile was determined at 24 h, by extracting lipid with chloroform/methanol solution (1:1, v/v) according to Folch et al. (1957). Fatty acids were derivatized following Chin et al. (1992) protocol, and analyzed by gaseous chromatography. Hydrolyzed soy and sunflower oils were individually added as substrate to obtain a final LA concentration of 200 and 400 µg/ml.
Results showed that growth rate and pH values were not affected during the milk fermentation, with or without vegetal oil addition. After 24 h of incubation (37ºC) CLA production in *L. paracasei* CRL 575 culture was 58 and 65 µg/ml, when sunflower oil was added as substrate (200 and 400 µg/ml, respectively). Higher levels of CLA (69 and 74 µg/ml) were determined in skim milk supplemented with soy oil at the same concentrations. *Lact. lactis* CRL 1434 showed a similar behaviour in milk enriched with soy or sunflower oils. CLA production was 65 and 87

µg/ml in presence of sunflower oil (200 and 400 µg/ml, respectively), and 59 and 101 µg/ml when soy oil was added at the same concentrations.
Both LAB strains showed a similar CLA production level in presence of hydrolyzed sunflower or soy oils, and a lower LA conversion rate than that previously observed in MRS or LAPTg broth. This fact could be due to the high protein concentration of milk which could neutralize the toxic effect of free fatty acids on bacteria metabolism. However, CLA concentration in fermented milk was higher when vegetal oils and LAB were used. This study demonstrates that LAB can be used as potential starter or adjunct cultures in the production of CLA-enriched milk.

3.5 Impact of hydrodynamics on yeast biofilms

[POSTER]

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In industrial processes, the geometry design of machines, pipes and tanks are usually complex, and biofilms are thus grown under a wide distribution of local hydrodynamic strengths. In order to adhere to surfaces and subsequently to form biofilms, microorganisms in flowing systems must overcome shear stress at the fluid-surface interface. Biofilms on juice processing plant are considered as serious sources for potential spoiling microorganisms. *Candida krusei* is one of the most frequently isolated yeast species in fruit concentrates: that might be due to its great potential for biofilm formation.
The aim of this study was describe the effect of hydrodynamic forces on adhesion and microcolony formation of *C. krusei* in a simulated industrial environment.
Different fluid dynamics were simulated in a parallel plate flow chamber and a rotating disk system: turbulent flow, transitional flow, laminar flow and no flow. The Reynolds (Re) number varied between 0 and 136,000, which determines shear stresses from 0 to 91 $N.m^{-2}$.
All experiments were carried out at 22 ± 1 ºC to accurately simulate the environmental conditions of production of concentrated apple juice in a processing plant. Stainless steel coupons (AISI 304, food grade) were used as substrate. A suspension of *C. krusei* was prepared to a final concentration of 10^5 cells/mL in sterile 12º Brix clarified apple juice. This suspension was circulated 2 h through the systems to allow cell attachment. Then, the coupons were rinsed to remove reversely attached cells and sterile 12 ºBrix clarified apple juice was circulated to observe the formation of microcolonies. The surfaces were stained with fluorescein diacetate and examined by epifluorescence microscopy.
In batch conditions (no flow) and laminar flow (Re=50, shear stress=0.047 $N.m^{-2}$), microcolonies were detected after 16 h. At Re between 15,000 and 136,000 (shear stress from 0.56 to 91 $N.m^{-2}$) the formation of microcolonies began at 48 h.
The morphology of the microcolonies and the disposition of *C. krusei* cells in laminar and transitional flow were markedly different. The microcolonies were longer in the direction of flow in biofilms subjected to high shear stress, showing the influence of hydrodynamic drag. Biofilms grown under batch and laminar conditions consisted of spherical mound-shaped microcolonies that showed no alignment relative to the direction of flow.
The effect of hydrodynamic forces on biofilm physical and microbial properties may be explained by physiological adaptation mechanisms of microbial species in the biofilm. One hypothesis would be that high shear environments select for populations that produce stronger biofilms. The presence of specific receptors on the wall or cell membrane seems to demonstrate that cells have prepared molecular level responses to mechanical stimuli, as might be caused by agitation.

3.6 Active efflux of bile acids in *Lactobacillus reuteri* CRL 1098

[POSTER]

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Bile acids (BA) are powerful detergents which disorganizes the lipid bilayer structure of cellular membranes. One of the main factors involved in bile tolerance in Gram-negative bacteria, which are inherently more resistant to bile than Gram-positive bacteria, are efflux pumps that remove bile that gets through the outer membrane. The defenses mechanism of Gram-positive bacteria against bile, however, is still poorly understood. The lactic acid bacteria *Lactobacillus (L.) reuteri*, a member of the intestinal microbiota currently used as probiotic, showed a significant degree of resistance to the toxic action of BA. We show in this work the presence of an active efflux of BA in the *L. reuteri* CRL 1098 strain and study whether the exporting mechanism is a primary transport mediated by ATP or by proton motive force (PMF). Everted vesicles of CRL 1098, prepared from stationary cells by disruption with several passages through a French pressure, were incubated for 20 min at 37 ºC in the presence of 2 mM conjugated and free BA [taurocholic (TCA) and cholic (CA) acids, respectively]. The experiments were carryied out at pH 5.2 and 6.5, using not energized vesicles, energized vesicles with 5 mM ATP, or deenergized vesicles (5 mM ATP plus 1 mM sodium vanadate or nigericine). In independent experiments, a proton gradient was generated trough the cell membrane (value of inside pH of 5.2 and outside pH of 6.5; and inversely). Remaining BA in the supernatant of samples removed after several times of incubation were determined by HPLC. Results showed that there was an ATP-dependent accumulation of BA in *L. reuteri* CRL 1098: both acids (CA and TCA) were incorporated by active transport and reached the maximum accumulation at 15 min (about 3 mm/mg protein). However, a small fraction of the CA was also able to diffuse passively through the vesicular membranes, even in the absence of ATP. These observations could be explained by differences in the pKa of both acids. The unconjugated BA, CA, has a pKa value of 5.5, so a significant portion of these compounds exists in lipophilic, uncharged forms, and should be able to traverse the lipid bilayer domains of the outer membrane. On the contrary, TCA, with a pKa value of 1.5, is a strong acid and is completely decoupled at the pHs tested, and, therefore, cannot diffuse freely across membranes. To our knowledge, our results are the first evidence of an active efflux of BA in lactic acid bacteria.

3.7 Relationship between carbon and nitrogen metabolism in the marine photosynthetic picoeukaryote *Ostreococcus tauri*

[POSTER]

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Picoeukaryotes are a taxonomically diverse group of microorganisms with less than 2 micrometers in diameter. In this group photosynthetic picoeukaryotes (PPEs) are widely distributed from temperate to polar marine habitats, being significant contributors to primary production. PPEs, Prasinophytes are marine planktonic green algae with a phylogenetic position branching near the base of the green lineage that evolved after the endosymbiosis event that gave rise to photosynthetic eukaryotes. Marine PPEs of the genus *Ostreococcus* thrive in ecosystems ranging from oligotrophic to eutrophic environments and could be key organisms for biogeochemical fluxes of modern oceans during climate change. In the green lineage the most important soluble disaccharide is Sucrose (Suc), which mainly occurs in

oxygenic photosynthetic organisms, including land plants, unicellular algae and cyanobacteria. Trehalose (Tre) is another important non-reductant disaccharide distributed in nature, is accumulated mainly in bacteria, fungi, and arthropods, but not in land plants, were occurs in trace amounts only. In addition to its role as energy and carbon source, accumulation of Tre has been associated with improved stress tolerance towards both biotic and abiotic stress situations. Its role in the green lineage is still controversial. The aim of this study was to investigate which are the important soluble carbohydrates in *O. tauri* and its relationship with nitrogen assimilation. Preliminary experiments showed that Tre but not Suc is the main soluble sugar in this Prasinophyte. Tre content increases in *O. tauri* cells grown under nitrogen deficiency in a time-dependent manner. Total reducing sugars and starch levels were measured in parallel. An opposite pattern was observed with total sugars reducing content. On the other hand, starch content also showed a high increase under nitrogen deficiency. Reversion of that condition restored carbohydrates to basal levels. The enzyme activity responsible of Tre synthesis (TPS, Tre-Phosphate Synthase) and expression at the mRNA level paralleled the accumulation of the disaccharide. We conclude that Tre plays an important physiological role as principal soluble sugar interconnecting carbon and nitrogen metabolism in *O. tauri*.
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3.8 Regulation of Unsaturated Fatty Acid synthesis in *Mycobacterium*

[POSTER]

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Unsaturated fatty acids (UFAS) are present in membrane phospholipids, mycolic acids, lypoglycans and triglycerides of mycobacteria. Double bonds are also substrate for cyclopropanation or modification by methoxy and keto- groups, which are all present in mycolic acids. Despite the relevance that double bonds have in mycobacterial physiology, biosynthetic pathways and regulatory mechanisms controlling desaturase expression are extremely scarce. Here we employed thin-layer chromatography and gas chromatography-mass sprectrometry to compare levels of UFAs of *Mycobacterium smegmatis* grown at different temperatures. We found that palmitoleic and oleic acids contents increased at low temperatures. *M. smegmatis* genome holds at least 7 desaturases. We selected three genes homologous to *desA1*, *desA2* (both believed to be involved in mycolic acid biosynthesis) and *desA3* (encoding an stearoyl desaturase) genes of *M. tuberculosis* to perform regulatory tests. Promoters corresponding to *desA1* (M_SMEG5773), *desA2* (M_SMEG 5248) and *desA3* (M_SMEG1886) genes were cloned upstream of the *B- galactosidase* reporter gene. Transcription of these genes seems not to increase with lowering temperatures. To confirm this findings Northern blots were performed. Both results suggest that either other enzyme is involved in UFA increase at low temperature or that UFA increase is due to a post-transcriptional control of desaturase activity. Accordingly, a putative desaturase encoded by the gene M_SMEG1211 was considered for this role. When the *desA3-1211* promoter was cloned and analyzed it showed a temperature- regulated expression. We also analyzed if *desA3-1886* transcription was regulated by end-product by adding oleic acid to the growth medium, finding that its expression was repressed upon oleic acid addition. Interestingly, an inverted repeat was located in the promoter region upstream of the *desA3*-M_SMEG1886 gene, as well as upstream M_SMEG1885 gene encoding the associated oxido-reductase, and this pattern is conserved in *M. tuberculosis*. We are now studying the role of these putative regulatory sequences in the regulation of the expression of this pathway.

3.9 En revisión

3.10 ROLE OF IRON ON BIOFILM FORMATION OF WILD TYPE AND FUR MUTANTS OF *Stenotrophomonas maltophilia*

[ORAL]

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Stenotrophomonas maltophilia (Sm) is an important nosocomial pathogen in patients exposed to invasive devices. A key virulence factor is its capacity to form biofilms. Iron limitation induces or inhibits biofilm formation depending on the species. Iron-dependent gene regulation in many bacterial species is mediated by Fur. The aim of this work was to study the role of iron on biofilm formation and phenotypic traits associated to biofilm development (twitching motility and exopolysaccharides, EPS). Studies were done on Sm K279a which full genome was sequenced, and a local isolate recovered from a patient with device-associated bacteremia (Sm13). Fur mutants were isolated by selection for a manganese-resistant phenotype. Several clones showed constitutive siderophore production and F71 derived from Sm13 and F60 from K279a were selected for this study. Biofilm formation was evaluated in microtiter plates for 48 h. Results were expressed as levels of cristal violet (CV) staining relative to the final culture density to avoid differences in bacterial growth generated by the addition of the iron chelator 2,2′-dipyridyl (Dip). Wild-type (wt) strains were more efficient in producing biofilms in the presence of Dip than in TSB. On the other hand, Fur mutants when cultured in TSB showed enhanced biofilm production compared to wt strains, and the presence of Dip did not affect this production. Evaluated by light microscopy, biofilms formed in TSB by Sm13 and K279a presented monolayers with microcolonies, while in the presence of Dip there were zones with larger microcolonies which coalesce to form three-dimensional bacterial structures typical of biofilms. Biofilms formed by Fur mutants in the presence or the absence of Dip showed bigger microcolonies and improved adherence and organization. Furthermore initial adhesion (2 h incubation) of F71 was higher than that of Sm13, and again F71 produced bigger microcolonies. Surface-associated twitching motility was assayed via subagar stab inoculations, and twitching zones were visualized by CV staining. F60 produced increased twitching motility zones compared to K279a. EPS production was quantified by ethanol precipitation and dry weight determination. Results, expressed as µg of EPS per mg of biomass, showed that F60 and F71 produced higher amounts of EPS than wt strains. Furthermore, the presence of Dip improved EPS production in wt strains but not in Fur mutants. Results showed that iron starvation improved significantly the adherence of Sm, its twitching motility and the production of EPS, an important component of biofilm matrix. Furthermore, the Fur mutants obtained for the first time in Sm showed improved adherence, produced higher amounts of biofilms and EPS, and showed enhanced twitching motility than wt strains. Thus, iron is a signal for Sm biofilm formation which is partially mediated by the Fur system.

3.11 GDH ACTIVITY AND GLUCONIC ACID PRODUCTION BY *Burkholderia tropica*

[POSTER]

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Burkholderia tropica strains have been reported to posses the ability to solubilize insoluble phosphates. Mineral phosphate solubilization (MPS) activity is usually related with the expression of a periplasmic glucose dehydrogenase (GDH), responsible for the conversion of glucose into gluconate. In the present work it was checked the ability of *B. tropica* to express an active GDH under different culture conditions and their relation with MPS activity. Batch cultures were carried out with glucose as C-source and different phosphate and nitrogen sources. Cultures using tricalcium phosphate and under nitrogen fixation showed the highest GDH activity and gluconic acid production. Soluble phosphorous concentration in the media increased together with the gluconic acid. These results indicate that phosphate solubilization was due to acidification via GDH activity and it seems that this activity is regulated by phosphate starvation.

3.12 High phosphate concentration impairs formation and stability of biofilm in *Escherichia coli*

[POSTER]

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Biofilm is the prevailing microbial life-style in most natural environments and it often serves as a strategy to overcome stress. The amount and structure of biofilm could be modified by the culture conditions.
In several organisms, such as *Escherichia coli*, *Pseudomona aeruginosa*, *Vibrio cholerae*, *Klebsiella aerogenes*, *Salmonella enterica* serovar *typhimurium*, etc., inorganic polyphosphate (polyP) formation was shown to be critical for attributes such as motility, quorum sensing, biofilm formation, resistance to stress, and stationary-phase survival. PolyP is a chain of tens or many hundreds of phosphate residues linked by ‘‘high-energy’’ phosphoanhydride bonds. The main enzymes associated to polyP metabolism in *E. coli* are polyphosphate kinase (encoded by *ppk*) and exopolyphosphatase (*ppx*). We have previously shown that *E. coli* cells grown in media containing a critical phosphate concentration >37 mM maintained a high polyP level in stationary phase (up to 96 h) and enhanced the cellular fitness. Moreover, we have observed that the decrease of polyP level in low phosphate media was reversible when phosphate was added at 24 h of culture.
The aim of this work was to analyze the effect of phosphate concentrations on *E. coli* biofilm. The cells were grown in minimal medium containing different phosphate concentrations in polystyrene microtiter plates at 30ºC. Biofilms assay was carried out by the O’Toole and Kolter method. At 48 h of growth, the biofilm formation in media with 40 mM phosphate (MT+P) was lower in respect to that in media with sufficient phosphate concentration (2 mM, MT). Furthermore, the amount of biofilm determined at 48 h was also decreased by the addition at 24 h of 40 mM phosphate to MT growing cultures. When a *ppk ppx* mutant was analyzed in the same conditions, opposite results were obtained. The wild type biofilm formation was maximal at 10 mM phosphate and start diminishing at 15 mM, whereas for the mutant a significant biofilm formation was observed from 15 mM. In addition, we analyzed the production of curli, swarming and swimming motility as possible mechanisms related to the observed phenotypes. Similar results were obtained with an environmental isolate of *Pseudomonas aeruginosa*. According to our results, we assume that internal Pi concentration is an important signal for

formation and stability of biofilm. This Pi concentration is influenced by the maintenance of polyP levels in stationary phase.

3.13 PHYSIOLOGICAL, BIOCHEMICAL AND STRUCTURAL DAMAGES IN CELLS OF *PROPONIBACTERIUM FREUDENREICHII* SUBJECTED TO *IN VITRO* GASTROINTESTINAL DIGESTION

[POSTER]

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Dairy propionibacteria have a long history as starter cultures in the food industry, and today, they are also studied as probiotic microorganisms. The successful performance as probiotics depends on achieving the intestine metabolically active and in high number. They must recover in short time their activity after exposure to the aggressive conditions of the gastrointestinal tract (GIT). For their protection various strategies have been developed, however, to design appropriate methods is necessary to identify the stages of the digestive process responsible for major damage to these bacteria. The aims of the study were to evaluate how the aggressive digestion conditions affect physiological, biochemical and structurally a strain of *Propionibacterium freudenreichii*, often used in the manufacture of Emmental cheese. *Propionibacterium freudenreichii* CRL757 was cultivated in LAPT broth with 1% lactose (LAPTL) for 24 hours at 37 °C. A suspension of 10^8 bacteria in phosphate buffer saline (PBS) was subjected to artificial gastric (DG) and intestinal (DI) digestion protocols and to the sequence of both digestions (DGI). The cells subjected to treatments were washed and the percentage of cells injury or death was determined by staining with propidium iodide (PI) and counted by fluorescence microscopy. An aliquot was used as untreated control. The count was repeated after subjecting the samples to an additional stress of heat (100 °C, 0 to 120 min). The cell injury was represented as IP (+) cells vs. heating time and the decimal reduction time (D) in each case was calculated. In other aliquots of cells subjected to digestion, we determined changes in physical-chemical properties of the cell wall through their affinity to organic solvents. We also assessed the biochemical activity of B-galactosidase and physiological changes by studying the recovery of cells subjected to digestion and cultured in LAPTL. The in vitro digestion increased the number of injured or death cells in a log unit, but the differences between treatments were evident by detection of D (without digestion> DG> DGI). DG increased the affinity to chloroform and DI to hexane and chloroform. The B-galactosidase activity was significantly decreased after DI, but not DG. Cultures inoculated with treated cells showed increased lag phase and arrested their growth in less time and with lower biomass production. The effects were similar in the DG and DI treatment, but greater delay and lower biomass was obtained with DGI. Therefore, both stages of digestion caused reduction on cells number or changes in the permeability of propionibacteria and modified properties related to adherence to epithelial cells. The DI, but not the DG affected the biochemical activity. The sequence of the DGI caused major bacterial growth delay than DG or DI. *Supported by ANPCyT PICT 2168 and CIUNT D429*.

3.14 Malic enzyme is involved in triacylglycerol metabolism in *Rhodococcus*

[ORAL]

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Rhodococcus bacteria, which belong to the non-sporulating and mycolic acid-containing actinomycetes, are able to accumulate variable amounts of triacylglycerols (TAG) as main storage lipids. In a previous transcriptome study, the *ro11316* gene was significantly up-regulated during cultivation of *Rhodococcus jostii* RHA1 cells with benzoate under nitrogen-limiting conditions. This condition promotes triacylglycerols (TAG) accumulation by cells of *Rhodococcus* members. The *ro11316* gene, which occurs in the megaplasmid pRHL3 of *R. jostii* RHA1, encode for a putative malic enzyme. This enzyme may contribute with the NADPH pool generation necessary for TAG biosynthesis in *Rhodococcus* members. In order to confirm this hypothesis, we analyzed in the model oleaginous bacterium *Rhodococus opacus* PD630 the *in vivo* effect of different concentrations (0, 1.5, 5 and 10 mM) of sesamol, an inhibitor of malic enzyme activity. TLC and GC analyses of cells cultivated in N-limiting mineral media (MSM0.1) with gluconate as sole carbon source showed a proportional decrease in TAG cellular content in the presence of different inhibitor concentrations (60, 42.8, 33.4 and 15.0 % w/w of TAG, respectively). No changes in fatty acid profiles of TAG were observed after cultivation of cells with sesamol. In the other hand, the *ro11316* gene of *R. jostii* RHA1 was cloned and expressed under a strong acetamidase promoter in *R. opacus* PD630. An increase of approximately 15% (w/w) of TAG content was observed in recombinant cells in comparison with wild type cells during cultivation in MSM0.1 with glucose as sole carbon source. The biomass production by recombinant cells was also increased. The results suggested that the product of *ro011316* gene and probably their orthologs may be involved in NADPH generation for the de novo fatty acid and TAG biosynthesis in *Rhodococcus* members.

3.15 *IN VITRO* FERMENTATION OF SUGAR CANE MOLASSES BY CHICKS NORMAL CROP BIOTA. EFFECT ON *SALMONELLA* SPP SURVIVAL

[POSTER]

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Salmonella is an important cause of clinical infections in poultry that act as a contaminant source in humans. The beneficial metabolic activity of the crop biota on the bird's feed increases lactic acid and short-chain fatty acids (SCFA) concentrations that reduce the pH associated with a lower concentration of enteropathogens. Molasses is used by lactic bacteria as a carbon source for its readily fermentable sugars like glucose and sucrose. In this context, the ability of molasses to modify the crop microbial community and the survival of *S.* Gallinarum and *S.* Enteritidis in this ecosystem were evaluated. Additionally, sucrose (main molasses component) and lactose were studied and compared. An *in vitro* fermentation system for broiler chick crop homogenates in a basal fermentation medium was used with the addition of 1% (w/v) carbohydrates or without them (control) in microaerophilia for 6 h at 42 ºC. The pH was recorded at different times and, after 6 h incubation, the number of microorganisms was determined as well as lactic acid and SCFA concentration by HPLC. Survival rate of both *Salmonella* was evaluated by count in SSA medium. Molasses fermentation of the crop biota caused a sharp pH decrease 4 h later (4.7), significantly lower than that obtained for sucrose (5.8), lactose (7.4) and control (7.8). At 6 h, sucrose fermentation equalled the pH value obtained for molasses in the same period (4.4). Fermentation of the sugars led to an increase in total aerobes, enterococci and lactobacilli, while enterobacteria viability decreased 4 log units in presence of sucrose and molasses. The enterobacteria count did not show any significant

differences in lactose and control. This result agrees with the low pH decrease during lactose fermentation as compared with those obtained in sucrose and molasses. Molasses fermentation generated the highest lactic, acetic, and formic acid concentrations that explain the pH reduction and the enterobacterial inhibitor effect. Sucrose fermentation led to a significant amount of lactic acid which was detrimental for the enterobacterial population. Lactic acid and SCFA contents, lactose fermentation products, were significantly lower than those found with the rest of sugars studied, which agrees with the results shown before. The inhibitory effect on *S.* Gallinarum and *S.* Enteritidis became evident after 6 h of molasses fermentation (reduction of 3 and 2 log units respectively). The fermentation products of sugars in molasses produced an enterobacterial population reduction and an important inhibitory effect on both *Salmonella* viability. In conclusion, the addition of small concentrations of molasses to the birds' diet emerges as an interesting alternative to control enteropathogens like *Salmonella*. *Supported by CONICET PIP 0996 and CIUNT D429*.

3.16 EFECT OF DIFFERENT CULTURE MEDIA AND THEIR COMPONENTS ON THE GROWTH OF BIOFILM-FORMING VAGINAL LACTOBACILLI

[POSTER]

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The vaginal microbiota of healthy women is composed by different species of lactobacilli, which form a protective biofilm on the mucosa. These bacteria have a beneficial effect by inhibiting the adhesion, growth or spread of undesirable microorganisms. In urogenital infections, indigenous lactobacilli are replaced by pathogenic microorganisms, some of them form biofilms. Probiotic lactobacilli could displace this pathogenic biofilms and potentially reestablish those protective biofilms. Different human vaginal lactobacilli (VL) have been previously isolated, characterized and selected for their beneficial properties in our research group (autoaggregation, inhibition of uropathogens by organic acids, hydrogen peroxide and bacteriocins). Eventhough these microorganisms are very exigent nutritionally to grow, they were able to form biofilm in culture media without Tween 80 (a source of fatty acids) in "in vitro" assays. Therefore, the objective of this work was to know if different culture media exert some effect on the growth of VL with different biofilm formation characteristics. Three biofilm-forming strains (*Lactobacillus reuteri* CRL1324, *L. rhamnosus* CRL1332 and *L. delbrueckii* CRL1510) and one non-biofilm-forming strain (*L. gasseri* CRL1263) were evaluated. The growth of the microorganisms was studied in LAPTg and MRS broths, with and without Tween 80. Growth kinetics were evaluated through optical densities (OD) at 540 nm, number of viable cells and pH. The strains tested showed a different behaviour under the different culture conditions assayed. *L. rhamnosus* CRL1332 was able to grow in the media with and without Tween (MRS and MRS without Tween OD=1.5; LAPTg and LAPTg without Tween OD=1). The growth of *L. rhamnosus* CRL1332 and *L. reuteri* CRL1324 was higher in MRS (OD=1.4-1.5) than in LAPTg (OD=1-1.1). *L. reuteri* CRL1324 growth was higher in complete media than in MRS and LAPTg without Tween (OD=1 and 0.75 respectively). *L. delbrueckii* CRL1510 growth was higher in standard media (OD=1.4) than in media without Tween (OD=0.82-0.88). *L. gasseri* CRL1263 growth was slightly higher in MRS (OD=1.4) than in LAPTg (OD=1.3), but did not grow in the media without Tween (OD=0.2). The pH decrease according to the growth in all the experiments (3.9-5.7).The different culture media assayed, used routinely for the growth of lactic acid bacteria, affect the growth kinetics of the evaluated microorganisms, showing a strain-specific response. Only the three strains previously selected for their ability to form biofilm in culture media without Tween 80, were able to grow under these conditions. However, the presence of Tween 80 in the culture media was essential for the growth of *L. gasseri* CRL1263. Further studies are being performed to determine the

effect of the individual components of the culture media on both, the growth and biofilm formation of these microorganisms.

3.17 A new stationary phase regulation of *ndh* gene expression dependent on glucose concentration in *Escherichia coli*

[POSTER]

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In *Escherichia coli* the main reducing equivalent synthesized by the cell central metabolism is NADH. This reduced dinucleotide is oxidized by the respiratory chain NADH dehydrogenases for energy production and as a potential source of NAD^+, the main cellular oxidant. The oxidation of one molecule of NADH by NADH dehydrogenase-2 (NDH-2) yields smaller amounts of ATP compared with that performed by NDH-1. Thus, NDH-2 activity is important when the [NADH]/[NAD^+] ratio is very high, precisely because it increases the flux of substrate.
E coli NDH-2 is encoded by *ndh* gene, which is highly regulated by global transcription factors. It has been described that this gene is expressed in exponential growth phase and repressed in late stationary phase. However, we have reported an unusual NDH-2 activity and *ndh* expression in the stationary phase when cells were grown in media containing at least 37 mM phosphate. In addition, the cells presented higher oxygen consumption rates, were more viable and had a lower NADH/NAD^+ ratio than cells grown in sufficient phosphate media. For those previous studies, cells were grown aerobically in minimal media (MT or MT+P containing 2 mM or 40 mM phosphate, respectively) supplemented with 28 mM glucose or 54 mM glycerol. The *ndh* expression in MT supplemented with 5 mM glucose is maintained in stationary phase as in MT+P. Here, the *ndh* expression was tested in MT under different glucose concentrations, 5 mM and 40 mM. In each culture condition, the cellular metabolism has been also determined measuring the oxygen consumption rate, the viability and the supernatant pH changes. In medium supplemented with low glucose concentration (5 mM), the cells grew slowly (DO=1 at 48 h). At 48 h of growth, they presented low oxygen consumption rate and high viability and maintained the pH at 7.5. By contrast, in MT medium supplemented with high glucose concentration (40 mM) where the *ndh* expression was negligible, a notable decrease in pH and viability were observed. The difference between both media may be due to an oxygen consumption rate. In order to investigate if glucose-dependent *ndh* expression, in stationary phase, was mediated by a known global transcription factor, the beta-galactosidase activity was assayed using i) chromosomal fusions of the complete promoter (-250/+165) or its deletions (-205/+165 or -105/+165), and ii) deficient strains in FNR, IHF, ArcA, PdhR and Fis regulators. Neither the deleted promoter region nor tested regulators could be identified as responsible for glucose effect. Thus, another factor that was not tested would be responsible for the absence of *ndh* expression and the metabolic effects observed. Additionally, the *ndh* expression in late stationary phase regulated at transcriptional level by diverse external stimuli is promising to postulate a not yet described NDH-2 role in this phase.

3.18 Characterization of *Burkholderia cepacia* complex strains isolated from a cystic fibrosis patient.

[POSTER]

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The *Burkholderia cepacia* complex (Bcc) is a group of closely related bacteria that are opportunistic pathogens for cystic fibrosis (CF) patients and inmunocompromised individuals. Infection of CF patient with Bcc members often has important negative consequences over patient's health. Bcc members are resistant to a great number of antimicrobials, so antibiotic therapy not always result effective.
When Bcc is detected in CF patient sputum, one strain usually is isolated and the antibiotic resistance profile of this strain is studied. We observed Bcc strains collected from the same chronic infected CF patient that belong to the same Bcc species but have not the same antibiotic resistance. This fact is well studied in infections with *Pseudomonas aeruginosa* but there are no data about Bcc species. The aim of this work was to characterize Bcc strains isolated from the same sputum sample of a CF chronic patient.
Two colonies with different morphology grown on *Burkholderia cepacia* selective agar (BCSA), were selected for this study. Both were identified as *Burkholderia contaminans* using amplification and sequence analysis. One of it grown as big colonies, produced biofilm, proteases but not lipases and exhibited important swarming motility. The other strain grown as very small colonies produced proteases but not lipases nor biofilms and exhibited few swarming motility. The antibiotic resistance profile of both strains was also different.
These results suggest that CF patients could be infected by strains belonging to the same Bcc species but with different characteristics. This fact would add complexity to CF treatments.

3.19 Analysis of mutants that are affected in phosphate solubilisation activity.

[POSTER]

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Phosphorus is a limiting factor for plant growth. Low levels are due to the fact that soluble soil phosphorus reacts with ions that cause the precipitation, reducing their availability to plants. Many soil microorganisms are involved in processes that affect the transformation of phosphorus from soil being an integral part of its cycle. The main mechanism by which microbial phosphate compounds are mobilized is the release of organic acids of low molecular weight with a significant decrease of pH values. Understanding the basic mechanisms related to the solubilization of these compounds will allow the application of microorganisms with an effective and high capacity to increase the available levels of phosphorus for plants. Objective: To identify genes involved in phosphate solubilising mechanism. Getting transpositional insertion mutants: The plasmid pUTminiTn5Km was transferred to the strain *Serratia* sp S119 by triparental conjugation using *Escherichia coli* CC118 λ pir (donor) and *E. coli* DH5α (helper) strains. Selection of mutants: in NBRIP-BPB containing tricalcium phosphate supplemented with appropriate antibiotics and determination of phosphate solubilization halo in NBRIP-BPB. Isolation of genomic DNA. ERIC-fingerprints. MiniTn5 detection by PCR. Viability of mutants in NBRIP broth determining CFU/ml. Quantification of soluble phosphorus released in NBRIP containing glucose or glycerol as carbon source and analysis of pH from 2 to 48 h of growth. There were selected 3 colonies (4A, 5A and 6A) that showed a significant reduction in their phosphate solubilization halo on NBRIP plates. These strains showed identical ERIC profiles being thus isogenic mutants of *Serratia* sp S119. In all of them it was possible to confirm the

presence of the insert. Viability of mutants 4A and 5A was similar to that of wt while mutant 6A showed a significant decrease in CFU/ml. The mutant 4A released significantly less amounts of soluble phosphorus compared to the wt after 8 and 10 h of growth, while in the mutant 5A this diminution was only observed after 8 h of growth. The quantity of soluble phosphorus released was negatively correlated with pH values of the medium for all strains. The presence of a non-fermentative carbon source such as glycerol significantly decreased the solubilisation of phosphate in both the wt and the mutant 4A strains after 8 and 10 h of growth. The pH values of the supernatants from wt or mutant 4A growing in presence of glycerol also differed respect to values obtained when glucose was used, showing a statistically significant increase at 8, 10 and 12 h of growth for the former whereas in the mutant 4A, difference was found only at 10 h. The results obtained suggest that the mutant 4A is affected in the phosphate solubilisation mechanism. Further studies are needed to characterize the affected gene and its role in this mechanism.

3.20 EFFECT OF ORGANIC ACIDS, GRAPE JUICE AND pH ON GROWTH AND AROMA COMPOUNDS PRODUCTION IN *Oenococcus oeni* FROM WINES

[POSTER]

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Lactic acid bacteria (LAB), preferably *Oenococcus oeni*, play an important role in winemaking by undertaking the malolactic fermentation (MLF), which improves the quality wine. Diacetyl produced during MLF at low concentration and in combinations with other compounds can impart yeasty, nutty, toasty aroma to wine. Nevertheless little information is available on this physiological aspect in LAB wine. So, the aim of this work was to investigate the influence of pH, organic acids and grape juice on growth and diacetyl, acetoin and 2,3 butilenglicol production in *O. oeni* strains from Argentinian wines. The microorganisms were grown in MRS broth with 15% tomato juice, pH 4.8 (control) and added individually or combined with (g/l): L - malate 2; citrate 0.7 and 10% grape juice in place of tomato juice. The pH 3.8 effect was also investigated.
In control medium the growth extent varied according to analyzed strain and it was related with the diminution of pH. The strains grew to final biomass of among $4.90x10^7$ and $3.09x10^8$ cfu/ml, while at pH 3.8 the final biomass ranged of among $1.10x10^7$ and $5.75x10^7$ cfu/ml.
Addition of organic acids and grape juice to control, in general, increased significantly growth, being L-malic and citric acids almost completely consumed. At pH 3.8 this stimulatory effect was also observed by approximately 50%. In control the production of diacetyl, acetoin and 2,3-butanediol by *O. oeni* strains ranged of among 0.2 and 7 mg/l. When pH was adjusted at 3.8 the MS21 and MS27 strains showed the highest aroma compounds production (5.1 ± 0.32 and 4.91 ± 0.53 mg/l) for 6 and 5 days, respectively. In the other strains diacetyl, acetoin and 2,3 butilenglicol formation was also detected but in concentrations lower than 4 mg/l. In presence of both organic acids and grape juice, pH 4.8, all strains except MS52, produced lower than 5 mg/l of the aroma compounds. In general, at pH 3.8 it decreased detecting values lesser than 3.65 mg/l in the MS21, MS27, MS29 and MS49 strains. In conclusion, although in general, a decrease in cell growth and aroma compounds was observed at pH 3.8 as regards to those obtained at pH 4.8, an interesting finding was that the majority of *O. oeni* strains were able to grow and to produce the aroma compounds in concentration lower than 4 mg/l at the lowest pH. So they would have adequate characteristics for potential use in winemaking. However, the variability of this production among strains makes convenient to considerer this as a point of strain selection to obtain starter which will give better sensory qualities to wine.

3.21 Optimization of the growth of lactic acid bacteria in different concentrations of pectin extracted from lemon peel.

Se sugiere presentación POSTER

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Probiotics are microorganisms (e.g., bifidobacteria and lactobacilli) that can reach the end of the digestive tract remaining viable; they have positive effects on consumer health, whether human or animals.
Pectin is a polysaccharide composed of galacturonic acid monomer units with different degrees of esterification and neutralization. Its applications include elaboration of jams, jellies, candies, essential oils, mayonnaise and cosmetics, among others. It is a protector and regulator of the gastrointestinal system, used in the treatment of hypercholesterolemic individuals.
The aim of this study was to evaluate the growth of probiotic lactic acid bacteria in a culture medium supplemented with different concentrations of pectin.
Lactic bacteria studied, *Enterococcus faecium* (strain RRC 14) and *Enterococcus faecium* (strain RRC 38), were provided by the Department of Public Health, School of Biochemistry, Chemistry and Pharmacy of the UNT. Bacteria were grown in LAPTG liquid culture medium, containing yeast extract (1.0 g), peptone (1.5 g), tryptone (1.0 g), glucose (1.0 g), distilled water (100 ml), pH = 7. They were incubated 24 h at 37 °C. After that, serial dilutions were performed and 0.5 ml were inoculated in liquid LAPTG medium supplemented with different pectin concentrations (1, 0.1, 0.5%). They were incubated at 37 ºC for 24 h and aliquots were extracted at different times (from 0 to 24 h) to measure the OD640 in spectrophotometer. At the same time, aliquots taken at different times were plated on solid LAPTG medium, supplemented with different pectin concentrations, as before. Plates were incubated 72 h at 37 ºC for viable cell count.
The results showed good growth in pectin concentrations of 0.5 and 0.1%, with the advantage to the latter concentration where the lag phase was reduced by 75% for strain RRC38 and 50% for strain RRC14. The viable counting when using pectin 0.1% was 1.03 x 10^{10} CFU/ml for strain RRC14, and 0.74 x 10^{11} CFU/ml for strain RRC 38. And when using pectin 0.5%: 0.82 x 10^{10} CFU/ml for strain RRC14 and 0.80 x 10^{11} CFU/ml for strain RRC38). CFU / ml and 38 respectively RRC14 . Values were lower when using pectin 1% in the grown medium. These results show the ability of the lactic bacteria assessed to grow at low concentrations of lemon´s pectin with an adequate number of viable cells in a short period of incubation, giving the possibility for using them in the formulation of a symbiotic diet.

3.22 Sucrose hydrolysis regulation in the filamentous cyanobacteria *Nostoc* sp. PCC7120 under nitrogen deprivation

[ORAL]

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Sucrose (Suc), a key sugar in plant life, is also present in cyanobacteria, oxygen-evolving photosynthetic bacteria. They were shown to present proteins for Suc synthesis and degradation similar to plant's enzymes. Particularly, the hydrolysis of Suc is performed by Alkaline/Neutral Invertases (A/N-Invs). Two A/N-Inv isoforms were biochemically and functionally characterized in *Nostoc* sp. PCC 7120, a filamentous nitrogen-fixing strain, and their

encoding genes (*invA* and *invB*) were identified and functionally characterized. Whereas both genes are expressed simultaneously in vegetative cells, only *invB* is expressed in heterocysts during diazotrophic growth and mutants lacking of *invB* were not able to grow under this condition, concluding that Suc metabolism plays an important role in relation to nitrogen fixation. On the other hand, previous results showed that NtcA, a global nitrogen regulator that is present exclusively in cyanobacteria, may be also regulating the expression of other Suc metabolism genes. NtcA (encoded by *ntcA*) belongs to the Crp-FNR family of bacterial transcriptional regulators. The aim of this study was to investigate the role of NtcA in the regulation of Suc hydrolysis in the filaments of *Nostoc* sp. PCC 7120. Levels of soluble sugars and polysaccharides were analyzed after 24 h of combined-nitrogen step-down in an NtcA deficient mutant (*CSE2*, a derivative mutant strain from *Nostoc* sp. PCC 7120 that bears an inactivated version of the *ntcA* gene). Besides expression at the activity and transcript level of A/N-Invs were investigated, showing to be differentially expressed in the *CSE2* mutant. Besides, the presence of putative NtcA-binding regions in the promoter region of *invB* suggested a possible physical interaction with the global regulator. Taken together, our results show that Suc hydrolysis through A/N-Invs is linked to nitrogen metabolism through the transcription factor NtcA.

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Keywords Cyanobacteria, Sucrose, nitrogen metabolism regulator

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3.23 S-layer protein protection to osmotic stress in *Lactobacillus acidophilus*

[POSTER]

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The Surface layer (S-layer) proteins are arrays of a single protein that constitute the outermost cell envelope in several Bacteria and Archaea. They have been considered to function as protective coats, maintenance of cell shape, be involved in adhesion and recently showed an antibacterial endopeptidase activity. Although many studies focus on the structure of the S-layer, their biological functions remains poorly understood.
Lactobacillus acidophilus is one of the major species found in human intestines and some strains are believed to have probiotic characteristics. Probiotics are live microorganisms, usually contained in food, traditionally regarded as safe for human consume that, when ingested in sufficient number, play an important role in control of the host intestinal microbiota and in the modulation of the host immune response.
Lactobacillus acidophilus possess an S-layer protein of approximately 50 KDa and a pI of 10.4 showing them as highly basic. This microorganism is extremely used in food industry, during which they suffer different stresses. This is why these bacteria have adopted mechanisms that enable them to cope with the challenges of changing environmental conditions, such as osmotic, acidic and other kinds of stress.
In this study we proved the S-layer protein is essential in the adaptation of the *Lactobacillus* during several kinds of stresses. In particular we analyzed the role of the S-layer by exposing cells to high salt conditions. We found an over expression of both the protein and the mRNA, in hiperosmotic condition that were demonstrate by Western blot and quantitative real time PCR. We also tested the growth aptitude of *Lactobacillus acidophilus* in high osmotic conditions. Cells with S-layer protein and cells treated with LiCl 6M, that remove completely the S-layer from the cell wall, were grown in different NaCl concentrations, their growth were follow by optical density and viable cell count. The differences in the both growth curves show the importance of the S-

layer protein in the adaptation to this kind of stress. We are currently studying the importance of this protein for other stress adaptations.

3.24 Effect of the addition of cysteine and phenylalanine on growth and hydrogen sulfide production in lactic acid bacteria isolated from orange

[POSTER]

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Lactic acid bacteria (LAB) are fastidious microorganisms towards nutritional. Furthermore, they have (in most cases) limited biosynthetic ability, requiring an exogenous source of amino acids or peptides for optimum growth. In a previous study we isolated and identified LAB from orange juice. Based on their capacity to produce hydrogen sulfide from cysteine added to orange juice strains of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* subsp. *mesenteroides* were selected. The aim of this study was to evaluate the effect of cysteine and/or phenylalanine on the growth of *Lact. plantarum* JNB21 y JNB25 and *Leuconostoc mesenteroides* subsp. *mesenteroides* JNB9 in a modified MRS medium, pH 6.5 (MRSM), from which the main sources of nitrogen were omitted. At the same time the hydrogen sulfide production was evaluated from cysteine catabolism.
Cysteine and/or phenylalanine were added to MRSM at 0.2 and 1g/l. Bacterial cultures were inoculated at a rate of 2%, and incubated at 30°C for 24 h. Growth was determined by counting cfu/ml at 30ºC. Hydrogen sulfide was determinated qualitatively under experimental conditions. In MRSM *Lact. plantarum* JNB21 y JNB25 multiplied in a significantly more extent than *Leuc. mesenteroides* strain, with a growth rate of 0.41 and 1.1 h^{-1} and final biomass of 8.33 and 10.04 log cfu/ml, respectively. Addition of 0.2 g/l of Cys or Phe stimulated *Lact. plantarum* JNB21 growth. By contrast, Cys and Phe in concentration of 1 g/l decreased its growth rate of about 19.5 %. For *Lact. plantarum* JNB25 strain the amino acids added to deficient MRSM medium retarded growth under all assayed conditions, reaching in presence of 1 g/l of Cys, the lowest final biomass (9.68 log cfu/ml) and growth rate (0.5 h^{-1}). Independently of growth conditions a terminal pH drop occurred. Only when Cys was included in the MRSM medium hydrogen sulfide was produced in all cultures, indicating that Cys was degraded.
Considering the growth responses under deficient nutritional condition, we infer that *Leuconostoc mesenteroides* subsp. *mesenteroides* strain could have higher nutritional requirements than *Lact. plantarum* strains. However, the addition of amino acids tested did not modify its behaviour suggesting that Cys and Phe would not be essentials for growth or their concentrations in the natural medium would be enough for development.
At low concentration Cys or Phe increased *Lact. plantarum* growth parameters, but at the highest one they inhibited growth possibly related to a controlling effect of biosynthetic pathways. These results suggest that the *Lact. plantarum* strains could show the highest possibility to growth in the natural medium orange juice without producing hydrogen sulfide.

3.25 Role of ppGpp in the protective action of previous sublethal UVA exposure against lethal doses in *Pseudomonas aeruginosa*

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P. aeruginosa is a versatile soil and aquatic microorganism whose high genome complexity explains the great adaptation to a wide range of environments with different stress factors, including solar UVA radiation. We reported that high doses of UVA lead to lethal effects mainly through oxidative stress in *P. aeruginosa*. When this bacterium is exposed to low UVA fluences, a growth delay is induced. This phenomenon was proposed as an adaptative mechanism related to bacterial protection against lethal and mutagenic effects of solar irradiation. Here we report the study of the ability of the pre-exposure to low UVA doses to protect *P. aeruginosa* of subsequent lethal doses exposure, and the mechanism involved in this phenomenon was evaluated.
Cell suspensions of the PAO1 strain were exposed to sublethal UVA dose (20 min at 28 W/m^2) while control cells were maintained in dark. No significant cell death was observed, however a radioinduced oxidative stress was detected by ultraweak chemiluminescence. Pre-irradiated and control bacterial suspensions were then transferred to LB medium, and a growth delay was observed in the irradiated sample. After growth, pre-irradiated and control cells were irradiated for 180 min at 28 W/m^2 and a survival increase of the pre-irradiated cells was observed, suggesting a protective mechanism.
Given the involvement of the quorum sensing (QS) mechanism in the response of *P. aeruginosa* to UVA exposure, we investigated the participation of QS on the protective action of the UVA pre-exposure. The level of the main QS signals, 4C-HSL and 3OC12-HSL, was monitored in supernatants of PAO1 previously irradiated or control cells, and no differences were detected. We also tested if UVA has a protective role in the PAO1 derivative PAO-JP2 strain, defective for the synthesis of both signals. Despite its high sensitivity to UVA, PAO-JP2 was protected by the pre-treatment, confirming that QS is not involved in this phenomenon.
The role of ppGpp (the main effector of the stringent response) in this adaptative mechanism was also investigated. When a *relA* derivative of PAO1 strain, defective for the main synthetase of ppGpp, was pre-exposed to low UVA doses, non increase in cell survival to lethal UVA exposure was observed, suggesting a protective role for ppGpp. This finding was confirmed by induction of the stringent response with serine hydroxamate, an analogue employed to initiate the RelA dependent accumulation of ppGpp. PAO1 and *relA* strains were treated with this compound before exposure to lethal UVA doses, and a restoration of cell viability was observed only in the wt.
It is concluded that sublethal exposure to UVA functions as a protective mechanism against subsequent lethal doses in *P. aeruginosa* and ppGpp effector plays a fundamental role, possibly inducing the transcription of genes involved in protective functions against the toxic effects of UVA irradiation.

3.26 ANALYSIS OF *SINORHIZOBIUM MELILOTI* NATIVE ISOLATES FROM ARGENTINA REVEALED A POSITIVE CORRELATION BETWEEN BIOFILM FORMATION AND AUTOAGGREGATION.

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Sinorhizobium meliloti is an alphaproteobacteria included in a group commonly known as rhizobia, with the potential to establish a symbiotic chronic infection inside plant-derived organs

known as root nodules. Besides their symbiotic way of life, rhizobia can live not only in a planktonic state, but also in a biofilm state. The *expR* regulator gene, in conjunction with a quorum sensing signal molecule, controls the production of the symbiotically active galactoglucan (EPS II), which is critical for biofilm formation on plastic surfaces and planktonic autoaggregation. Wild type *S. meliloti* reference strains carrying non-functional *expR* loci (and thus, unable to synthesize EPS II) fail to autoaggregate and develop a relatively small biomass adhered to plastic surfaces. In an attempt to characterize indigenous, undomesticated *S. meliloti* strains, we isolated bacteria from alfalfa root nodules growing in fields without previous known inoculation procedures. All isolates developed mucous colonies (indicating EPS II production) and after inoculation, they were able to elicit root nodules formation on alfalfa. *16S rRNA* sequencing revealed a high homology with *S. meliloti* strains. Unlike some reference wild-type strains in which the *expR* locus is interrupted with an insertion sequence, these native isolates showed a normal-sized *expR* locus, as revealed by PCR analysis. Planktonic autoaggregation percentages and biofilm formation indexes were obtained from each isolate. Interestingly, we found a positive correlation between both variables. In order to confirm the participation of EPS II in intercellular interactions for native isolates, the *expA*::Tn5 mutant allele (blocking EPS II synthesis) was mobilized to three highly autoaggregative native strains, and the resulting transductant strains showed a dry colony phenotype, reduced biofilm formation on plastic, and a poorly autoaggregative phenotype. Taken together, these observations indicate that the strength/stability of cell-cell interactions in the context of planktonic and biofilm cells are quantitatively related. In the particular case of highly autoaggregative and biofilm-proficient native bacteria, mutant analysis confirmed that EPS II plays a critical role in cell-cell interactions in both, sessile and planktonic cells.

3.27 ESTEROLYTIC ACTIVITIES OF INDIGENOUS LACTIC ACID BACTERIA ISOLATED FROM GOAT DAIRY PRODUCTS

[POSTER]

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During cheese ripening, several flavor compounds arise from carbohydrate, citrate, protein and lipid metabolism. Lactic acid bacteria (LAB), the predominant microflora of many cheeses, have enzymatic systems (esterases and lipases) capable of hydrolyzing lipids. Esterases hydrolyze carboxyl ester linkages of water-soluble substrates, such as short-chain fatty acid (SCFA) esters and triglycerides composed of SCFAs. These enzymes contribute to flavor compound generation in dairy products by catalyzing not only the release of SCFAs, but also the synthesis of esters. These metabolites are responsible for piquant (SCFAs) and fruity (esters) flavors in goat's milk cheeses. The aim of this study was to evaluate the esterolytic activities of ten LAB strains isolated from artisanal goat dairy products from Santiago del Estero. Esterolytic activity was determined in cell free extract (CFE) of *Lactobacillus plantarum* (UNSE66, UNSE230, UNSE287), *L. bulgaricus* (UNSE308, UNSE309), *Pediococcus* sp. (UNSE22, UNSE216, UNSE 253) and *Streptococcus thermophilus* (UNSE314, UNSE315) strains, using (α and β-naphthyl derivatives of fatty acids (α-, β-NA) of 2-12 carbon atoms (C2-12) as substrates. A unit of esterolytic activity (U) was defined as the amount of enzyme that released 1 µmol of α- or β-naphthol per minute. Specific esterolytic activity was defined as units per milligram of protein. Results showed the presence of esterolytic activity in the CFE of all the strains assayed on α- and β-NA of C2-C8, and α-NA of C10. *Streptococcus thermophilus* UNSE315 was the only strain displaying activity on α-NA C12. The highest activities were observed in lactobacilli, mean activities on α-NA C2 being 1.92 ± 1.10 U/mg and 1.19 ± 0.39 U/mg in *L. plantarum* and *L. bulgaricus* strains, respectively. Lower activities were observed on longer-chain substrates, a 10-fold lower activity (0.13 ± 0.10 U/mg) on α-NA C8 being detected in *L. bulgaricus*. In general,

pediococci showed 2-fold higher activities compared to streptococci (0.43 ± 0.17 U/mg and 0.23 ± 0.02 U/mg on α-NA C2, respectively). The evaluated strains preferentially release fatty acids from 2 to 8 carbon atoms. These fatty acids, such as butyric and caprylic are responsible for piquant flavors in cheese. Therefore, the use of these strains could improve the organoleptic characteristics of goat's milk cheeses.

3.28 Antioxidant and antibacterial properties of phenolic compounds of fruit juices from northwestern Argentina

Aclarar abreviaturas. Citas de procedimiento entre paréntesis

[POSTER]

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In the recent years, nutrition specialists agree that fruits and their transformation products, contribute to reducing risks of certain diseases, including cardio and vascular diseases as well as to posses the ability to act as antioxidant and antibacterial agents. The aim of this study was to investigate the phenolic content of fruit juices consumed in northwestern Argentina, and correlate with their antioxidant and antibacterial properties. The fruit varieties used were, three varieties of lemon: *lisboa*, *eureka* and *genova*; one mandarin variety: *murcott*; three orange varieties: *tangerinas*, *valencia late* and *criollas jaffa*; two strawberry varieties: *camarosa* and *albion*. Total phenolic determination was based on the procedure of Singleton and Rossi. The ascorbic acid content was measured by HPLC. The antioxidant activity was carried out by FRAP, DPPH and ABTS assays. The bacterial strains used as test organism were *Escherichia coli* ATCC 25922 and *Listeria inocua 7*. Percentage of viable, injured and dead bacteria was obtained by flow cytometric analysis. Strawberry juices present the highest amount of total phenolic compounds and flavonoid fraction and the lowest value was found in *genova* lemon juice. The clarification process with activate carbon was effective to remove phenolic compounds. The highest concentration of ascorbic acid was found in lemon varieties. *Camarosa* and *albion* strawberry juices posses the highest ferric reducing power, whereas, *lisboa* and *genova* lemon posses the lowest. All juices posses a DPPH free radical scavenging activity higher than 50% and contain an important scavenging activity of ABTS radical (> 67%). With clarified juices, the antioxidant activity decreased around 80% and 98%. The high correlation between antioxidant capacities and total phenolic concentration indicate that phenolic compounds are the major contributors of these capacities. *Lisboa* lemon juice produces a cellular damage of *E. coli* and the death of around 100% of *L. innocua* cells. Clarified juice produces similar effect, being the antibacterial effect probably related to ascorbic acid. With both orange juice varieties, *E. coli* and *L. innocua* showed the major cells killed or damage. *Albion* strawberry produces the death of *E. coli* and *L. innocua* cells. The antibacterial effect was lower with its respective clarified juices. The antimicrobial properties of the fruit juices are related with phenolic compounds concentration but also with its profile.

3.29 Effects of a double mutation *sbmA tolC* on the physiology of *Escherichia coli*

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E. coli inner-membrane protein SbmA transports the peptide antibiotics microcin B17, J25 and bleomycin to the cell cytoplasm. Homologs of *sbmA* have been found in a wide variety of bacteria; however, the natural function of SbmA remains unknown. The outer membrane-protein TolC is an essential component of several efflux pumps in *E coli*. We have previously demonstrated that an *E. coli* K-12 double mutant *sbmA tolC* carrying a Tn10 transposon, which should confer high resistance to tetracycline, is hypersusceptible to that antibiotic. In addition, previous studies on the role of SbmA in microcin J25 entry led us to the fortuitous finding that a *sbmA tolC* double mutant rapidly loses viability four to five days after entry in stationary phase. Moreover, the combined loss of SbmA and the outer membrane protein TolC results in a heat-sensitive colony formation phenotype. The double mutant is impaired in its ability to form colonies when plated in LB medium at temperatures above 37ºC. Strikingly, this thermal growth defect was only observed in solid medium, since when cultivated in liquid LB the double mutant showed a wild-type growth profile in the temperature range between 37-42°C. In the present study we have discovered that these phenotypes are reversed by a diffusible factor present in the stationary-phase supernatants from liquid cultures of laboratory strains of *E. coli* K-12. The extracellular factor, designated VSPF (for “Viability Stationary Phase Factor”) has not been described previously. It was purified by reverse phase HPLC, and its molecular mass was determined, but its chemical nature remains unknown. Interestingly, VSPF is produced by both wild-type and the double mutant *E. coli* strains.
Although the exact mechanism underlying the temperature-dependent phenotype of *sbmA tolC* mutants remains to be clarified, this is to our knowledge the first description of a requirement of SbmA for growth at high temperatures, and it should provide a groundwork for understanding the physiological role of this highly conserved and enigmatic protein. Our results are particularly interesting with regard to the processes which enable cells to switch from growth in liquid to growth on agar surfaces, and may also open the way to a better understanding of the physiology of colony formation.

Sección 4 - Microbiología Molecular

4.1 STABILITY OF PLASMID pRC18 MEDIATED BY A TOXIN-ANTITOXIN SYSTEM

[POSTER]

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A plasmid-borne toxin-antitoxin (TA) system is composed of a stable toxin protein and an unstable antitoxin component and helps in plasmid maintenance by killing of plasmid-free segregants. The lactic acid bacterium *Lactobacillus curvatus* CRL 705 harbors plasmid pRC18, which is associated with the production of lactocin Lac705, a two-component bacteriocin. Recent sequencing studies describe the presence of a putative TA module (partitioning locus) on plasmid pRC18, which is organized in a single operon that produces a 92-amino-acids antitoxin (orf4) and a 118-amino-acids toxin (orf3); these proteins belong, respectively, to the superfamilies PhdYeFM and COG3041. Plasmid curing experiments showed that plasmid pRC18 was relatively stable: only 4% of CRL705 cells were plasmid free after cell growth at 39 ºC. In contrast, 68% of CRL1597 cells, a CRL705-derivative harboring plasmid p3.3, a plasmid similar to pRC18 but that does not has the TA system, has lost their plasmid after similar growth conditions. These results suggest that plasmid maintenance functions of the putative TA locus on pRC18 may explain the remarkable stability of this plasmid. However, the TA system of

pRC18 seemed to be not functional in *Escherichia coli* since plasmids pBlueScript SKII$^+$ and pJR, a pBlueScript SKII$^+$. Derivative which contains the pRC18-TA functions cloned in its *EcoRI* site, showed the same stability in *E. coli* DH5α cells. The minimal region of the TA system involved in plasmid pRC18 stability is currently evaluated in *Lactobacillus curvatus* AR3, a plasmid-cured derivative of CRL705.

4.2. Distinctive properties of ADP-glucose pyrophosphorylases from Gram-positive bacteria

[ORAL]

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Glycogen or similar α-1,4-polyglucans were reported in more than 50 different bacterial species. This is not restricted to any class of bacteria, as many Gram-negative and Gram-positive bacteria as well as archaebacteria have been reported to accumulate the polysaccharide. The process occurs through ADP glucose (ADPGlc) as glucosyl donor for elongation of the polyglucan. ADPGlc is produced via ADPGlc pyrophosphorylase (ADPGlcPPase, EC 2.7.7.27) in a key regulatory step of the biosynthetic pathway. ADPGlcPPase has been purified from a number of microorganisms, and as a rule is was found homotetrameric in structure. The one exception is the enzyme from *Bacillus* sp., as in *B. stearothermophilus* it is also a tetramer, but composed of two subunits: GlgC (active) and GlgD. Also, ADPGlcPPases from bacteria are allosterically regulated enzymes. In general, ADPGlcPPase effectors are main metabolites of the major pathway for carbon assimilation in the respective organism. Studies of enzymes involved in glycogen metabolism in Gram positive bacteria are scarce, which represents an important deficit for the understanding of carbon metabolism in these organisms. We characterized the kinetic, regulatory and structural properties of ADPGlcPPases from *Mycobacterium tuberculosis* (*Mtu*), *Streptomyces coelicolor* (*Sco*) and *Streptococcus mutans* (*Smu*). *Mtu* and *Sco* ADPGlcPPases share 60% identity at the protein level and both were activated by PEP and Glc6P, the latter being a novel activator for bacterial ADPGlcPPases. In contrast, we found differences concerning affinity for substrates and enzyme activity, as well as sensitivity to allosteric effectors. Particularly, the *Sco* enzyme is 20-fold less active than the *Mtu* counterpart, but the former has 5-fold more affinity for substrates and exhibits more promiscuity to effectors, being also inhibited by NADPH and Pi. Concerning the *Smu* ADPGlcPPase, it is composed by two subunits: GlgC and GlgD [this being a characteristic of the enzyme from firmicutes], and we determined that GlgC, but not GlgD, is the catalytic one. Also, the heteromeric conformation GlgCD has activity one order of magnitude higher than homomeric GlgC and both conformations have distinctive allosteric effectors. Fru-1,6-bisP activated 2-fold GlgC activity, but it was ineffective on GlgCD. As well, PEP inhibited GlgCD but not GlgC activity. Our results represent key differences regarding the enzyme from *B. stearothermophilus*, which is an unregulated ADPGlcPPase exhibiting no difference in V_{max} between GlgC and GlgCD conformations. The whole view supports that regulation of the key regulatory enzyme for glycogen synthesis in Gram-positive bacteria shows peculiarities. These seem closely related to particular metabolic routes in the respective organism operating in carbon and energy accumulation as well as in Glc1P partitioning.

4.3. ISOLATION AND MOLECULAR CHARACTERIZATION OF POLYHYDROXYALKANOATES-PRODUCING BACTERIA FROM AGRO-INDUSTRIAL WASTES IN COLOMBIA

[POSTER]

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Polyhydroxyalkanoates (PHAs) are thermosetting plastics, synthesized by bacteria as carbon and energy reserves accumulating as inclusion bodies. These compounds are a biotechnological option for replacing non-biodegradable synthetic polymers. There are several genetic arrangements that contain the necessary genes for biosynthesis of PHAs, named as types I to IV PHAs synthases and are usually identified by the most representative microorganism of each group (eg. I: *Cupriavidus necator*, IV: *Bacillus megaterium*). In this study, we evaluated the presence of potentially PHA producing (ppPHAs) microorganisms from agro-industrial wastes, as an environmentally sustainable alternative that generates added value to their disposal. Samples were obtained from cattle and goat whey; molasses, cachaza and sugar cane bagasse, as well as waste edible oils. Isolation was made in Mineral salt medium (MSM) supplemented with 2% glucose and 1 µl/ml of Nile red (0.1%). Colonies showing fluorescence at 366 nm were purified in nutrient agar (NA) and tested for presumptive PHAs accumulation by colony-staining with Nile blue A (1%). DNA was extracted from ppPHAs positive strains by SDS lysis, direct boiling and, depending on Gram-stain, lysozyme treatment. Small subunit ribosomal DNA was subsequently amplified with primers PA and PC5B; amplicons were purified and directly sequenced in both directions. Sequences were compared with GenBank and a phylogenetic analysis based on maximum parsimony was conducted. Bacteria previously reported as ppPHAs were further evaluated using specific PCR and gene sequencing of the *pha*C gene with primers GD / G-1R (semi-nested with G-2R) and phaCF1 / phaCR4 (semi-nested with phaCF2) for type I PHA synthases; I-179L / R-179R for type II PHA synthases; and B1F / B1R for type IV PHA synthases. A total of 35 ppPHAs bacterial strains were obtained. The predominant ppPHAs genera were: *Lactococcus*, *Klebsiella*, *Enterobacter*, *Enterococcus* for whey, and *Bacillus*, *Enterobacter*, *Klebsiella* and *Gluconobacter* for sugar cane wastes. *pha*C sequences, confirmed the presence of PHA synthases I and IV in selected bacteria. ppPHAs bacteria obtained from oils have not yet been identified. In the future, the production of PHAs by selected strains will be evaluated in different fermentation systems.

4.4. Enterocin CRL35 is active against Gram-negative bacteria regardless of a specific receptor when is anchored in the membrane

[POSTER]

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Enterocin CRL35 is a pediocin-like bacteriocin (subclass IIa) produced by *Enterococcus mundtii* CRL35. Subclass IIa bacteriocins act on the cell membrane of gram positive bacteria through a pore formation. Apparently, the peptides bind to the cytoplasmic membrane through a very specific receptor, the mannose phosphotransferase system. There are two theories on the mechanism of pore formation: i) they would induce a conformational changes in the receptor to form a channel that remains open or , ii) they would use the receptor simply as an anchor and then form the pore in the membrane. In both cases, the pore formation leads to the leakage of ions, dissipation of proton motive force and release of ATP and essential metabolites. In order to study the mechanism by which enterocin CRL35 induces the loss of membrane integrity, we led the bacteriocin to the cell membrane of *E. coli*, by fusing it with EtpM, a protein of the type II

secretion system from *E. coli* O157: H7. Previously in our laboratory, we carried out a transcriptional fusion between structural genes of enterocin CRL35 and colicin V thus obtaining the chimera MunA-CvaC active against Gram-positive and Gram- negative bacteria. In this work we constructed the fusions *etpM-munA* and *etpM-munA-cvaC* under the tight control of the P_{BAD} promoter (repressed by glucose and induced by arabinose). In addition we carried out the co-expression of EtpM-MunA and EtpM-MunA-CvaC with the enterocin CRL35 and colicin V immunity proteins. This result is surprising because it is shown for the very first time that enterocin CRL35 is capable of acting on Gram-negative bacteria when it is anchored in the plasma membrane, independently of its specific receptor.

4.5. New antimicrobial strategies: inhibition of *ftsz* vital gene by EGS technology

Se sugiere comunicación ORAL. Comunicar aceptación al mail de la Secretaría (secretaria_samige@leloir.org.ar)

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FtsZ is a highly conserved bacterial tubuline-like protein whose correct dosage is necessary for proper cell division. A relatively small decrease in *ftsZ* expression is enough to interfere with septation and concomitantly with cell division. Therefore, this gene could be used as target of novel antimicrobials. External Guide Sequence (EGS) technology consists of inducing degradation of the target RNA by RNAse P in the presence of the EGS, an appropriate complementary oligonucleotide or analog. We have recently showed that EGSs composed of a mix of deoxynucleotide and locked nucleic acid (LNA) residues efficiently induced RNase P cleavage of a target mRNA in vivo. In this work we define EGSs that induce RNase P-mediated decay of *ftsZ* mRNA. These EGSs could be the foundation for a new kind of antimicrobial agents or could be used in combination with existing antibiotics and other EGSs that inhibit expression of antibiotic resistance genes.
We designed EGSs complementary to four *ftsZ* mRNA regions available for interaction as determined by *in silico* secondary structure modeling. Our initial analyses were carried out using oligoribonucleotide EGSs (RNA-EGSs). The RNA-EGSs were used in electrophoresis mobility shift assays (EMSA) to determine their ability to bind the mRNA target. Selected RNA-EGSs were then tested to assess their efficiency in eliciting RNase P-mediated cleavage of *ftsZ* mRNA in vitro. For this, radioactive 5´-end labeled mRNA was incubated with the appropriate ratio of the catalytic M1 RNA and the cofactor protein C5.
RNA-EGSs targeting two of the selected regions (RNA-EGS2 and RNA-EGS4) showed high affinity of binding the *ftsZ* mRNA and elicited RNAse P-mediated cleavage in vitro. Since RNA-EGS4 showed the highest cleavage inducing efficiency, a nuclease resistant isosequential EGS, LNA1-EGS4, consisting of a mix of deoxynucleotide and LNA residues was designed and tested. The results of these assays indicated that LNA1-EGS4 mediates cleavage of the target mRNA with an efficiency similar to that of the RNA-EGS4.
Our results indicate that there are at least two regions of the *ftsZ* mRNA accessible for productive interaction with EGSs composed by either RNA or nuclease resistant analogs. Future experiments will determine if these later compounds also interfere with expression of *ftsZ* in vivo.

4.6. COMPLETE NUCLEOTIDE SEQUENCE OF A NOVEL PLASMID pAT23 FROM *Halopiger* sp. AT23 ISOLATED FROM LIVING STROMATOLITES IN THE ARGENTINEAN PUNA

[POSTER]

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Living or 'modern' stromatolites are mostly found in hypersaline lakes and marine lagoons with extreme conditions. The most recently found and as yet oldest living stromatolites were discovered by our lab in February 2009, in Laguna Socompa and Tolar Grande, in Salta province, Argentina; at about 4,000 m. A collection of Archaea isolated from these stromatolites has been established. Among the domain of the Archaea, haloarchaea are considered suitable for the study of archaeal genetics and a rich source of plasmids as well.
The main of this work was the isolation, sequencing and bioinformatic analysis of plasmid pAT23 from *Halopiger* sp. strain AT23, a haloarchaea isolated from living stromatolites.
Plasmid DNA was isolated by QIAGEN Plasmid Mini Kit and sequenced by Sanger technology followed by standard bioinformatic analyses.
DNA sequence of a novel plasmid pAT23 (7,843 bp) from haloarchaeal *Halopiger* sp. AT23 was determined. A total of 15 open reading frames (ORFs) were identified; putative functions were assigned to 4 of them among others with no annotatable function. Regarding the replication region, one ORF was found with significant homology to the RepA of *Haloarcula marismortui*. Analysis of the nucleotide sequence also revealed the presence of type 1 fimbriae regulatory protein FimB as well as a protein PemK, responsible for mediating cell death through inhibiting protein synthesis through the cleavage of single-stranded RNA. The largest ORF encodes a putative RloA protein which shares homology with *Lutiella nitroferrum*.
pAT23 is a novel plasmid isolated from the halophilic archaeon *Halopiger* sp. strain AT23, which has little homology with those haloarchaeal plasmids reported so far. To our knowledge this is the first report of the sequencing of a plasmid for the genus *Halopiger*.

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4.7. Study of the *crtZ* gene in *Pseudomonas fluorescens* isolated from grape roots as related to bacterial synthesis of abscisic acid

[POSTER]

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Plant growth-promoting rhizobacteria (PGPR) colonize the root system of several plants with the effect of increasing yield. Among the mechanisms proposed to explain such beneficial effect is the production of phytohormones by the bacteria, including abscisic acid (ABA). In this regard *Pseudomonas fluorescens* has received particular attention. We have previously isolated *P. fluorescens* from roots of *Vitis vinifera* (cv. Malbec L.) and demonstrated that it produces ABA in

chemically-defined medium. The aim of this work was to study if the *crtZ* gene, which encodes a crucial enzyme in the biosynthesis of zeaxanthin in bacteria, is present in *P. fluorescens*. The *crtZ* gene encodes the enzyme β carotene hydroxylase, an oxidoreductase involved in the biosynthesis of zeaxanthin from β carotene hydroxylation. In plants, zeaxanthin is a key intermediate in the biosynthesis of ABA. Specific primers were designed from the *crtZ* gene sequence presents in *Pseudomonas putida KT 2440*. The expected gene was amplified by PCR and then cloned into competent cells. Plasmidic DNA was extracted and sequenced. Homology searches were done using the basic local alignment Search Tool BLAST program showing 98% homology with the *crtZ* gene found in *Pseudomonas putida KT 2440*. This result shows that *crtZ* gene is present in *Pseudomonas fluorescens*, which strongly suggests that the ABA biosynthetic pathway in bacteria is via 1-deoxy-D-xylulose-5-phosphate (DXP) by carotenoid breakdown, as it happens in plastids of plants.

4.8. IDENTIFICATION OF NOVEL RCSB-REGULON GENES IN *S.* Typhimurium

[POSTER]

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The Rcs phosphorelay system is a complex two component system that consist of the sensor protein RcsC, the response regulator RcsB and the phosphorelation intermediary RcsD. Even when the signal remains unknown, several conditions can activate the system such us the *tolB*, *pmrA* and *rcsC11* mutants, as well as the *rcsB* overexpression and polymyxin B treatment. The Rcs regulatory system controls the colanic acid biosynthesis, the flagellar biosynthesis, the O-antigen chain length determinant, the cell division and osmoregulation, between others. Previously, we found that the *rcsB* gene is transcribed from two promoters: P_{rcsDB} and P_{rcsB}. The first promoter is located upstream of *rcsD* gene and high induced during the exponential growth phase, while the second promoter is located at the end of *rcsD* coding sequence and is lower induced during stationary phase. In the present study, we analyses the expression of certain genes that, according to microarray assay results, appears to be regulated by the RcsCDB system activation. We selected the *dps* and *osmY* genes, regulated during stationary growth phase and involved in the stress response, respectively. The activity of its promoters cloned into the pRS415 plasmid was measured by β-Galactosidase assays. The genes analyzed were modulated by the RcsB regulator under different RcsCDB system activation conditions. In the other hand, we observed that these new identified genes were controlled differentially when RcsB is produced by one of the two *rcsB* promoters. Here, we report several genes as new members of the RcsB regulon that contributes to the Rcs system study and allow us to postulated the implication of this system in others physiologically roles.

4.9. c-di-GMP enhanced biofilm formation in *Bordetella bronchiseptica* is not BvgA regulated.

[ORAL]

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Cyclic diguanylate (or bis-(3'-5') cyclic dimeric guanosine monophosphate; c-di-GMP) is a ubiquitous second messenger that regulates diverse cellular functions, including motility, biofilm formation, cell cycle progression, and virulence in bacteria. C-di-GMP is produced from two molecules of GTP by diguanylate cyclase. Enzyme activity is conferred with the GGDEF functional domain whereas c-di-GMP-specific phosphodiesterase activity is carried out by EAL or HD-GYP domains. Notably, bacterial genomes frequently encode numerous GGDEF and EAL/HD-GYP proteins, implying that the c-di-GMP network is a highly complex and tightly regulated intracellular signalling system. Most GGDEF and EAL domains are linked directly to a two component phosphorylation cascade.
Bordetella bronchiseptica is a pathogenic bacterium that causes respiratory infections in a wide variety of host. Most known virulence factors in *Bordetella* are regulated by the BvgAS two-component signal transduction system. In response to external signals, BvgAS undergoes a series of phosphorelay signal transduction events that ultimately lead to differential transcription of genes. This phenotypic modulation results in virulent, intermediate and avirulent phases. This system also regulates biofilm formation in *B. bronchiseptica* and intermediate phase is the one with maximum formation of biofilm. In a previous work we separately transformed *B. bronchiseptica* with heterologous genes from *Pseudomonas aeruginosa* coding for reported EAL or GGDEF proteins (PA3947 and PA1120) and determined that high c-di-GMP enhances biofilm formation and decreases motility in *B. bronchiseptica*.
In the present work we evaluate a possible relation between c-di-GMP network and BvgAS system. PA3947 and PA1120 were introduced in a *B. bronchiseptica* mutant blocked in avirulent phase (BbΔBvgA), and thus unable to modulate between phases. Biofilm formation was evaluated in the recombinant and parental strain in presence of different concentrations of known BvgAS system regulating signals (nicotinic acid and magnesium sulfate), which induce expression of different phases. Parental strain with a functional BvgAS system presents hyperbiofilm formation in intermediate phase as expected. In absence of BvgA in BbΔBvgA, biofilm phenotype corresponds to the observed in avirulent phase despite modulators presence. Surprisingly, BbΔBvgA expressing PA1120 corresponding to high c-di-GMP concentration, exhibited a biofilm formation behavior similar to the parental strain, with maximal formation in presence of signals concentration corresponding to intermediate phase. These results showed that although both systems are necessary for biofilm formation, diguanilate cyclase expression in *B. bronchiseptica* allowed biofilm formation in BvgA regulation absence.

4.10. Transcriptional regulation of *ppx* gen that encodes the exopolyphosphatase of *Pseudomonas aeruginosa*

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Most polyphosphate (poly P) have been conserved in different cells as bacterial, fungal, plant, and animal. The enzymes involved in the metabolism of polyP are polyphosphate kinase and exopolyphosphatase. The accumulation of polyP in many microbial cells at stationary phase is in response of different kinds of stresses as desiccation, heat, nitrogen starvation, etc. The stringent response is triggered by low phosphate or nitrogen starvation which stimulates the guanosine nucleotides ppGpp, inhibiting the hydrolysis or polyP by PPX, and thereby increase their accumulation. PPX was described in *Pseudomonas aeruginosa* and in many microorganisms. The first aim of this work was to identify and characterized the promoter of *ppx* gene in *P. aeruginosa*, its transcriptional organization and the factors that affect its expression.

Then, we will recognize which are the signals to wakes up the *ppx* gen, in order to obtain energy used for growth.
In order to locate the promoter controlling the *ppx* expression, we carried out experiments to map the transcriptional start site using a modified 5´RACE methodology. The unique initiation event is at C residue located 140 bp upstream from ATG start codon. This +1 site is 13 bp downstream of the putative σ^{54}-12 element, detected by the Promscan program, with a score of 0.69. By bioinformatics predictions and confirmed experimentally by transcriptional fusion, it was found that the region comprised between 350 bp upstream and 18 bp downstream of the ATG initiation codon, had all the elements necessary for optimal expression of *ppx* σ^{54} promoter: two putative consensus regions, to Integration Host Factor and for the Enhancer Binding Protein. To test the physiological role of nitrogen starvation, *P. aeruginosa* PAO1 wt strain was grown in a minimal medium with succinate as sole carbon source, without a nitrogen source. When the growth stopped by nitrogen starvation, different nitrogen sources were added: nitrate, arginine, histidine, choline, betaine or dimethylglycine, all of them metabolized by the activation of the two component system NtrBC. The expression of the *ppx* promoter was approximately 550 ±75 MU. The promoter expression decreased at least a 70 % when ammonium, a preferential nitrogen source, was added to the starved cells. The hyperosmolarity obtained with the addition of NaCl 0.5 or 0.7 M in a minimal medium, was also tested in two different conditions: choline as carbon, nitrogen and osmoprotector or with succinate, ammonium plus 1 mM of choline as osmoprotectant. The promoter expression increased proportionally as the osmolarity of the culture medium increases only when choline was added as nitrogen and carbon sources, and as osmoprotectant. In conclusion, *ppx* gen is regulated by a σ^{54}– dependent promoter and its full expression depends on the NtrC response regulator of two components system.

4.11. Site-directed mutagenesis by using a "megaprimer" PCR method allowed to get an active variant of the global anaerobic regulador, Anr

[POSTER]

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The metabolic flexibility of bacteria is essential to survive in a wide range of environments. Switching from aerobic to anaerobic conditions in *Pseudomonas* species is controlled by Anr, the global regulator of anaerobic metabolism. This regulator is homologous to Fnr from *E. coli*. Both regulators have a $[4Fe–4S]^{2+}$ cluster able to sense changes in O_2 levels generating a transcriptionally active dimeric form under anaerobic conditions. Exposure to O_2 results in conversion of this cluster leading to dissociation of the protein into inactive monomers. It has been reported that by changing one aminoacid is possible to obtain a protein active under laboratory atmosphere. In this work, we constructed an *anr* variant (*anr**) active in aerobic conditions using a PCR strategy to be applied in DNA binding assays in *Pseudomonas* species. This strategy involves two PCR steps and the utilization of a "megaprimer". The *anr** variant was able to complement the deficiency of *E. coli* MG1655 in the regulator Fnr. We tested the restitution of classical phenotypes controlled by Fnr, nitrate reduction activity and gas production during glucose fermentation. These results demonstrated the functionality of the *anr**. The *anr** variant was cloned in pQE32 to add a 6xHis tag and expressed in *E. coli* to allow protein purification. Anr* obtained in this study will be useful to analyze the effect of this global regulator in different physiological processes in *Pseudomonas extremaustralis* using DNA-binding experiments, production antibodies, etc. We propose that this rapid and efficient site-directed mutagenesis "megaprimer" PCR method could be used to generate mutations in other genes.

4.12. Characterization of the *Sinorhizobium meliloti* gene *sm8* encoding a putative trans-encoded small RNA

[POSTER]

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Small regulatory non coding RNAs (sRNAs) are key players in post-transcriptional regulation of gene expression in prokaryotes. Hundreds of putative sRNA genes have been identified in several species, but the biological function of most sRNA genes remains unknown. We have computationally identified and experimentally detected a set of candidate sRNA genes in the nitrogen-fixing and root nodule symbiont, *Sinorhizobium meliloti*. Here, we present data on the characterization of the sRNA gene *sm8*. Northern blotting revealed that *sm8* encodes a transcript of *ca*. 80 nt that accumulates in stationary phase. The Sm8 RNA achieves a higher cellular level in minimal medium than in rich medium, whereas it is drastically reduced in a mutant lacking Hfq, the protein that chaperones sRNA – mRNA interactions. This effect is partly due to increased Sm8 instability in the Hfq- background. Multiple sequence alignment of the Sm8 transcript revealed a strongly conserved core of 29 nucleotides (positions 24-52), of which the first 15 nt would fold into a stable hairpin and the last 14 nt would be a single stranded and exposed stretch. Based on the fact that trans-encoded sRNAs as Sm8 would base pair with target mRNAs to control their translation and/or stability, we performed *in silico* target mRNA prediction using two different algorithms (TargetRNA and IntaRNA) and the 29-nt conserved sequence as input; both approaches rendered a set of putative target mRNAs mostly encoding periplasmic and membrane transport systems; some of these hits (e.g., LivJ and DppA2) are up-regulated in the Hfq- background. This suggests that Sm8 RNA may negatively control expression of these mRNAs with the participation of Hfq. In order to experimentally verify these predictions and to identify additional cellular phenotypes regulated by Sm8 RNA, we have constructed a set of *S. meliloti* 2011 isogenic strains with altered Sm8 sequence or intracellular levels. Two types of *sm8* mutants were generated by allelic exchange: 1) an Sm8 variant with base replacements within the strongly conserved stretch possibly involved in mRNA target binding; 2) an insertional *lacZ-accC1* mutant to knock down *sm8* expression. On the other hand, the *sm8* locus has been cloned in a multicopy vector for overexpression. All recombinant strains were confirmed by PCR and *sm8* expression was checked by Northern blotting. The impact of altering Sm8 sequence or intracellular level on *S. meliloti* physiology and its cellular protein pattern is currently under evaluation.

4.13. REGULATION OF DIFFERENT GENE EXPRESSION UNDER *Salmonella* Typhimurium STRESS CONDITIONS.

[POSTER]

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Salmonella Typhimurium is an intracellular pathogen or free-living bacteria. This microorganism is able to adapt to different microenvironments, some of whom are limited in essential nutrients for bacterial development or presented extremes physical-chemical conditions. In a previous work, we study the regulation of *narZ* and *asr* the "acid shock RNA" gene expression by the phosphorelay RcsCDB system. The *narZ* gene is required for bacteria survival in anaerobiosis,

at low concentrations of carbon source, acid pH and thermotolerance; while the *asr* "acid shock RNA" gene is expressed at low pH. The goal of this work was to study the expression of the genes listed in bacterial stress conditions such as growth with low carbon source and anaerobiosis. The results of bioinformatics analysis of the selected genes promoters region revealed potential binding sites for the RcsB regulator. The analysis was conducted with *lacZY* fusion to chromosomal *narZ* and *asr* genes, and determined under anaerobic growth conditions. Moreover, an RcsB direct effect was confirmed using the gel shift assay. Our results show that RcsB binds to promoter regions, affecting the regulation of these genes when the regulator *rcsB* is present at high levels, suggesting the important role of the RcsCDB system in the bacterial stress response.

4.14. From continuous culture to the proteome of *Gluconacetobacter diazotrophicus*.

[POSTER]

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The *Gluconacetobacter diazotrophicus* proteomes were studied under batch culture conditions. Nonetheless, this approach does not reflect the bacterial protein expression response to only one variable. *G. diazotrophicus* is an acid-tolerant endophyte, which associates and may contribute with fixed N_2 to sugarcane and other non-leguminous crops. Although the interaction between the plant and endophytic bacteria is not fully understood, several plant growth promotion mechanisms have been attributed to it. The proteomic analyses of cellular and extracellular protein contents (exoproteome) under defined growth condition is currently one of the most promising tools to unravel the mechanisms involved during the plant-bacteria interaction. Besides, when combined to bacteria growth in continuous culture mode can provide samples that represent the influence of only one variable for comparative reliable proteomic analysis. However, factors such as excess of salts and exopolysaccharide productions can interfere to 2D-protein analysis from these sample types. Each continuous culture of *G. diazotrophicus* PAL5 was grown in a fermentor, containing 1L of LGIM modified medium, at 30 ºC and dilution rate adjusted to 0.05 h^{-1}. To each treatment, the pH and dissolved oxygen were automatically monitored and controlled. Treatments evaluated consisted of continuous cultures that varied in the concentration of carbon source supply (sucrose 20 g/l or 100 g/l) in combination with two N-status. When the bacteria growth occurred at BNF, the dissolved oxygen was maintained at 2% maximum to simulate microaerophilic conditions. In contrast, when $(NH_4)_2SO_4$ (3 g/l) was supplied the O_2 level was maintained under air saturation during the growth. At steady-state, samples were collected, centrifuged to obtain cellular and supernatants fractions and 0.2 mM of PMSF solution were added to each samples prior to storage. Protein extraction protocols from cellular and extracellular fractions obtained under continuous culture conditions were developed. The 2D-protein analysis of supernatants showed that the use of ethanol precipitation and dialysis against mixed resin aqueous suspension previous to protein precipitation protocol improved the quality of protein preparation to the isoelectric focusing step. The protein extraction from cellular fraction was improved by the use of CTAB precipitation for removal of insoluble cell debris, DNA and polysaccharides, resulting in reduction or elimination of horizontal streaking or incomplete focused spots.

4.15. Analysis of interaction between GbdR, a choline metabolism regulator, with DNA and RNA polymerase

[POSTER]

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GdbR, a protein encoded by the gene *pa5380* of *P. aeruginosa* is important because activates the genes of choline metabolism, and regulates many genes encoding proteins involved in the pathogenesis of this bacterium such as phospholipase C and phosphorylcholine phosphatase. The aim of this work was to study the relationship between structure and function of *P. aeruginosa* GbdR. For this, *gbdR* was cloned; the protein was expressed as an N-terminal fusion to intein-tag and purified as soluble form by affinity chromatography. The interaction DNA/GbdR was detected through the utilization of gel shift assays.
Bioinformatic analysis revealed that GbdR was homologue with MarA, a transcriptional activator of the AraC family that activates at least 24 promoters of *E. coli*. The structure of MarA is known, and determined by NMR in complex with a synthetic DNA and the C-terminal domain of the &alpha subunit of RNA polymerase (&alpha -CTD-RNAP) (PDB: 1XS9).
Using threading techniques, we have modeled *P. aeruginosa* GbdR using as template the atomic coordinates of MarA of *E. coli* (PDB:1XS9) for the C-terminal domain and, the protein Atu0886 from *A. tumefasciens* for N-terminal domain. The obtained model, evaluated using the assessment tools available in Swiss-Model workspace, indicated the presence of two DNA binding motifs helix-turn-helix (HTH), suggesting that these are the critical regions of GbdR that interact with DNA.
To understand the function of the DNA-regulator-polymerase complex, the &alpha -CTD domain of *P. aeruginosa* RpoA was also modeled by comparative modeling in Swiss-Model workspace using atomic coordinated of the same protein of *E. coli* (PDB:1XS9). The complex &alpha -CTD-RNAP-GbdR-DNA was obtained and two &alpha -helix of GbdR that interact with DNA might be: ^{254}RRQLERLFQKYL^{265} and ^{304}TPHFSKCYREYF^{315}. In addition, we also propose that the regions interacting in the protein-protein interface are: ^{19}V, ^{20}R, ^{23}N, ^{24}C, ^{49}N, and ^{84}D for RNAP; ^{219}L, ^{228}E, ^{231}A, ^{232}L, and ^{235}A for GbdR.
In conclusion, we were able to identify the residues of GbdR that might be involved in the interaction with DNA and with RNAP. So, with all these bioinformatic results we have opened a way to discover the relevance of these regions which will be tested experimentally by site-directed mutations.

4.16. Cyt b5 dependent-C5(6) sterol desaturase from *Tetrahymena thermophila* complements ergosterol biosynthesis in *Saccharomyces cerevisiae*

[ORAL]

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Biosynthesis of sterols involves several enzymatic steps, such as squalene cyclization, demethylations, reductions and desaturations. C-5(6) sterol desaturases are important enzymes in sterol-synthesizing organisms and catalyze the introduction of $(\Delta)^5$ double bound. Previous studies in plants, mammals and yeasts have shown that desaturation at C-5(6) involves an electron transfer from NADH to the terminal oxidase (the desaturase itself) via a cytochrome b_5 reductase and the cytochrome b_5. These features are typical of fatty acid hydroxylase superfamily (FAHS).

Although the ciliate *Tetrahymena thermophila* can not synthesize sterols, it performs several modifications in the sterol moiety. Four activities have been described: C-5(6), C-7(8) and C-22(23) sterol desaturations and removal of C-24 ethyl group. We have recently identified two of them, C-5(6) sterol desaturase, *DES5A*, and C-24 sterol desaturase-like, *DES24*. Moreover, previous characterization of sterol desaturases of *T. thermophila* has revealed the typical features of these enzymes. The C5(6) sterol desaturase of yeasts is an endoplasmic reticulum enzyme encoded by *ERG3* gene, its disruption creates the interruption of ergosterol synthesis. In order to evaluate the functionality of cytochrome b_5 dependent enzymes of the ciliate and to confirm the activity of C-5(6) sterol desaturase, Des5Ap, we performed a heterologous expression in *ERG3* deficient strain, *erg3-*. Thus, *erg3-* was transformed with a p425-C5T plasmid, carrying *DES5A* gene of *Tetrahymena*. The sterol profile of the complemented strain was analyzed by HPLC and GC-MS. The results showed that complementation restored ergosterol synthesis.
Therefore, this work shows for the first time that Cyt b_5 dependent enzyme of the FAHS of the ciliate is active in *S. cerevisiae*, indicating that yeasts has the necessary requirements to restore the activity with a foreign gene. Moreover, these data suggest that *S. cerevisiae* is a good system to be used for both the study of membrane protein complexes of FAHS and the identification of putative sterol related genes of ciliates, such as the C-7(8) sterol desaturase which has biotechnological applications.

4.17. Plasmid pSmeLPU88b is able to entry in *Ensifer meliloti* LPU26 when pSmeLPU26b is absent

[POSTER]

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Horizontal gene transfer is mediated by several mechanisms; bacterial conjugation is the most widespread of them. Bacterial conjugation is a specialized process involving unidirectional transfer of DNA from a donor to a recipient cell by a mechanism requiring specific contact between bacteria. The genetic information for conjugation is generally encoded in a double-stranded, circular piece of DNA called plasmid. The plasmids exist in the bacterial cell entirely separated from the bacterial chromosome. Conjugation systems are very efficient in mediating transfer between a wide range of bacterial genera and, in some cases, conjugation goes beyond prokaryotes e.g. plant cells.
The biology and biochemistry of plasmid transfer in soil bacteria is currently under active investigation because of its central role in prokaryote adaptation and evolution. In a previous work, we examined the conjugal properties of the cryptic plasmids present in a collection of the N2-fixing legume-symbiont *Ensifer meliloti* in order to investigate how frequently conjugative/mobilizable plasmids are found within the diversity of the population, and the degree of cross-complementation among the helper functions. The results show that isolates of nearly 14% of the tested strains hosted transmissible plasmids and that isolates of 29% of the tested strains were able to retransfer the previously characterized mobilizable-cryptic plasmid pSmeLPU88b to a third recipient strain. The proportion of rhizobia carrying transmissible replicons was inferred from experiments in the laboratory. It is noteworthy that isolates belonging to 14% of the tested strains proved to be refractory to the entrance of the model plasmid pSmeLPU88b, suggesting either the presence of surface exclusion phenomena or the occurrence of restriction incompatibility with the incoming replicon.
In this work we approached the identification of rhizobial surface/entry exclusion functions through the Tn5-B13 mutagenesis of LPU26 strain, and the subsequent selection of those mutants that had lost the ability of exclusion of pSmeLPU88b plasmid. In one Tn5-B13 clone the

entrance of pSmeLPU88b produced the lost on a resident plasmid (pSmeLPU26b). Further analysis showed that pSmeLPU26b was responsible for surface/entry exclusion.

4.18. BIOCHEMICAL CHARACTERIZATION OF TWO UNRELATED MICROBIAL PROTEINS INVOLVED ON AN *IN VITRO* EVOLUTION SYSTEM

[POSTER]

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The amino acid sequence determines the three-dimensional structure of proteins. However, the relationship between sequence and structure is very complex and poorly understood. We have hypothesized that a sequence could in principle attain any structure, but the observed native conformation corresponds to the most energetically favorable structure. If this is true, any fold may be obtained from any sequence provided that additional appropriate mutations are introduced to stabilize the final desired structure. As a proof-of-concept, we set to obtain by *in vitro* evolution the chorismate mutase (CM) fold from the sequence of an unrelated hydrolase, the *Bacillus licheniformis* beta-lactamase (ESP). By genetic engineering, we placed six catalytic residues of *B. subtillis* CM into ESP and reduced the length of the latter from 265 to 157 residues. The resulting polypeptide (ESPCM) has the catalytic residues of CM properly spaced but in the context the ESP sequence. The conformational properties of ESPCM protein were characterized using biophysical and functional techniques. It is a molten globule devoid of tertiary structure and inactive for both, CM and lactamase, activities. Thus, ESPCM possesses all the prerequisites for the intended *in vitro* evolution. If ESPCM was to fold into the CM fold, it would have the catalytic residues of CM in the correct position in space and it would, in principle, possess CM activity. CM catalyzes the rearrangement of chorismate to yield prephenate, and this reaction is the first step of tyrosine and phenylalanine synthesis in bacteria and fungi. Exploiting this, we were able to complement a CM(-) *Escherichia coli* strain by transformation with an expression vector carrying the CM gene from the yeast *Yarrowia lipolytica* (YLCM), building a selection system that should allow us to detect and isolate *in vitro* evolved ESPCM mutants with CM activity. YLCM protein catalytic activity was also characterized by measuring its kinetics parameters. Along with the data presented, the implications of a positive result at the first round of evolution will be discussed.

4.19. The presence of choline activates the expression of *gbdR* which encodes for a choline metabolism regulator in *Pseudomonas aeruginosa*

[POSTER]

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Pseudomonas aeruginosa is a ubiquitous and opportunistic Gram-negative bacterium and the cause of many urinary-tracts, respiratory, corneal, and surgical wound infections. It is the primary cause of chronic lung infections and mortality in patients with cystic fibrosis. This bacterium has high metabolic versatility and the ability to exploit very different environments. It grows in iso- or hyper-osmotic media, with choline or its derivatives as nutrients. Choline is widely distributed in the environmental, and as it can be used as source of carbon, nitrogen or both, it contributes in the colonization of the pathogen. *gbdR* gen (*pa5380*) of *P. aeruginosa*

PAO1, encodes for the regulatory protein GbdR, wich is involved as specific regulator of choline metabolism. The aim of this work was to study the physiological conditions that might regulate the expression of *gbdR*. For this, the promoter of this gen (P*gbdR*) was cloned, fused to the reporter gen *lacZ*, and transformed in *P. aeruginosa* to observe its activity on different nutritional and physiological conditions.
We demonstrated that the expression of this promoter was inhibited by the preferential nitrogen source, ammonium. While, if choline is used as the only substrate for bacterial growth, the P*gbdR* was activated. The full expression was observed if the preferential source of carbon, succinate, was added jointly with choline that was used as sole source of nitrogen.
In order to investigate which was the real responsible of *gbdR* full expression, choline was replaced by alternative sources of nitrogen as histidine, betaine, dimethylglycine, etc. None of them reached the level of expression observed with choline. As we assumed that choline was the trigger by its mere presence, the choline concentration added to the culture went down from 20 mM to 0.5 mM. It was determined that the latter concentration was sufficient to activate the promoter when succinate was present. Among other tested carbon sources, only succinate, the preferential carbon source of *P. aeruginosa* was essential for the maximum promoter expression. To test if the two-component-system CbrAB or NtrBC are involved as global regulator of *gbdR*, the transcript P*gbdR::LacZ* was cloned into the mutant strains Δ*cbrB* and Δ*ntrC*; β-galactosidase activity was measured, showing that both systems were involved in the regulation of this promoter.
In conclusion, the presence of choline in low concentration is sufficient to promote the *gbdR* expression and possibly the intracellular balance C:N is the responsible for the wakes up of several genes included *gbdR*. As consequence, NtrC and CbrB are also involved in this regulation because these two component systems are the responsible to maintaining the N:C intracellular level in *P. aeruginoasa*.

4.20. Effects of microcin J25 on the gene expression profile in *Escherichia coli*

[POSTER]

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Based on the idea that the primary action of most organic compounds of low molecular weight secreted by microbes in their natural habitat, including antibiotics, is to serve as signal molecules that regulate gene expression in microbial populations we investigated the effect of the peptide antibiotic microcin J25 (MccJ25) on gene regulation in *E. coli*.
In a preliminary experiment, we examined the effect of microcins J25, B17, C7 and H47 on the expression of plasmid-borne *lux* fusions to the *phoB, fadB, yrbC, tsr, ilvIG* and *lasI* promoters. We observed that the expression of the *lasI* promoter is induced in the presence of subinhibitory concentrations of microcins B17 and J25, the latter showing the highest inducing effect. These experiments were done with supernatants from microcin-producer strains. Subsequently, in order to analyze the effect of MccJ25 on the gene expression profile of *E. coli*, we tested a genomic library of *E. coli* promoters which employs the gene encoding the Green Fluorescent Protein (GFP) as a reporter. First, we determined that the minimum inhibitory concentration (MIC) of MccJ25 for the host strain MG1655 was 0.46 μM. Then, in preliminary experiments, we examined the effect of MccJ25 on gene expression, using the antibiotic at a concentration ten times lower than the MIC. Under these conditions, it did not significantly alter gene expression of *E. coli*. When the experiment was repeated using a MccJ25 concentration four times lower than the MIC we were able to identify *E. coli* promoters whose expression was selectively affected by MccJ25. Some promoters were induced while others were repressed by the antibiotic. In order to determine whether the effect of MccJ25 on the expression of the target promoters depends on the bacterial growth phase we performed growth curves in the presence

and absence of the antibiotic. This showed that the effect of MccJ25 intensifies during the stationary phase of growth. In sum, in the present study we have identified 14 genes that respond specifically to the antibiotic MccJ25. These results could provide clues on the natural function of MccJ25. Particularly interesting is the effect of the antibiotic on the expression of the genes *tig* and *mraZ*, both involved in cell division. It has been previously reported that alterations in the expression levels of these genes lead to cell filamentation, which is one of the phenotypes observed when bacterial cells are exposed to MccJ25, and whose mechanism has not yet been clarified.

4.21. Genome sequencing of the broad-host-range *Rhizobium* sp. LPU83 and characterization of its chromosomal phylogenetic relationships

[POSTER]

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Alfalfa (*Medicago sativa*) is the most widely cultivated forage legume for cattle and animal feeding over the world. In Argentina, over 6 million hectares of land are cultivated with alfalfa, where the productive symbiosis with N2-fixation efficient rhizobia represents a crucial condition for insuring the entry of fixed atmospheric nitrogen into agricultural soils (1). A distinctive feature of alfalfa is the marked specificity of this legume towards its microsymbiont partner's *E. meliloti* or *E. medicae*, but a different population of rhizobia represented by the strains *Rhizobium* sp. Or191 and LPU83 are present in fields and are able to nodulate alfalfa (2, 3). These rhizobia are acid-tolerant and have an extended host range; moreover, they possess the ability to nodulate *Phaseolus vulgaris* (3) and *Leucaena leucocephala* (2), among other legumes, and are highly competitive for the nodulation of alfalfa in acid soils. A phylogenetic analysis of the 16s rRNA gene showed that the Oregon-like strains were related to the bean-, pea-, and clover-nodulating rhizobia. In contrast, the phylogenetic analysis of the *nodC* gene indicated that the *E. meliloti nodC* gene, is the most closely related one, thus suggesting that both *nodC* genes originated from a common ancestor. Moreover, the collected genetic information has revealed that the genomic structure of these rhizobial isolates is complex in terms of sequence similarities shared with other rhizobia. Such a "patched" genetic composition has obviously imposed severe restrictions to the classical taxonomy of these rhizobia. In order to more completely characterize the genetics of the Oregon-like strains, *Rhizobium* sp. LPU83 was fully sequenced. The draft nucleotide sequence of this strain was established by a *de novo* sequencing approach carried out on a GS FLX system (454 Life Sciences, Roche) using the Titanium sequencing chemistry. An in-depth phylogenetic study was performed involving concatenation of these seven genes (*dnaK, glnA, gltA, gyrB, recA, rpoB*, and *thrC*). The LPU83 strain is located close to a clade where *Rhizobium etli* and *Rhizobium leguminosarum* were situated, as had been observed previously for other housekeeping genes. Interestingly, in this tree LPU83 appears to be a precursor of this linage.
The complete LPU83 genomic sequence together with future information from new, likewise entirely sequenced rhizobial genomes will constitute key information towards a more complete characterization and reconstruction of the Oregon-like genomic genealogy.

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4.22. A C-terminal single point mutation in *Escherichia coli* NADH dehydrogenase-2 affects enzymatic activity

[POSTER]

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Respiratory NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is a membrane-bound flavoprotein, encoded by *ndh* gene. In our laboratory, four NDH-2 mutants were constructed (named Trun-1 to Trun-4, lacking the last 13, 28, 43, and 57 aminoacids, respectively). When enzymatic activities were measured, it was observed that only Trun-1 was active similar to the wild type. The rest of the mutants restored part of the activity upon the addition of 10 μM FAD in the reaction mixture. In order to investigate this behaviour, we studied the region between Trun-1 and Trun-2 (from A406 to G420). Three single mutants were constructed, substituting H408, Y410, and K412 by alanine. These versions were then transformed into strain GNB10608 (*nuo-ndh-*), which lacks NADH dehydrogenase activities. As a rapid screening for NDH-2 activity, an *in vivo* test was performed in minimal medium supplemented with mannitol as a sole carbon source at 37ºC, where it was described that NADH dehydrogenases null mutants are not able to grow. This phenotype was reverted by wild type NDH-2, Y410xA and K412xA versions, while the mutant protein H408xA was not able to grow under this condition. Moreover, enzymatic activities were measured in membrane fractions. Y410xA and K412xA samples were active, whereas H408xA was less active in respect to the wild-type enzyme. However, in the last mutant, activity was restored by the addition of FAD in the mentioned fraction. Taking together, these results give us an idea that, within the region comprised by aminoacids 406 to 420, the H408 participates in NDH-2 activity, whether by a direct involvement in the FAD binding or by the induction of a conformational change.

4.23. RapA1 secretion by the PrsDE type I system contributes to biofilm formation in a CPS/EPS dependent mode in *Rhizobium leguminosarum*

[ORAL]

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Rhizobium leguminosarum is capable of forming biofilms in vitro that depend on the production of the acidic exopolysaccharide (EPS). The type-I secretion system PrsDE is also required for the development of a typical structured biofilm. Among the proteins secreted by PrsDE are the nodulation protein NodO, the glycanases PlyA and PlyB that modulate the length of EPS chains and members of the Rap (Rhizobial adhering proteins) family. The Raps are present only in *R. leguminosarum* and *R. etli*, and were identified by their ability to bind the bacterial cell surface at one pole. It was originally proposed that RapA1, the only characterized member of the Rap family, has affinity for the EPS and would act as adhesin by bridging between bacterial EPS associated to the bacterial surface and other bacteria or a plant cell surface polysaccharide. Mutation of *rapA1* in *R. leguminosarum* bv *viciae* strain A34 produced no clear adhesion or biofilm phenotypes. In order to investigate if RapA1 is required for in vitro biofilm formation, we overexpressed RapA1 in different backgrounds and studied the bacterial surface properties and biofilm formation. Overexpression and secretion of RapA1 in three different wild type backgrounds produced distinctive macroscopic phenotypes; the colonies became dry and

wrinkle and bacterial sedimentation was prevented, suggesting that cellular interactions were altered. Observation of static biofilms by confocal microscopy showed that RapA1-overproducing strains had altered cell-to-cell interactions when compared to isogenic wild-type strains and cells within the biofilms have lost close contact and had more void space between them. By Immunofluorescence (IF) we observed that in the wild type strain, RapA1 localized to one bacterial pole as described previously. Intriguingly, IF showed that RapA1 localizes diffusely around the cell in RapA1-overexpressing strains, providing evidence of disruption of intimate contact between bacteria; however, microtiter plate biofilm assay showed that RapA1 overexpression enhanced attachment to polystyrene in comparison to wild type. Interestingly, RapA1-overexpression had no effect on the adhesive phenotypes of an EPS-deficient strain, reinforcing the idea that the EPS is required for RapA1 to confer a particular adhesion phenotype. To determine if RapA1 overexpression was beneficial for biofilm formation under more stringent conditions, the development of a biofilm was assessed under continuous flow conditions. We observed that RapA1-overexpressing strains showed increased adhesion and biofilm formation respect to wild type strains, and reached a “mature biofilm” status in a shorter time. This effect was also dependent on EPS production. We propose that interaction of RapA1with the EPS influences attachment to abiotic surfaces and cell-cell interactions during biofilm formation.

Sección 5 - Microbiología Ambiental

5.1. Structure and abundance of ammonia-oxidizing bacteria and archaea in no-till soils under contrasting crop management

[POSTER]

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Ammonia oxidation is central to the global nitrogen cycle. It is presently recognized that other group of microorganisms, besides the ammonia-oxidizing bacteria (AOB), are capable of carrying out autotrophic ammonia oxidation. The ammonia-oxidizing archaea (AOA) have widespread distribution in terrestrial environments, suggesting an important role for them in the nitrification process. Whereas both the presence and activity of soil microorganisms are profoundly influenced by the characteristics of the environment where they grow and develop, it is important to analyze whether differences in soil management influence the abundance and activity of these populations, and inversely how does the microbial community structure affect soil quality. This issue has particular ecological relevance when it is addressed within the framework of crop productivity, as assessed by farmers’ records. The aim of this work was to determine the effect of contrasting agricultural management practices on the abundance and structure of AOB and AOA in no-till systems. Four soils with documented history of no-till management located across a west-east transect in the central region of Argentina were taken as the basis for replication. Three treatments were defined according to land use: 1) Sustainable agricultural management, subjected to crop rotation and nutrient amendment, 2) Non-sustainable agricultural management with crop monoculture and reduced nutrient reposition, 3) Non-cultivated areas adjacent to the sampled fields, as references for natural soil environments.
To determine the bacterial community structure, ammonia oxidation (*amoA*) genes were used to fingerprint AOA and AOB soil communities by terminal restriction fragment length polymorphism analysis (t-RFLP). According to canonical correspondence analysis, AOA communities clustered by sampling site. Envfit permutation test showed that correlations between AOA community structure and soil total C were highly significant ($p = 0.007$). Correlation between AOA structure and soil total N was also significant ($p = 0.014$). AOB structure did not correlate to any of the measured relevant environmental variables. The ratio between AOA and AOB abundance, assessed by quantitative real time PCR, displayed a linear correlation with carbon to nitrogen (C/N) ratio. The nitrification potential, a proxy for ammonia oxidizing activity,

correlated to total nitrogen and with the abundance of ammonia oxidizing bacteria, but not with abundance of ammonia oxidizing archaea. In conclusion, in no-till systems the abundance and compositionof AOA communities exhibit a biogeographical pattern, whereas AOB appears to be shaped largely by environmental factors.

5.2. Characterization of *Pseudomonas spp.* from soil and rhizosphere with biocontrol activity against fungal pathogens and plant growth promoting properties (PGPP).

[ORAL]

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Bacteria from *Pseudomonas* genus are known for their ability to colonize the rhizosphere of different plant species, and for displaying a wide range of properties related to plant growth promotion. Solubilization of inorganic phosphorous, mineralization of organic phosphorous, synthesis of phytohormones, and production of secondary metabolites and extracellular enzymes involved in antagonism to fungal pathogens, are some of the activities already described for several species belonging to this genus. These characteristics make pseudomonads interesting candidates as components of biological agricultural inputs to stimulate plant development and/or promote crop health.
This research line is part of a large and multidisciplinary consortium dedicated to study soil biology in relation to crop productivity under sustainable agricultural management (BIOSPAS; http://www.biospas.org). In this context, we decided to get plant-probiotic pseudomonads isolates, which are already adapted to local conditions of soil and rhizosphere. In particular, the search is focused on isolates able to inhibit the growth of fungal pathogens that occur in the same plots where samples are taken.
To this end, serial dilutions of soil or rhizosphere suspensions were plated on S1 agar, a pseudomonads selective medium. About 100 colonies were selected by their differential growth aspect on S1. They were purified on nutrient broth agar, confirmed as *Pseudomonas spp.* by carrying out a PCR of the genus marker gene *oprF*, and cryopreserved at -80oC. A first selection step was a general screening of antagonistic activity of all isolates against 12 fungal pathogens isolated during the same sampling, including *Fusarium spp.*, *Colletotrichum graminicola*, *Phomopsis sp.*, *Macrophomina phaseolina* and *Cercospora sojina*. Finally, we chose 20 isolates that confirmed their antagonistic potential in individual assays, and we determined their taxonomic position by partial sequencing of 16S rDNA and *oprF* genes.
In vitro assays showed that these 20 isolates also possess interesting PGPP. The following percentages were obtained regarding the presence of each activity or gene: 10% could grow on ACC as the sole N source (putative ACC deaminase activity), 45% produced hydrogen cyanide, 50% produced IAA, 95% secreted proteases, 50% solubilized hydroxyapatite, 30% were *phzF*+ (phenazine synthesis) and 10% were *phlD*+ (DAPG synthesis). Some isolates displayed several activities and antagonistic potential to different fungi. For example, *P. chlororaphis* isolate SMMP3 solubilizes inorganic phosphorous, produces IAA, HCN, exoprotease and possibly phenazines (*phzF*+), and antagonizes the growth of 8 out of 12 tested pathogenic fungi. Thus, we have generated a collection of *Pseudomonas* isolates with PGPP demonstrated *in vitro. In planta* tests will be required to confirm their biocontrol potential.

5.3. Role of two flagella of *Bradyrhizobium japonicum* in Competition for Nodulation of Soybean

[POSTER]

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The agronomic use of *Bradyrhizobium japonicum* in symbiosis with soybean is via inoculation of seeds or seed furrow with rhizobia. As a result, the roots of soybean plants develop nodules that once became occupied by *B. japonicum* can fix atmospheric N_2 to NH_4^+, reducing it to an extent that would satisfy all the needs of the plant. Therefore, the capacity of rhizobia to move from the soil or from seeds to the sites of infection on the roots results determinant for the establishment of the symbiosis and consequently motility of *B. japonicum* is very interesting to study.
B. japonicum has two flagellar systems. One system is composed of a 33 kDa flagellin (thin filament) and the other consists of a 65 kDa flagellin (thick filament); it has been proposed that both play different roles (Kanbe et al., 2007). From the parental strains *B. japonicum* LP 3004 (derived from USDA 110, Sm-resistant) and LP 3008 (LP 3004 with an increased motility) (Althabegoiti et al., 2008), we generated mutants lacking thin flagellum (LP 6865 and LP 6866), mutants lacking thick flagellum (LP 5843 and LP 5844) and mutants that lack flagella (LP 6543 y LP 6644), respectively. Strains were observed by TEM and mutations and morphology were confirmed for each type of flagellum. After that, liquid cultures of each mutant were observed in a phase-contrast microscope and only strains that have thick filament were capable of swim straightly whereas strains with thin filament tumbled more frequently. Finally, those that not have flagella did not swim.
With the purpose of studying contribution of each flagellum in colonization of soybean roots competition experiments were done. Competitiveness was examinated at field capacity and soybean was inoculated with mixtures containing each parental strain together with each derived mutant. After twenty one days of growth in greenhouse the occupation of plant nodules was determinated by their antibiotic resistance and a statistical analysis was done. As a result, we observed that mutants lacking thick flagellum and non-motile double mutants were less competitive that parental strains, but there were not completely displaced. However, mutants lacking thin flagellum were more competitive for nodulation. This event suggests that thin filament is not necessary for *B. japonicum* movement to the roots, showing a complex role of these flagellins in competitiveness. They could also have an additional function involved in interaction with the plant, opening interesting aspects for further analysis.

5.4. Water quality in agricultural watersheds of Córdoba: effect of pesticides on native microbiota

[POSTER]

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Modern agriculture depends on the use of pesticides to ensure the production of crops. Their continued and excessive use has led to the contamination of differents ecosystems. The persistence of pesticides in aquatic systems depends on biotic processes in which the microorganisms play an important role. Natural microbial degradation over a wide range of

pesticides exposes catabolic capabilities for their use in bioremediation processes. In this work we monitored different organochlorinated and organophosphorous pesticides on environmental surface waters samples of the south-central region of Córdoba, Argentina. We also studied the effect of 2 pesticides on culturable heterotrophic microorganisms. Samples were taken from 6 stations located on Tercero River and Cuarto River watersheds during Febrary 2011 (summer), with high river-flow conditions. The analysis of 9 organophosphorus and 30 organochlorinated pesticides was performed using capillar gas chromatography with electron capture detector (GC-ECD). The effect of both Atrazine and 2,4D (Atanor, Argentina) on culturable heterotrophic bacteria was assayed from water samples of Pampayasta and Carcarañá River (López, 2005). Experiments were also conducted to isolate in minimal medium potential indigenous bacterial strains capable of utilizing either 2.4D or Atrazine (3000 ug/ml) as a sole N and C sources, respectively. Since high amounts of pesticides were added to cultures and soil, water quality was expected to be deteriorated. Preliminary results did not detect the presence of pesticide concentrations on the screneed water samples. In agreement with Cooman (2005) we considered that during high-flow conditions pesticides in running surface waters may be present at relatively high concentrations for only a short period of time after which a rapid decrease in concentrations takes place. Future studies will be encouraged in low river-flow conditions. The plate-count data indicated that total viable bacteria in the water samples from Pampayasta River amended with 2.4D or atrazine (10 ug/ml) were significantly higher by almost 0.83 log and 0.35 log, respectively, than unamended controls during the first 3 weeks of the experiments. In contrast, after the treatment with both pesticides in water samples of Carcarañá River bacterial microbiota did not increase the number of control. The results indicated that Pampayasta River harbor indigenous microorganisms with enhanced 2.4D and Atrazine catabolic potential. Three indigenous strains were isolated from water samples amended with 2.4D and 12 from Atrazine. In the present study no pesticide were detected in the aquatic environments at the time of sampling indicating no contamination derived from human activities. The indigenous bacteria isolated capable of utilize high concentrations of both 2.4D or Atrazine for growth are actually under study in our laboratory as potential biocatalysts for degradation of these compounds.
Supported by MinCyT Cba; UNVM

5.5. Microbial biomass carbon and soil respiration as biological indicators in pastoral bovine systems with Chloris gayana cv Finecut

[POSTER]

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The quantification of microbial biomass and activity contributes to understand the immobilization and mineralization process, and its role like sink and source of plant nutrients. Therefore, these variables can be used as indicators to compare temporal and spatial changes among systems. The objective of this work was to assess the influence of different management practices at different soil depths on microbial activity and biomass after three years of assessment. The treatments applied were: 1) Grazed plots without fertilization (GPWF). The paddock was grazed rotationally with 2.5 steers ha-1 year-1, 2) Grazed plots with nitrogen fertilization (GPNF), applying 50 kg urea ha-1; 3) Hayed plots without fertilization (HPWF). The animals did not access to these paddocks and the pasture was clipped; 4) Hayed plots with nitrogen fertilization (HPNF), applying 50 kg urea ha-1. The soil samples were collected in two different dates: september 2007 and september 2009, from 0-5, 5-20 and 20-40cm depth and then were packed in polyethylene bags and transported to the laboratory to be air-dried and sieved (2 mm). Soil respiration (SR), like CO2 production from the heterotrophic soil biota after ten days of incubation was determined by return titration with NaOH. Microbial biomass carbon (MBC)

was determined by fumigation-extraction method using the extraction factor of 0.45. All data were analyzed by one-way ANOVA at $p < 0.001$, followed by Tukey test at $p < 0.05$ using the software package Infostat/P (2008) for Windows. SR and MBC showed significant differences among treatments, depths and dates ($p < 0,0001$). The mean values of SR and CBM showed stratification, finding the highest values in the first five centimeters of soil. This was related with continuum depositions of animal and vegetal residues (dung, orine, litter and rhizodeposition), principal sources of nutrients for microorganisms. For all depths and treatments, the microbial activity increased after three years of assesment. These increments in CO2 flux could be explained, in part, with a high and active root system development after ten years of perennial grass cultivation. This extensive root development ensures the presence of O2 (biological pores), and the supply of nutrients that promotes the microbial activity. In contrast, means values of MBC presented a slight decrease between dates. This situation would be linked with higher moisture soil content during 2007. The values of SR and MBC were significant higher in GPNF for all depths in both dates. We related this latter with higher C and N inputs, and a continuum nutrients recycling. By assessing MBC and SR as biological indicators in a pastoral bovine system, we observed that the nutrient supply favors the activity and development of microbial biomass. Furthermore, it is important to supply external sources of nitrogen in systems in which perennial grasses are cultivated placed for many years, to ensure growth and activity of microorganism.

5.6. EN REVISION

5.7. Tolerance to pro-oxidant agents of actinomycetes isolated from Andean Wetland

[POSTER]

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High-Altitud Andean Wetland (HAAW) is located at more than 4,400 m above sea level and is characterized by extreme conditions such as high UV radiation, elevated heavy metal content, and high salinity. Desiccation is one of the main factors occurring in these extreme environments and the oxidative stress is one of its consequences. For these reasons, bacteria need to develop efficient mechanisms and physiological strategies to survive in these extreme environments. We investigated the responses of four actinomycete bacterial strains to the pro-oxidants hydrogen peroxide (H_2O_2) or methyl viologen (MV). We used in this study two isolates from HAAW; *Rhodococcus* sp. CH13 and *Microbacterium arborescens* CH5, and two model bacteria; *Rhodococcus jostii* RHA1 and *Rhodococcus opacus* PD630. Tolerance of cells to pro-oxidants was studied in agar plates. Aliquots of 10 µL (OD600 of 0.8) were inoculated onto NB agar or mineral salts media plates and supplemented with hydrogen peroxide (H_2O_2) or methyl viologen (MV). Lipid accumulation was investigated by thin layer chromatography after extraction with organic solvents. Superoxide dismutase (SOD) activity was analyzed after polyacrylamide gel electrophoresis. The four studied strains posses Mn-SOD type as suggested the inhibition profile of the SOD activity after treatment with H_2O_2 and KCN. In general, the extremophile strains CH5 and CH13 were more tolerant to oxidant agents than the model strains PD630 and RHA1. Strains CH5 and CH13 were able to grow in the presence of 0.5 mM MV at the 10^{-4} and 10^{-2} dilutions, respectively; whereas strains PD630 and RHA1 showed limited growth at the 10^{-1} dilution. CH13 was the most tolerant strain when cells were challenged with H_2O_2 (visible growth at 10^{-1} dilution at 0.5 mM); whereas strain PD630 was the most

sensible to this challenge (no growth at 0.25 mM). Since the biosynthesis of triacylglycerols (TAG) by strains PD630 and RHA1 is a NADPH-dependent process as well as the defense mechanisms against the oxidant agents, we investigated the influence of TAG biosynthesis on the antioxidant response of these strains. Cells of both strains were more tolerant to 0.05 mM H_2O_2 in nitrogen-limiting mineral salts medium (MSM); which promotes TAG accumulation, than those cells cultivated on nitrogen-rich MSM.
The results indicated that the extremophile actinomycetes strains CH5 and CH13 isolated from HAAW were more resistant to oxidant agents than the model strains used in this study. Moreover, our results suggested that the TAG-accumulating conditions, such as the cultivation of cells in N-limiting media, enhance the antioxidant response of cells probably due to a high NADPH pool availability.

5.8. Diversity of *Burkholderia* species in Argentinian soils with different agricultural management

[POSTER]

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In the context of the BIOSPAS global project, we performed a survey to recognize the biodiversity of *Burkholderia* in soils with different agricultural management within the main agricultural production area of Argentina. The *Burkholderia* genus is widely distributed in the environment, including in some associations with plants, which could be beneficial to crop production. Three sites (Bengolea, Monte Buey y Pergamino) and three treatments: natural environment (AN), sustainable agricultural practices (BPA), and non- sustainable practices (MPA), were chosen for soil sampling. *Burkholderia* was selected on PCAT medium, and confirmed by *recA* amplification. Diversity was determined by both RFLP and sequencing of a *recA* amplicon. We identified 7 different species (*B. cepacia*, *B. ambifaria*, *B. gladioli*, *B. cenocepacia*, *B. caribensis*, *B. caledonica*, *B. terricol*a). Besides, we also identified several strains, which match strains described as ”uncultured” and others to which no species could be assigned. To analyze the effect of agricultural managements, the Shannon biodiversity index was obtained at each location. In general, the index evolved until reach a stable value, showing that sufficient samples were analyzed. We observed that in 2 out of 3 sites, AN showed a higher diversity, according to the Shannon Index. We also observed an increasing proportion of *B. ambifaria* from AN to BPA and MPA. This observation suggests that agricultural practices decrease the incidence of some *Burkholderia* species. Comparation of these results with metagenomic data obtained by other group within the BIOSPAS consortium will allow us to determine if changes in the biodiversity profiles is exclusive of culturable species, or if it is a general behavior of the *Burkholderia* pool in soils (culturable and non culturable species).

5.9. Biodegradation of phenol by *Penicillium chrysogenum*: degradation abilities and kinetic model

[POSTER]

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Phenol is one of the most common organic water pollutants, usually present in the industrial wastewater generated by the production of resins, petroleum refinery and petrochemicals. Various techniques are used for the elimination of phenol present in aqueous wastes and, among them; biotechnology methods are relevant because they have the potential to mineralize toxic compounds at a relatively low cost. Most treatments are based on the biodegradation ability of bacteria, but only a few studies have involved fungi. Most of them, using plant pathogenic fungi, which results in a high risk to the environment or fungi that have highly nutritional requirements. Strains of the genus *Penicillium* have been reported as good hydrocarbon-assimilating and there are many reports showing their ability to transform xenobiotic compounds into less mutagenic products.
The bioremediation potential of *Penicillium chrysogenum* was studied in batch culture using synthetic phenol in water as the sole carbon and energy source. In order to describe the substrate biodegradation, a suitable kinetic model, which relates specific growth rate μ and the phenol concentration S, was formulated. Dynamic mass balance equations for biomass and phenol during the exponential and stationary growth phases were solved and contrasted with experimental outcomes.
Degradation was performed at room temperature and without a previous acclimation period. Studies were conducted in liquid mineral salt medium supplemented with initial phenol concentration of 5, 30, 60, 200, 350 and 400 $mg.l^{-1}$. The effect of initial phenol concentration on the degradation process (growth and phenol degradation) was investigated over several days. Phenol was completely degraded at different cultivation times for the different initial phenol concentrations. An inhibitory effect was observed on the specific growth and degradation rates. The growth rate was inhibited at phenol concentrations higher than 30 $mg.l^{-1}$, while the degradation rate was inhibited at 200 $mg.l^{-1}$. Several mathematical models have been developed to quantify inhibitory effects of toxic substrates on microbial growth kinetics. Therefore, experimental results were fitted to Haldane, Yano, Aiba, Webb and Teissier models using least squares fitting method analysis. Among the five inhibition models tested, the Haldane model was found to give the best fit. Calculated kinetic values for μmax, K_S, and K_I were: 1.306 ± 0.200 d^{-1}, 9.434 ± 1.54 $mg.l^{-1}$ and 64.912 ± 17.88 mg. l^{-1}, respectively. These values are in agreement with literature results. The variation of the observed yield coefficient YX/S with the specific growth rate μ was represented by Pirt's maintenance energy model. Dynamic mass balance equations for biomass and phenol were formulated and solved for different initial phenol concentrations. Model predictions are satisfactory

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5.10. EFFECT OF SALINITY ON GROWTH, NODULATION AND N_2 FIXATION OF *Prosopis alba*

[POSTER]

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Algarrobo blanco (*Prosopis alba* Griseb) is a legume tree native from arid zones of centre and north Argentina. It has been reported as tolerant to salinity. The ability of plants to adapt to saline soils can be strongly dependant on the establishment of effective symbiosis. In a greenhouse experiment, seedlings were inoculated with two rhizobia isolates. One was obtained from nodules of *P. alba* growing in Santiago del Estero province, and was used as a reference nodulating strain (R strain). The other was isolated from nodules of *P. flexuosa* growing on a substrate of a saline soil from Salina del Bebedero, San Luis province (S strain). After two months growing in a mineral solution without added salt, the seedlings were watered adding different concentrations of NaCl to the mineral solution: 0, 50, 100 and 200 mM.

Inoculated plants were watered without added N while non-inoculated controls were watered with 5 ppm N. At the end of the experiment, N_2 fixation activity of intact plants was measured by the Acetylene Reduction Activity (ARA) method, then plants were harvested and the different parts (shoot, root, nodules) were weighed to estimate plant biomass. Non inoculated plants showed an almost null growth rate for every salt level, suggesting a deficiency in nitrogen more than a salt stress. Inoculated plants showed a sustained growth rate along 40 days, even for the highest salt level. However, the shoot growth rate and the biomass decreased regularly when the salt concentration was increased. Plants inoculated with R strain grew more in height and biomass than the inoculated with S strain, specially without salt addition, suggesting that the symbiotic pair Rstrain x *P. alba* was more efficient, and specific for plant growth, than the pair with S strain, which was isolated from another *Prosopis* species. The Nitrogenase activity per plant (ARA) decreased regularly as the salinity level increased for both strains. However, when plants were inoculated with the S strain, isolated from saline soils, the Nitrogenase specific activity (ARA/nodule biomass) was rather constant and unchanged until a salinity level of 100 mM. This activity slightly decreased at the highest salt level (200 mM). A different response was shown by plants inoculated with the R strain, since the specific activity of those nodules decreased with salinity. In conclusion, both strains were able to fix nitrogen actively still at the highest salinity level, allowing a sustained plant growth. The salt stress reduced the nodulation or the activity of the nodules, but this effect was weaker in the strain isolated from a saline soil, suggesting a bigger potential of this rhizobia strain for projects of reforestation of saline lands with native trees.

5.11. INCURSION IN THE STUDY OF FUNCTIONAL INTERACTIONS BETWEEN BACTERIAL STRAINS FROM A PHENANTHRENE-DEGRADING CONSORTIUM

[POSTER]

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In bioremediation of aged contaminated soils, the applications of bioaugmentation with bacterial consortia is of special concern since syntrophic interactions are an extremely interesting example of “fitness support”, specially in the biodegradation of aromatic compounds where the biochemical steps are shared among community members in order to completely mineralize recalcitrant and/or toxic substrates. In a previous work a phenanthrene degrading consortium was obtained from an aged contaminated soil and characterized in terms of structure, diversity and functionally, finding that it shows an effective phenanthrene degradation (59%) with the concomitant accumulation of 1-hydroxy 2-naphtoic acid. Four strains were isolated and identified by PCR-sequencing as *Sphingobium sp.* (AM), two *Pseudomonas sp.* (Bc y T) and one *Enterobacter sp.* (B1). In the present work some physiological properties of the isolates, related with the phenanthrene degradation, were investigated with the aim of revealing bacterial interactions that take place in the consortium during degradation of the contaminant. Also a metaproteomic approach was performed with the objective of studying the functional dynamics of the consortium. The four isolates showed growth in LMM with 200 mg/l of phenanthrene as sole carbon and energy source, although only AM strain was confirmed to be a phenanthrene degrader, showing a percentage of degradation of 55 %, after 48 h of incubation, with the concomitant accumulation of 1-hydroxy 2-naphtoic acid (74.47 ± 7.5 mg/l). This is consistent with a previous result that showed that AM was the predominant strain at the beginning of the phenanthrene degradation by the consortium. When the four strains were cultivated in 1-hydroxy 2-naphtoic acid as sole carbon an energy source only the T and Bc isolates showed significant increase in cellular density but none showed 1-hydroxy 2-naphtoic acid degradation after 96h of incubation. In addition, combinations of the strains in pairs (AM+Bc, AM+B1 y AM+T) were cultivated in MML with phenanthrene in order to study bacterial cooperation and degradation capacities. In the tree combinations, all the strains showed an increase in cellular

density, and the phenanthrene degradation was higher than 80 %. The metaproteomic study by 2DE of the consortium during phenanthrene degradation was performed at days 4 and 15 of incubation. A significant higher diversity was observed in protein expression after 15 days of incubation, this could be correlated with previous findings where, a significant degradation of phenanthrene occurred between the first 7 days of incubation, after what no degradation was observed. These results could be indicating that whereas in the first days of incubation the consortium metaproteome is dominated by the specific phenanthrene degradation enzymes, after 15 days the metaproteome would reveal the diversity of phenanthrene by-products available as carbon source.

5.12. Construction of a stable *A. brasilense* REC3 transconjugant expressing *gfp*mut3.1 gene

Se sugiere presentación POSTER

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Nitrogen-fixing bacteria of the genus *Azospirillum* are known as PGPB (Plant Growth Promoting Bacteria) for their ability to exert beneficial effects on plant growth. Despite several succesful transformations of the wild type strain *A. brasilense* REC3 (previously isolated and characterized in our laboratory) with plasmids constitutively expressing *gfp* gene, stability of these constructions was not achieved resulting on a gradual lost of fluorescence within 3 generations cultured under selective pressure.
The purpose of this work was to create a highly stable *A. brasilense* REC3 transconjugant expressing the *gfpmut3.1* gene to monitor the colonization of strawberry roots. To obtain a stable *A. brasilense* REC3::*gfp* we modified the pRU1156 plasmid, containing the promoterless *gfpmut3.1* and *gusA* reporter genes, by introducing the *ChsA* gene promoter upstream of these genes, thus obtaining high levels of expression on *A. brasilense*. Also, the pJP2-based plasmids, such as pRU1156, constitutively express tetracycline resistance gene and *parADCDE* genes, responsible of the segregation of these plasmids during cell division. The resulting plasmid was transformed into *E.coli* DH5&alpha and then mobilized into *A. brasilense* REC3 strain by triparental matting using *E. coli* pRK2013 as helper. *A. brasilense* transconjugants expressing GFP fluorescence were easily observed by fluorescence microscopy. To determine the stability of the pRU1156*ChsA* plasmid, these transconjugants were grown in liquid medium for several generations and plated onto solid medium with and without selective pressure (Tc), checking the GFP expression of each generation by fluorescence microscopy.
A. brasilense REC3::*gfp* fluoresced when it was excited by short-wave blue light showing the successful transformation of the wild type strain. The constructed plasmid was stably maintained inside the GFP-labeled strain under antibiotic pressure for 25 generations and in absence of selective pressure for 10 generations, so far. These results confirm that the vector is very stable upon repeated subculturing. The vector-stability among the high levels of GFP expression in the present construct makes it suitable for ecological studies such as monitoring root colonization both in the laboratory and in natural environments.

5.13. Identification of free indigoidine pigment in aquatic environments of Andean Patagonia

[POSTER]

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Reddish-deposits of iron hydroxides can be frequently observed in superficial bodies of water in Andean Patagonia (El Bolsón- Lago Puelo). The abundance of bacteria able to metabolize iron leaching from the ground; which is rich in pyrite in that place, is responsible for these deposits. An oily sheen on the water surface that reflects a rainbow of colors (principally blue) like an oil spill frequently occurs. It is unknown the exact origin of this oily sheen, although it is presumed that this sheen probably contains iron and manganese compounds produced by bacteria of the genus *Leptothrix*. We collected water samples from natural aquifers in El Bolsón for chemical and microbiological analyses. We identified *Leptothrix*- like bacteria from neutral-water samples containing high amounts of iron (3.8 mg/ml). In addition, we were able to identify heterotrophic associated bacteria, belonging to the genera *Microbacterium*, *Arthrobacter*, *Janthinobacterium*, *Pseudomonas*, *Exiguobacterium*, *Micrococcus*, and *Flavobacterium*, as revealed the 16S rDNA sequencing. In one of the samples of the oil-like film collected from the surface of water, we isolated a bacterial strain belonging to *Vogesella* genus (strain EB), which produced blue colonies and was able to excrete extracellularly a blue pigment. The free pigment in the culture medium showed a similar appearance than the oil-like film observed in the natural water samples. The pigment was purified and identified as indigoidine by visible spectral and IR analyses. Indigoidine is a blue pigment produced by different Gram-negative bacteria, which may be involved in neutralization of free radicals during oxidative stress. We were no more able to isolate indigoidine-producing strains from successive oil-like film samples. However, we identified free indigoidine in the oily sheen samples collected from water surfaces as revealed the comparison between IR spectra of the blue pigment purified from *Vogesella* cells (strain EB) and the pigments directly extracted from natural water samples. All these results suggest that the oil-like blue film usually associated with iron-oxidizing bacteria in aquatic environments, can contain free indigoidine and probably other pigments produced by heterotrophic bacteria. Indigoidine may be excreted by heterotrophic bacteria occurring in iron-oxidizing ecosystems as response against oxidative stress in water environments of the Andean Patagonia.

5.14. Differential expression of carotenoids under copper overload and H_2O_2 in Rhodotorula mucilaginosa RCL-11

[POSTER]

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There are enzymatic and not enzymatic systems involved in cells defense under diverse stresses. Superoxide dismutase (SODs) and catalase represent the primary enzymatic defense against ROS. Nonenzymatic antioxidants can be carotenoids, glutathione, ascorbate, tocopherol, flavonoids, and alkaloids. Carotenoids are isoprenoid membrane-protective antioxidant pigments with the capacity of to interact with reactive oxygen species. Organisms subject to routine metal exposure in their natural environments generally have had to develop resistance mechanisms. *Rhodotorula mucilaginosa* RCL-11, yeast isolated from a copper filter

plant at the province of Tucumán, Argentina, has the ability of supporting high amounts of copper metal. Also, RCL-11 showed high carotenoids contents, obtained by absorption spectrum (330nm-600nm). We observed that copper overload and inhibition of carotenoids synthesis (by diphenylamine, DPA) have a negative effect in viability of *Rhodotorula mucilaginosa* RCL-11, in addition combination of cooper and DPA showed the lowest growth rate. We found that when the cells grow in 0.5mM copper, carotenoids synthesis is strongly stimulated. Accordingly, carotenoids content is triplicate in copper overload. Moreover, superoxide dismutase activity and catalase activity was assayed in the same copper concentration. We observed an increased in both antioxidants enzymes as a response of copper excess. Total carotenoids content, as well as its spectral characteristics differs when cells are exposed to copper or 5mM H_2O_2 (oxidant agent). A qualitative study was carried out using HPLC equipped with an online diode-array detector. Detection was performed at 490 nm, and UV–vis absorption spectra were recorded online with the photodiode-array detection system. We obtained quantitative and qualitative differences in carotenoids characteristics depending on the content of copper, hydrogen peroxide, both or neither in the culture medium. For example, in presence of H_2O_2 were mostly expressed torulene, alfa-carotene and beta-carotene, while copper stimulated production of torularhodin. The results obtained in the present work show that when exposing *R. mucilaginosa* RCL-11 to prooxidant agents as copper and/or H_2O_2 it is produced an antioxidant enzymatic response and a carotenoids synthesis stimulation.

5.15. ANTIFUNGAL COMPOUNDS FROM *Zuccagnia punctata* LEAVES ACTIVE AGAINST CEREAL EAR ROT PATHOGENS

[ORAL]

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Argentina is both an important cereal producer and consumer. Diseases restrict the grain yields, specially ear rotting and grain mold caused by *Fusarium* species. These fungi also contaminate grains with mycotoxins which are a serious risk for human and animal health. We showed that the ethanolic leaf extracts from *Zuccagnia punctata* Cav. have antifungal activity against several phytopathogenic fungi, including *Fusarium* species. The aim of this work was to isolate and identify the bioactive compounds responsible for the antifungal activity of leaf extracts from *Z. punctata*. The dry residue of the ethanolic leaf extract from *Z. punctata* was sequentially extracted with diethyl ether, ethyl acetate, and methanol. The obtained extracts were dried and assayed by the microdilution broth method on strains of *Fusarium* species isolated from cereals of Argentina (*F. thapsinum*, *F. verticillioides*, *F. graminearum sensu stricto*, *F. subglutinans* and *F. bothii*). Diethyl ether extract was the most fungitoxic. Their constituents were separated by thin layer chromatography (TLC) and visualized under short and long wave UV light, and after spraying with $FeCl_3$, NP-PEG, and vanillin-sulphuric acid. The detected bands were scraped from the TLC plates and assayed by bioautography. TLC visualization reagents, UV/Vis spectrophotometric analysis and gas chromatography coupled to mass spectrometry indicated that the most fungitoxic band was constituted by the known 2´, 4´-dihydroxy-chalcone and a new compound (2´, 6´-dihydroxy-4´-methoxy-chalcone). Further research is in progress to determine the antifungal activity of these compounds alone, in mixtures and as additives of commercial fungicides.

5.16. STUDY OF THE BACTERICIDAL ACTION OF DIVERSE DISINFECTANTS ON *Alicyclobacillus acidoterrestris*

[ORAL]

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The exigency of the world-wide markets with respect to the presence of acidophilic termophilic bacterias such as *Alicyclobacillus* genus, potentially deteriorantes juices microorganisms originating of the ground, it has taken to the industry to the necessity to guarantee the quality throughout the producing chain.The objective proposed for the present work was to prove the bactericidal efficiency of 4 commercial disinfectants on a *Alicyclobacillus acidoterrestris* strain.In order to carry out the efficiency tests we work with the following biocides: Vortexx, Inspexx 200, Triquart BA y Bioplan Q122. The *Alicyclobacillus* strain used was yielded by the Malbran Institute of Buenos Aires. We work with the techniques of 1) agar diffusion: on a layer of YSG agar we deposit a 100 µl carpet of a culture of approximately 70000 CFU/ml of the issue strain; in small cups, 30 µl of three concentrations of each biocide were applied : the recommended one by the manufacturer (0,1%), a concentration by above (1%) and one underneath (0,01%); 2) determination of the minimum bactericidal concentration (CBM) by dilution in YSG broth double concentration by means of serial dilutions to the half from a 1% solution of each disinfectant plus 100 µl of the inoculate and later count in plate and 3) count after application of the contact time indicated for each disinfectant: counts were realised in YSG agar before and after the contact of diverse dilutions of the strain culture in 0.1% peptone water along with the 4 biocides to the concentration indicated by each supplier of 0,1% to the times indicated for each of them (1 and 10 minutes). In the agar diffusion test, inhibition halos were only obtained when the bacterium was faced the Triaquart BA disinfectant; until de 0.1% cocentration, a 19 mm halo were found, and a 39 mm halo for the 1% concentration of the biocide; whereas the strain is resistant for the rest of the biocides. The CBM determination shows that the most effective biocide against the strain is Triquart BA with a CBM of 0.125%; 0.5% for Vortexx and Bioplan Q122 and no inhibition for Inspexx 200. Finally, the greater percentage of inhibition, 95%, is observed for Triquart BA product against a 70000 CFU/ml inoculate concentration; 50% for Vortexx, 26% for Bioplan Q122 and 2% for Inspexx 200.
It is possible to be concluded that 1) Triquart BA product, with quaternary ammoniums, seems to be the most adapted disinfectant for the elimination of *Alicyclobacillus acidoterrestris* to the concentration and time indicated by the supplier and 2) chemical agents Vortexx and Bioplan Q122, of similar behavior, can be used of rotating way to complement Triquart BA; not therefore the biocide Inspexx 200.

5.17. PRODUCTION OF CELLULOSE AND CURLI FIMBRIA BY SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM RECREATIONAL WATERS

[POSTER]

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The high persistence of Shiga toxing-producing *Escherichia coli* (STEC) cells in soils and water appears to be an important risk factor for contamination and has been increasingly studied during the last years. Cells of *E. coli* may need to live for a considerable length of time during their life cycle outside animal hosts where the conditions may be less than optimal. Association with solid surfaces in an aqueous environment is a strategy that cells use to survive under suboptimal conditions. *E. coli* O157 and other pathogens can form biofilms when they are in nutritionally deficient media (water, soil and inert surfaces).

In members of the Family *Enterobacteriaceae*, biofilm formation is associated with the expression of curli and exopolysaccharide (EPS), such as cellulose. In this Family, the co-expression of thin aggregative fimbriae and cellulose leads to an aggregative colony phenotype (red, dry, and rough [rdar]) when grown on medium containing the dye Congo red. The rdar morphotype produces an extracellular matrix consisting of cellulose and curli fimbriae, which is the major determinant of cell-cell interactions and cell adherence to hydrophilic and hydrophobic abiotic surfaces.
The aim of this study was to recognize the capacity to express the curli and cellulose operon in four STEC considered non-O157, isolated from recreational waters in the region of Sierra de la Ventana (Buenos Aires Province, Argentina). These strains amplified the *stx*2 gene and two of these carried the *eae* factor.
The isolations were made onto McConkey agar. After incubation overnight at 37ºC, colonies were streaked onto Luria–Bertani (LB) agar plates without salt supplemented with Congo red. The plates were either incubated for 72 h at 28 ºC.
All STEC strains showed the morphotype rdar: red colony, expresses curli fimbriae and cellulose on LB agar without salt, which is coupled to the biofilm-formation capability of the organism.
The rdar morphotype has been linked to increased virulence. This work suggests the presence *E. coli* non-O157 with virulent characteristics in rivers and streams which are used for recreational purposes in Buenos Aires Province.

5.18. MICROBIOLOGICAL CHARACTERIZATION OF MILK FROM TAPIA-TRANCAS (TUCUMÁN, ARGENTINA) IN 2010

[POSTER]

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Tucumán dairy region is located in Trancas department, in the north of the province, where 60,000 liters/day are produced. In the Laboratorio de Calidad de Lácteos (Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán) we perform periodic controls of milk from the region since 2010. Milk quality is a key parameter which has a major impact on public health; it determines the payment for milk and indicates the health status of the udders. The milk is assessed mainly by chemical, physico-chemical and microbiological parameters. Once the milk is extracted from the udder, a contamination produced by environmental microorganisms begins. It is a nutrient-rich medium, so it is essential to achieve low contamination levels with good health practices and minimize growth of microorganisms by immediate refrigerating. Microbiological parameters considered to evaluate the hygienic-sanitary quality of milk are the aerobic mesophilic count, coliform count and somatic cell count, being the latter an udder infection indicator. Argentinean Food Code allows up to 200,000 cfu/ml for aerobic mesophilic and up to 400,000 cells/ml for somatic cell counts, although the dairy industries are more exigent with these parameters. The aim of this study was to monitor the hygienic-sanitary quality of milk from Tapia-Trancas region during 2010 through the environmental contamination of the product. Four samplings were conducted (1 for each season of the year). Homogenized milk was extracted from aseptic cold tanks of 38 dairies and transported under refrigeration conditions to the laboratory where aerobic mesophilic and total coliform counts (Plate Count Agar and Violet Red Bile Agar respectively, incubated at 30 °C for 48 hours) were performed. Somatic cell counts were also examined by the official technical FIL 148A:1995. The average values were calculated by multiplying the value of each dairy by the percentage of the total production. The average results from autumn, winter and spring were: aerobic mesophilic = 140,000 cfu/ml; total coliform = 8,000 cfu/ml; somatic cells = 430,000 cells/ml. In summer there was a low increase in these parameters, showing the following values:

aerobic mesophilic = 370,000 cfu/ml; total coliforms = 19,000 cfu/ml, somatic cell count = 440,000 cells/ml. Further studies are conducted to evaluate the microbiological evolution of the milk.

5.19. Eu-endolithic algae developing in modern stromatolites at High-Altitude Lakes in Argentinean Puna (4,000 m asl)

[POSTER]

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High-Altitude Andean Lakes (HAAL) at Argentinian-Puna-High Andes (between 3,000 to 6,000 m) are exposed to extreme conditions: volcanic settings, high UV-irradiation, hypersalinity, desiccation, high pH. An outstanding microbial biodiversity has developed, most of them ordered in multi-layered sedimentary mats formed by algae, bacteria and sediments called microbialites. Within them, stromatolites are of world-wide interest as they were common in ancient marine environments but today restricted to few lacustrine and perimarine settings; i) Shark Bay in Western Australia, ii) Exuma Sound in the Bahamas, and iii) Cuatro Ciénagas basin, México. All of these locations are situated at the sea level where microorganisms cope with little or no stress conditions. In turn, in the dessertic region of Salta, Northwestern Argentina, near the border of Chile we have found characteristic stromatolite-like ecosystems laying and developing in shallow hypersaline lakes located above 4,000 metters, under the pressure of harsh conditions, very similar to the ones present in the Early´s Earth atmosphere. The aim of this work is to make the first description of extreme algae developing within modern stromatolites at Laguna Socompa (4000 masl) in Salta, Argentina, Northwest-Argentina. Samples from the stromatolites were taken aseptically and fix in situ to perform further mineral characterization by X-ray diffraction and optical and scanning electron microscopic.
Socompa microbialites can be classified as typical stromatolites as the main complexing-mineral was Aragonite. They are exposed to basic conditions (pH 9) hipersalinity (8.3%), and high arsenic content (35 mg/L). Great quantities of Silicon (450 mg/Kg) and chlorophyll (70 mg/L) were present, which agreed with the fact that diatoms and cyanobacteria were abundant in the samples. Among diatoms, we could recognize different genera using both, optic and electron microscopy, i.e.: *Cymbella* sp., *Navicula* sp., *Hantzschia* sp., *Nitzschia* sp., *Synedra* sp., *Surirella* sp., *Rhopalodia* sp., *Asterionellia* sp., *Eucocconeis* sp., and *Pinnularia* sp. Filamentous and nonfilamentous cyanobacteria were represented by: *Scytonema* sp., *Microcoleus* sp. and *Phormidium* sp. among others. Both, bacteria and algae were agglutinated by exopolysaccharides and associated to minerals, presenting typical microboring patterns produced by bacteria.
The extreme stromatolites found at the highlands are of utmost interest as they provide a model to study ecology and biogeochemistry of their Precambrian counterparts in a very similar environment to the Early Eath´s one. Further research will help to clear up the metabolic process from these algae involved in the biogenesis of the stromatolites.

5.20. Characterization of environmental stress response of *Exiguobacterium* sp. isolated from high altitude Andean wetlands corregir idioma!

[POSTER]

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Andean Wetlands are characterized for their extreme environmental conditions such as high ultraviolet (UV) radiation, elevated heavy metal content and salinity. We present here the first comparative study on tolerance against extreme culture conditions of two strains *Exiguobacterium* sp. strains N30 and S17 isolated from Lake Negra and Lake Socompa, respectively, both lakes located at 4000 meters above sea level.
Exiguobacterium sp DSM6208 from the German microbial collection was used for comparison as a control strain.
In the present work we evaluate the effect of temperature, sodium cloride and sodium arsenite on growth rates. Bacterial survival after UV radiation challenges was also studied. To evaluate the antioxidant defense of the studied strains the detoxifying enzymes superoxide dismutase (SOD) and catalase were visualized by *in situ* staining after native poliacrilamide gels after electrophoresis and, moreover, tolerance to pro-oxidants agents was measured.
For all three studied strains the highest growth rates were obtained at 30ºC.
S17 and N30 isolates showed better growth than the collection strain in culture media supplemented with 5% NaCl.
Exiguobacterium sp N30 isotale presented a good tolerance to sodium arsenite while the colection strain DSM6208 exhibited a 70% inhibition of growth. Interestingly, the S17 isolate displayed an increase of growth rate after addition of 5 mM of sodium arsenite to culture media.
UV radiation hardly decrease survival counting of N30 isolate, while 50-60% of bacteria of S17 and DSM6208 strains resisted the challenge.
Bacterial resistance to pro-oxidant agents as H_2O_2 and methyl viologen was evaluated finding no significant differencies in tolerance among the studied strains.
A single SOD species compatible with Mn-type was visualized in all three strains. On the other hand, a more diverse electrophoretic pattern was found after catalase activity staining, since *Exiguobacterium* sp DSM6208 and N30 strains presented three and two activity bands, respectively, while a single species was detected for S17 isolate.
This is the first report on tolerance against extreme environmental conditions of *Exiguobacterium* sp. strains from Andean wetlands.

5.21. Microbial fuel cells prepared with Rio de la Plata river freshwater sediments. Current production and its relationship to changes at anodophilic microbial community.

[POSTER]

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In a Microbial Fuel Cell (MFC) microorganisms are used to generate an electrical current. At the anodic compartment, the bacteria oxidize organic matter and in some way, electrons are transfer to the anode. An external circuit leads the electrons to the cathode, originating an electrical current. The operating principle of a “Sedimentary Microbial Fuel Cell” (SMFC) is simple; an anode is embedded in anaerobic sediment, and a cathode is exposed in the oxygenated aqueous phase over the sediment; in this condition a redox gradient between the electrodes take place. In this work two types of electrodes have been examined in a lab-scale SMFC: plain graphite (disks) and graphite reinforced with (rods). Three SMFCs were prepared with different characteristics: SM1: plain sediment; SM2: sediment + NaC2H3O2;, SM3: sediment + formaldehyde. Analysis was by denaturing gradient gel electrophoresis (DGGE) and scanning electron microscopy (SEM) to analyze the native bacterial communities in sediments

and study their development as the SMFC matured. SM2 disk electrode reached a current density was maintained at average of 4 mA/m ^{2} and PDmax was 8.72 ± 1.39 mWm $^{-2}$. For rod electrodes the values obtained was 9 mAm $^{-2}$ on average, and the maximum power density (PDmax) was 13.93 ± 3.87 mWm $^{-2}$. The PDmax obtained for SM1 was approx. 19 mWm $^{-2}$ using rod electrodes and approx. 12 mW m $^{-2}$ for disk electrodes. With SM3 fuel cell (the control of "non-current") the PDmax reached during the entire experiment was 0.20 ± 0.02 mWm $^{-2}$. The DGGE allowed a comparison of band profiles corresponding to the sediment samples used for the assembly of the SMFC and anodes SM2 and SM1. Through a cluster analysis of the obtained profiles, we show that the clustering of the electrodes added with acetate are more similar to the sludge, presenting, however, greater diversity associated with the addition of an extra carbon source. In the case of anode belonging to SM1, there is a lower diversity compared to initial inoculum. This could be due to the enrichment with species capable of adhering to the electrode surface and exchange electrons with it. The SEM micrographs obtained showed a dense biofilm embedded in an extracellular substrate surrounding the electrodes. All the microorganisms seemed to have the same morphology, bacilli of 1.25 and 2 µm approximately. Our SMFC data shows that these freshwater sediments can be useful to provide an electric power comparable to SMFC values obtained with marine sediments. This is the first study of a SMFC from Rio de La Plata river sediment.

5.22. BIOLOGICAL EFFECTIVENES OF LIGARIA CUNEIFOLIA AND JODINA RHOMBIFOLIA EXTRACTS AGAINST PHYTOPATHOGENIC BACTERIA: MICROBIOLOGICAL AND TOXICOLOGICAL TESTS

[POSTER]

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Introduction: phytopathogenic bacteria are one of the most serious hazards faced by plants, resulting in major economic losses worldwide. The main genera implicated are: *Agrobacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. These strains, under favorable environmental conditions cause devastating diseases. One promising alternative is the use of plants employed in folk medicine to obtain chemical agents with high antimicrobial activities and low toxicity. *Ligaria cuneifolia* y *Jodina rhombifolia* are argentinean northwestern species used in folk medicine with several purposes, that may be sources of those kind of compounds. Materials and Methods: Bacterial strains: [phytopathogenic] *Xanthomonas campestris*, *Agrobacterium tumefaciens*, *Pseudomonas corrugata*, *Pseudomonas syringae* and *Erwinia carotovora*, [non phytopathogenic] *Escherichia coli* and *Staphylococcus aureus* (reference strains). Plant extracts: dichloromethane, methanol and aqueous extracts were prepared from the plant material dried powdered. Antimicrobial activity tests: Agar dilution (macrodilution) and broth dilution (microdilution) assays were employed to attain Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs). Oxytetracycline and Sptreptomycine were used as reference drugs. General toxicity: *Artemia salina* larvae lethality test (Soberón et al., 2007) was employed to attain the Lethal Concentrations 50 (LC50s). Results and discussion: *L. cuneifolia* extracts generated higher yields of extracted material (EM) and higher content of phenolic compounds (37.8 mg/mL) than *J. rhombifolia* extracts. *L. cuneifolia* methanolic extract showed inhibitory effects on all assayed strains, at concentrations below 0.16 mg EM/mL. This extract exhibited bactericidal effect on five of the seven strains, with MBCs ranged from 0.078 to 0.312 mg/mL. The infusion had inhibitory activity on the strains at concentrations above 5 mg ME/mL. *J. rhombifolia* extracts showed no significant antibacterial activity on the assayed strains, as nor dichloromethane extract presented any inhibitory effect. The more active antibacterial extracts were also the more toxic on *A. salina*, but the concentration required to exert antibacterial activities were significantly lower than LC50s,

because LC50s and MICs ratios were near 70, i.e. the concentrations required to inhibit bacterial growth were 70 times lower than toxic concentrations. According to the results we conclude that *L. cuneifolia* synthesizes active metabolites against the assayed strains at low toxic concentrations.

5.23. REMOVAL OF ENTERIC BACTERIA FROM A FRUIT JUICE PROCESSING WASTEWATER TREATMENT SYSTEM

[POSTER]

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Argentina is the dominant apple juice producing country in the south hemisphere, and exports large amounts of concentrated apple juice. The “Alto Valle de Río Negro y Neuquén” area of Argentina, located across two states in the southwest of the country, is a region where numerous processing industries are located. These industries release its effluents, previous treatment, to Río Negro River.
This treatment plant has a system of three lagoons: two aeration lagoons (6,250 m^2, with an incoming waste of about 15,000 m^3) and one facultative lagoon (18,750 m^2, with an incoming waste of about 55,000 m^3) arranged in series which received different amounts of discharge according to the season. The total retention time in the plant is 14 days.
One of the main objectives of the use of wastewater treatment lagoons is pathogen removal. This, in turn, determines compliance with the pertinent legislation and establishes the allowable uses of the effluent and receiving water body, such as irrigation, bathing, aquaculture, water supply and others. Prediction of bacterial effluent quality is essential to provide an indication of health risks posed by enteric contamination and to determine whether the reused lagoon water will reliably meet current health regulations.
The purpose of this work was to evaluate the removal of enteric bacteria in a fruit juice processing wastewater treatment. With this purpose, samples were periodically collected in different seasons (peak season: January to July, off-peak season: August to December).
In the aerobic lagoons the samples were taken from superficial zone and the facultative lagoon was divided in a superficial zone (0.30 m below the surface) and a middle zone (1.20 m below the surface). At each zone, the samples were taken at inflow and outflow of the lagoon, and then stored at 4°C until processed in the laboratory.
The samples were diluted and the dilutions were plated in triplicate onto selective medium, VRBG (Violet Red Bile Agar with Glucose). The selective media plates were incubated at 35°C for 24 hours. Typical colonies were count and the result was expressed as Colony Forming Units (CFU)/mL.
Along the treatment plant a reduction in enteric bacterial isolates was observed (percentage of removal between 90-99%), demonstrating the efficiency of the wastewater treatment process regardless of the season. We must keep in mind that this water will suffer a chemical treatment (chlorination) before reusing, and that it will also contribute to the reduction of enteric bacteria numbers.

5.24 FUNGAL DIVERSITY OF AN APPLE AND PEAR PROCESSING WASTEWATER TREATMENT SYSTEM

[POSTER]

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Aerated and facultative lagoons are commonly used for domestic and industrial wastewater treatment due to their low cost and minimal need of operational requirements. The microbial diversity growing in these lagoons largely depends on the nature of the effluent and geographical conditions, being temperature and pH the major selective factors.
The fruit juice industry generate large volumes of wastewater seasonally, and the major problem of this effluent treatment are the low pH, imbalance of nutrients and the very considerable fluctuations in the amount of effluents and waste matter produced.
In this work, we studied fungal diversity associated with the effluent treatment systems of juice producers belonging to the industrial park of Villa Regina, Río Negro (Argentina).
Samples were taken monthly to determine the effect of the fruit processing seasons (peak and off-peak season).
In the aerated lagoon (deep=2.5 m, volume=15,000 m^3, area=6,250 m^2) the samples were taken from superficial zone. The facultative lagoon (deep=3.0 m, volume=55,000 m^3, area=18,750 m^2) was divided in superficial zone (0.30 m below the surface) and middle zone (1.20 m below the surface).
We performed total fungal counting (filamentous fungi and yeasts) using the method of dilution in plates by surface spreading in Yeast extract-Glucose-Chloramphenicol agar medium (YGC agar). The plates were incubated at 25 ºC for 5 days. The different yeast colonies were identified by their morphological and physiological characteristics as described by Kurtzman and Fell (1998). The filamentous fungi were identified by their morphological characteristics.
A total of twenty five yeast strains and five filamentous fungi were isolated and identified. Ninety six percent of yeast strains and eighty percent of filamentous fungi were isolated in peak season. Among the filamentous fungi, the identified genera with their corresponding percentage of occurrence were: *Aspergillus* 41%, *Cladosporium* 25%, *Penicillium* and *Mucor* 17%. More than 91% of isolates were obtained from aerated lagoons and the superficial zone from facultative lagoon. Between the unicellular fungi, the genus *Candida* represents the majority of the isolates (47%) with a great variation of species. Species of *Cryptococcus*, *Rhodotorula*, *Kloeckera* and *Geotrichum* were isolated in lower percentages. The major yeast diversity was observed in aerated lagoons, with fermenting and non- fermenting strains. In the middle zone of facultative lagoons, only yeast belonging *Candida* genus was isolated. More than 50% of *Candida* isolates showed a seudomicelial configuration. This invasive pattern could explain its high occurrence in fruit juice wastewater treatment lagoons. The general trend clearly showed the impact of the peak season in fungal diversity.

5.25. ARSENITE RESISTANCE AND REMOVAL FROM AQUEOUS SOLUTION BY *RHODOCOCCUS* SP. AW3 ISOLATED FROM SOYBEAN RHIZOSPHERE IN AN AGRICULTURAL FIELD FROM CÓRDOBA

[POSTER]

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Arsenic (As) in groundwater and soil, and its fate and transport in the environment have become matters of great concern in several countries. In agricultural environment, besides domestic use (drinking and cooking), huge quantities of water from As contaminated aquifer are usually used for irrigation purposes which increases As accumulation through the food chain. Moreover, with increased arsenic in soil and water, sustainable agriculture development is hindered by As phytoxicity and long-term impact on agricultural yield. Simple, environmentally-friendly and novel methods are needed for its removal from water and soil. For this purpose, the potential of different microbes in As removal has gained interest.

In this work we isolated an arsenite-resistant bacterium from the rhizosphere of soybean (*Glycine max*) plants grown in an intensive agricultural soil (Paraje La Escondida, Alcira Gigena, Córdoba, located at 32º40'83"S, 64º33'40"W). This strain was selected, among others, due to its high resistance to arsenite (As^{+3}). On the basis of its morphological, cultural, physiological, biochemical characteristics, and supported by phylogenetic analysis based upon their 16S rRNA gene sequence, it was identified as *Rhodococcus* sp. and named as *Rhodococcus* sp. AW3. This strain grew in the presence of high sodium arsenite ($NaAsO_2$) concentrations, over 38 mM, in solid YEMA (Yeast Extract Mannitol Agar). Induction experiments in YEM liquid media indicated that resistance mechanisms of this bacterium are constitutive. Qualitative oxidation experiments suggested that *Rhodococcus* sp. AW3 is not able to oxidize As^{+3}. In preliminary arsenite (As^{+3}) removal assays liquid medium containing 5 mM $NaAsO_2$, *Rhodococcus* sp. AW3 was able to remove about 62% of the contaminant. Because As^{+3} is more difficult to eliminate than As^{+5}, currently, further studies are being conducted to establish the genetic factors that determine As resistance and the mechanisms responsible for As removal.
Selection of appropriate bacteria for soil and water treatments will widen the perspectives of sustainable management of agricultural areas affected by As. In particular, the isolated native *Rhodococcus* sp. AW3 could prove to be useful in forthcoming experiments of As bioremediation in agricultural soils or eventually contaminated waters

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5.26. Biodegradation of phenol and 2, 4, 6-trichlorophenol by a new soil isolate of *Penicillium chrysogenum*: degradation abilities and phytotoxicity assays after fungal treatment

Pasa a BIORREMEDIACION y Biocontrol

[POSTER]

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Industrial wastewaters frequently contain high concentrations of phenolic compounds and its chlorinated derivates, which represents a serious ecological problem due to their toxicity. Therefore, the removal of phenol from industrial wastewater and soils is of great practical significance to the environmental protection.
Several fungal strains have been reported to degrade phenol as only source of carbon and energy, but many of them are pathogens or have high nutritional requirements and will not be useful to apply in soil bioremediation or water treatments. Strains of the genus *Penicillium* have been reported as good hydrocarbon-assimilating and there are many reports showing their ability to transform xenobiotic compounds into less mutagenic products.
In this work, we study the degradation of phenol and 2, 4, 6-trichlorophenol (TCP) by a *Penicillium chrysogenum* strain isolated from crops soils. Batch cultures were conducted in liquid mineral salt medium (LMS) supplemented with different concentrations of phenol or TCP, as the only source of carbon and energy. Phenol and TCP contents were measured by HPLC. *P. chrysogenum* degrades completely phenol concentrations ranging from 5 to 400 $mg.l^{-1}$ at room temperature and without shaking. It took 28 days to degrade 400 $mg.l^{-1}$ of phenol. Lower phenol concentrations were degraded in shorter times.
On the other hand, *P. chrysogenum* did not degrade TCP under these conditions. For this reason, new batch cultures were conducted LMS with 10 $mg.l^{-1}$ of TCP and 2 $g.l^{-1}$ of sodium acetate as co-substrate, at 30 °C with agitation. Under these conditions *P. chrysogenum* was able to degrade 85% of the TCP in 26 days. New experiments with higher concentrations of TCP are underway.
In addition, phytotoxic assays on wheat seeds were conducted to evaluate the toxicity of degradation products of phenol and TCP. Seed germination and root elongation were measured

to evaluate the germination index (GI). The products from phenol degradation were not phytotoxic for wheat (GI= 100%), while phenol showed high phytotoxicity (GI= 0.4%). Seed germination was 100% for wheat in LMS used as a control, 95% for seeds treated with the products of phenol degradation and 6.25% for 400 mg.l^{-1} of phenol. No root elongation was observed for the latter case. Seeds treated with the products of phenol degradation and control seeds showed similar root elongation about 14 ± 7.1 mm.
Because an increase in root elongation was reported for low concentrations of TCP, this parameter was not taken into account to calculate the GI in TCP phytotoxicity assays. Therefore, only seed germination was evaluated. This was about 85.7% for TCP degradation residues and 5% for TCP, these results showed that the fungal treatment reduces the TCP phytoxicity.
Finally, degradation potential of *P. chrysogenum* makes this strain attractive for using it in remediation

Sección 6 - Interacciones Procariota-Eucariota

6.1. *Azospirillum brasilense* modifies root architecture of *Arabidopsis thaliana*

[ORAL]

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Azospirillum sp is a plant growth promoting rhizobacteria (PGPR) that improves growth and yield of many important crops. The main effect of root colonization by *Azospirillum* sp is the increase of the radical system, which enhances mineral and water absorption. The mechanism proposed to explain the root growth promotion is the production phytohormones of the bacteria. These hormones include indole-3-acetic acid, gibberellins and abscisic acid (ABA) in which may alter metabolism and morphology of plants that leads to better absorption of minerals and water uptake. Drought is among the main adverse environmental conditions that reduce crop yield worldwide. In plants experiencing drought, ABA is produced by roots and acts as the signal that prepares the plant to resist the water restriction stimulating stomatal closure and thus helping water conservation by plants during the drought period. The purpose of this work was to evaluate the morphological and physiological changes produced in *Arabidopsis thaliana* plants inoculated with *A. brasilense*, using two different experimental models. 1) Petri dishes with MS agar, and 2) plastic pots with peat:perlite (3:1). In experiment 1, 7 days-old *A. thaliana* plants inoculated with *A. brasilense* in PBS or PBS (control) were cultivated under a photoperiod of 12 h. After 30 days, leaf area (LA), root length (RL), root and shoot fresh and dry weight (RFW, SFW, RDW, SDW) of inoculated and control plants were measured. Inoculated plants retarded water loss after cutting aerial part, anticipating water deficit detection as compared to control plants. In experiment 2, 15 days-old *A. thaliana* plants inoculated or not with *A. brasilense* C with 12 h light were grown at 24. The rosette growth and rod length were measured weekly and the seed production at the end of the experiment. In addition, ABA was measured by GC-MS in both experiments. *A. brasilense* significantly increased the aerial part and root surface of the plants assessed as LA, RL, RFW, SFW, RDW and SDW. Root-growth promotion by *A. brasilense* posses developmental (increasing lateral-root number) and growth bases (enhancing lateral-root length). Also, the plants inoculated anticipated the phenological stages respect non inoculated ones, with a significant increase in rosette growth, rod length and number of seeds per plant. The inoculation with *A. brasilense* in *Arabidopsis* caused an increase in the plants ABA content.
Taken together our results show that *Arabidopsis* inoculation with *A. brasilense* enhances plant biomass and yield. Increments in LA could enhance water transpiration per plant. Nevertheless the parallel enhancement in root surface and ABA levels, that could explain the higher

sensitivity of inoculated plants to induce stomata closure when experienced water deficit, suggest that *A. brasilense* inoculation could enhance drought tolerance.

6.2. EVALUATION OF THE INTRAVAGINAL ADMINISTRATION OF BENEFICIAL HUMAN VAGINAL LACTOBACILLI IN A MURINE EXPERIMENTAL MODEL

[POSTER]

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The female urogenital tract infections (UGTI) are the result of an imbalance of the urogenital ecosystem, resulting in a decrease of protective lactic acid bacteria (LAB). One of the alternatives proposed for the prevention and/or treatment of UGTI is the application of probiotic products containing LAB. The objective of the present work was to evaluate the degree of colonization of vaginal lactobacilli selected by their potentially probiotic properties, and if they produce some type of adverse effect on the vaginal tract of mice. Two-months-old female BALB/c mice were inoculated intramuscularly with estradiol (48 h before the inoculation of lactobacilli), in order to induce the estrogenic phase of estrous cycle. In the first stage, five different strains of human vaginal lactobacilli (*Lactobacillus gasseri* CRL1263, *L. gasseri* CRL1509, *L. reuteri* CRL1324, *L. salivarius* CRL1328 and *L. rhamnosus* CRL1332) were intravaginally administered (10^7-10^8 CFU/50 µl) to different groups of mice, two times every day during two days. The number of viable lactobacilli in vaginal washings and the production of adverse effects on vaginal tract (by standard cytological and histological techniques) were determined at 2 and 4 days post-inoculation. In the second stage, *L. gasseri* CRL1263 and *L. salivarius* CRL1328 were administered as follows: a) two inoculations every day during two days (scheme 1), or b) two inoculations every day, during four days (scheme 2). The number of viable lactobacilli was evaluated in vaginal washings and homogenates, at 2, 5 and 7 days post-inoculation. On the day 2 post-inoculation, the five strains were recovered in vaginal washings (10^3-10^4 CFU/50 µl). On day 4, a decrease of 1-2 logarithmic units was observed for all the strains. The inoculation of lactobacilli did not produce modifications in the histological structure of the vaginal tract; the characteristics of the different layers of the epithelial and connective tissues were similar both in control and lactobacilli-treated mice, at all the days evaluated. When the inoculation assay 1 was performed, the number of viable cells of *L. gasseri* CRL1263 and *L. salivarius* CRL1328 were around 10^3 CFU/50 µl (in vaginal washings) and 10^3 CFU/70 mg (in homogenates). Only *L. salivarius* CRL1328 was able to persist up to day 5 in the vaginal tract, being the counts higher in vaginal washing (10^2 CFU/50 µl) than in homogenates (10 CFU/70 mg). The administration of lactobacilli at higher doses (scheme 2) did not produce a better recuperation of bacteria in the vaginal tract. The results of this work support the safe use of beneficial vaginal lactobacilli as probiotics to restore the ecological balance of the UGT, based in the no production of adverse effects at cytological and histological levels.

6.3. Tracking *Escherichia coli* infection of lettuce by dual-fluorescent labelling of bacterial cells

[ORAL]

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Escherichia coli is a major cause of hemorrhagic colitis and the hemolytic-uremic syndrome worldwide. Although most outbreaks have been associated to undercooked ground beef and dairy products, fresh vegetables are also vectors of food-borne illness. It is of special public-health concern that as few as 10-100 cells are required for this pathogen to cause illness in humans. The mechanisms by which the pathogen is introduced into plants are not fully understood; however, it is presumed that plants become contaminated when grown in fields fertilized with improperly treated manure, watering with contaminated irrigation water and (or) improper post-harvest manipulation.
The purpose of our study is to expand our understanding of the infection pathway of lettuce by *Escherichia coli*. To accomplish this objective we have prepared *E. coli* reporter strains expressing green- or red-fluorescent proteins to visually track the infection process and made viable-cell counting determinations to confirm fluorescence microscopy information. In roots, we have observed a preferred localization of bacterial cells clusters in zones of elongation of lateral roots and the apoplastic space between parenquima cells. During plantlet development, at least 2 completely unfolded true leaves, infection was more prominent in parenquima cells of leaf tissue, especially in young, still folded leaves. Using this infection system, *E. coli* typically formed cell-patches in leaves surrounding the vascular tissue. The general infection pathway was modified according to the nutritional status of the plants: while a richer medium favored proliferation of bacteria in the leaves, starvation changed the pattern towards root colonization. By simultaneous infection with reporter *E. coli* strains with different fluorescence properties we only observed green-patches and red-patches in the same leaf area (no mixed patches), strongly suggesting that true micro-colonies developed from single cells that might reach the leaf parenquima through the vascular tissue
Conversely, cells in the apoplast of roots were more randomly arranged. A similar approach was used to investigate whether bacterial cells that reach the leaves were more infective than cells maintained in culture medium for enterobacteria. Thus, reciprocal competition experiments showed that in planta cultured cells were nearly 10-fold more infective to lettuce.
Additionally, we have evaluated the effect of the commonly used growth-promoting rhizobacterium *Azospirillum brasilense* on lettuce infection by *E. coli*. We found that the infection was reduced by several orders of magnitude both in roots and leaves.
These results may help understanding the infection and colonization mechanisms of fresh produce by *E. coli* and to model agricultural practices towards food-born illness-risk amelioration. CONICET-ANPCyT-FIBA.

6.4. SURVIVAL CAPACITY OF *Arcobacter butzleri* INSIDE *Acanthamoeba castellanii*

[POSTER]

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The genus *Arcobacter* has become increasingly important because some of their species have been considered emergent enteropathogens and potential zoonotic agents. This genus

comprises Gram negative, curved and motile bacteria with a wide diversity of habitats and hosts. Some *Arcobacter* species, mainly *A. butzleri* have been isolated from cases of human diarrhea, animal fecal samples, foods and aquatic environments.
The free-living amoeba, *Acanthamoeba castellanii*, is a unicellular heterotrophic eukaryotic organism, ubiquitous in natural aquatic and terrestrial environments and present in diverse anthropogenic surroundings, where it can share habitat with *A. butzleri*.
In the last years it was demonstrated that some pathogenic bacteria can survive inside free-living amoeba, which could play a role in the transmission of that bacterial infectious agents.
The aim of this study was to establish the survival capacity of three *A. butzleri* strains (one isolated from chicken meat, one from human stool and the reference strain) inside *A. castellanii*.
Axenic cultures of *A. castellanii* were inoculated with the strains and incubated at 26 ºC aerobically for 240 hours. The interaction bacteria/amoeba was monitored by phase contrast microscopy and the bacterial survival rates inside the amoebas were assessed by colony forming unit (cfu) count before and after amoebal-lysis.
At 5-10 min after infection, bacteria surrounded the trophozoites adhering to their membrane. At 30-35 min a polar bacterial aggregation was formed and after 50-60 min internalized, motile bacteria, inside cytoplasmic vacuoles were seen. Intracellular bacteria were confirmed by optical and electronic transmission microscopy.
All *A. butzleri* strains were able to survive, at least, for 240 hours inside the amoebas with cfu counts varying between 49000 and 12500 cells/ml.
Our results allow us to infer that *A. butzleri* is able to establish endosymbiotic relationships with *A. castellanii* surviving, at least, for a 10 days period inside the amoebas, thus suggesting that free-living amoebas could be a potential environmental host for this emergent and zoonotic pathogen.
FINANCIAL SUPPORT: Grants FONDECYT 1110202 and DID-UACh S-2007-37

6.5. INFLUENCE OF LACTIC ACID BACTERIA ON SERUM LEPTIN SECRETION AND IMMUNE PROPERTIES IN MICE

[POSTER]

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The leptin plays multiple homeostatic functions in the organism. The information about the influence of lactic acid bacteria (LAB) on the leptin secretion is scarce and contradictory. Aim: The objective of this study was to compare the influence of different doses of LAB oral administration on leptin secretion and its relationship with immunological parameters in mice. Material and methods: adult Swiss mice received during 5 consecutive days, 10 E8 ufc/ml of Lactobacillus casei CRL 431 (Lc431); and 10 E6, 10 E7, and 10 E8 ufc/ml of Lactobacillus plantarum 236 (Lp236) and Lactobacillus rhamnosus ETC14 (ETC14) administered in drinking water. Serum leptin concentration, number of IgA+ B lymphocytes in mucosal small intestine and phagocytic activity were determined. Results: We observed a significant increase on serum leptin after Lc431 and Lp236 administration. Whereas that ETC14 induced a significant decrease of this hormone. No differences between doses of the same microorganism were observed. We only showed significant increase of peritoneal phagocitosis with Lc431 administration. When we analyzed a count of number of IgA+ cells, we viewed that 10 E8 ufc/ml of Lc431 and 10 E6 ufc/ml of ETC14 induced significant increases of this cells. While different doses of Lp236 did not induce changes in IgA +cells.
These results suggest that the oral administration of LAB has influence in serum leptin secretion. This effect was strain dependent. The modulation of leptin secretion in addition to the immunomodulatory properties of some lactic acid bacteria may be part of the criteria of selection

for probiotic microorganisms. We suggest as new potential probiotic to Lactobacillus rhamnosus ETC14.

6.6. Differential pattern of response to *Lactobacillus reuteri* CRL 1098 between two *in vitro* eukaryotic cell culture systems: relevance on probiotic strain selection with specific applications

[POSTER]

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Probiotics are live microorganisms which when administered in adequate amounts confer health benefits to the host. Among these beneficts, probiotics are able to modulate immune responses and they have been extensively studied as immunomodulatory agents in the fields of inflammatory diseases. Certain strains from *Lactobacillus* and *Bifidobacterium* genera have the capacity to modify the profile of pro- and anti-inflammatory mediators released from immune cells in *in vitro* and *in vivo* inflammation experimental models. The aim of this work was to compare the capacity of *L. reuteri* CRL 1098 to exert an anti-inflammatory effect in two cellular *in vitro* models: human peripheral blood mononuclear cells (PBMC) and mouse macrophage cell line (RAW 264.7). *L. reuteri* or its supernatant were incubated for 4 and 24 h with control or LPS-stimulated PBMC and RAW 264.7 cells at 37 °C and 5% CO_2. TNF-α, IL-10 and nitric oxide (NO) producion was then measured in the co-cultures supernatants. The results showed that after 4h incubation *L. reuteri* and its supernatant diminished TNF-α production by 33 and 34%, respectively in control PBMC and by 68 and 37%, respectively in LPS-stimulated PBMC. In RAW 264.7 cells *L. reuteri* increased 147 times the TNF-α production while *L. reuteri* supernatant had no effect on TNF-α production by control and LPS-stimulated RAW 264.7. NO production decreased by effect of *L. reuteri* supernatant similarly than TNF-α after 4 and 24h incubation in both LPS-stimulated PBMC and RAW 264.7. In contrast, *L.reuteri* or its supernatant did not affect the IL-10 production by PBMC and RAW 264.7 cells in control and LPS-stimulated cells. Our findings prove that a single bacterial strain can generate different immune patterns of responses depending on which *in vitro* model was evaluated. Therefore, anti-inflammatory properties of *L. reuteri* CRL 1098 might be tested in a number a cell cultures before extrapolation to *in vivo* trials in an experimental animal model.

6.7. Blue ligth sensing of *Xanthomonas axonopodis* pv. citri during citrus canker

Se sugiere comunicación ORAL. Por favor comunicar aceptación al mail de la Secretaria (secretaria_samige@leloir.org.ar)

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Light is a major signal that regulates plant physiology. Recent studies demonstrated that an appropriate light environment is required to establish efficient resistance responses in several

plant-pathogen interactions. On the other hand, the participation of light in the regulation of bacterial virulence was found recently in non-photosynthetic bacteria. Data obtained from bacterial genome sequences revealed the presence of photosensory proteins of the BLUF (blue-light sensing using FAD), LOV (light, oxygen, voltage) and phytochrome families. The light-sensing capacity of these proteins is mediated by their association with specific light-absorbing molecules or chromophores, such as flavin mononucleotide (FMN) in the case of LOV proteins. The LOV photoreceptors are related to the plant phototropins which are involved in phototropic bending, light-induced stomatal opening and light-directed chloroplast movement. *Xanthomonas axonopodis* pv. citri (Xac) is a gram negative bacterium responsible for the citrus canker. This disease affects all citrus cultivars damaging fruits, leaves and stems. The *in silico* analysis of Xac genome sequence showed the presence of the gene *fixL*, which encodes a LOV protein that contains an N-terminal LOV domain associated to a C-terminal histidin kinase and a response regulator domains (hybrid HK-RR). The LOV domain contains the highly conserved motif GNNCRFLQ that includes the reactive cysteine essential for the photochemical cycle initiated with the absorption of blue/UVA light. To study whether environmental light modulates the virulence of Xac, we have constructed a knockout mutant by replacing the *fixL* gene with an antibiotic resistance cassette. First, we showed that the absence of this gene did not affect the bacterial viability and division. Then we analyzed several physiological features of the bacteria, particularly those related with pathogen's ability to colonize its host plant. We found significant differences in physiological features like motility, biofilm formation, exopolysaccharide production and bacterial adhesion between both strains which indicate that the LOV protein has a role in the regulation of these processes. We also observed that the adhesion of the mutant strain was significantly diminished compared to the wild type strain. This response showed a strong dependence on the presence of light during bacterial growth. Altogether these results suggest a light-dependent regulation of Xac virulence during the host colonization.

6.8. Association with an ammonium-excreting bacterium allows diazotrophic culture of a eukaryotic microalga

[ORAL]

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The industrial production of nitrogen fertilizers has greatly increased agricultural productivity worldwide with a dramatic impact on human population. It is likely that further population increases and the use of biofuels as alternative sources of energy will further increase the demand of fertilizers in the future. However, the use of fertilizers also entails adverse economic and environmental effects partly due to low nitrogen use efficiency and the corresponding fate of excess fertilizer altering most ecosystems on Earth.
On the other hand, biological nitrogen fixation is a natural way of converting nitrogen from the air into biologically active nitrogen. By interacting with diazotrophic bacteria in the soil most plants take part of the nitrogen they need. It has been shown that either naturally occurring or mutant bacterial strains with enhanced properties of ammonium excretion improve the performance of plants in nitrogen poor soils.
We are interesting in studding and developing the nitrogen bio fertilization concept applied to microalgae culture as a promising feedstock for next generation biofuels.
We have obtained an *Azotobacter vinelandii* mutant strain with a deletion of the nifL gene that is impaired in sensing ammonium sufficiency and thus expresses nitrogenase and accessory nif genes constitutively. This strain accumulates several times more ammonium in the culture medium than the wt cells and presents a reduced rate of growth. Ammonium excreted by the mutant cells is bioavailable to promote growth of another *A. vinelandii* non-diazotrophic mutant strain (delta-*nifA*) when cultured onto solid medium in close proximity. Similar results were obtained with the non-diazotrophic microalgae *Pseudokirchneriella* sp. After co-cultured with the

ammonium-producing bacterium, the microalga grew onto diazotrophic plates even when the bacteria were eliminated by dilution. Co-culturing experiments in liquid medium confirmed the previous results and show that, assisted by the ammonium excreting bacterium, the microalgae achieves growth rates approaching those of the ammonium-amended cultures. *Pseudokirchneriella* sp. accumulates up to 35 % of its dry biomass as oil under nitrogen starvation. By using a convenient fluorometric assay we observed that, when in co-culture, microalgae lipids accumulation roughly matched the likely availability of nitrogen, but with some apparent modulation due to the mode of ammonium delivery. Although nitrogen fixation is energetically expensive to diazotrophs, glucose feeding experiments show no evidence that, under the current conditions, growth and nitrogen fixation of *A. vinelandii* were carbon and/or energy limited. The bacterium grew and produced excess ammonium in the microalgae medium supplemeted with sugars. However, it did it much slower in the presence of the microalgae preventing the bacterium outcompites the microalgae at the end of the cultivation. CONICET, ANPCyT, FIBA.

6.9. Role of type IV secretion system of *Xanthomonas axonopodis* pv. citri in citrus canker

[POSTER]

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Adherence to eukaryotic cells is an important early step during the infection of host cells by many bacterial pathogens. Adherent pathogens typically have one or more extracellular molecules or structures that interact with various types of surfaces. One of the most widespread adherence structures among Gram negative bacteria are the filamentous appendages called pili or fimbriae. Type IV pili (Tfp) is among the most common and best studied. In addition to their role in adherence, Tfp mediate a form of flagella-independent surface translocation called twitching and this structure can be important for autoaggregation, biofilm formation, natural transformation competence and muticellular development. Twitching motility also is required for host colonization and pathogenesis, including the activation of host cell responses. *Xanthomonas axonopodis* pv. citri (Xac) is the phytopathogen responsible for citrus canker. Adhesins have an important function in Xac attachment to host cell surface. The objective of this work is to determine if Tfp of Xac participates in the virulence process during citrus canker. A Xac mutant strain defective in Tfp was constructed by conjugation with the mobilizable suicide vector pKmob. No differences were observed in the growth curves in liquid medium between Xac wild-type (wt) and the mutant strain. Differences were observed in twitching motility on agar plates. Xac wt formed colonies with a characteristic rough appearance in the peripheral twitching zone consisting of a thin layer of cells. On the other hand, mutant strain produced smooth colonies on agar plates. Also, adhesion and biofilm assays showed differences between Xac wt and the mutant strain. These results indicate that bacterial growth was not affected in the Tfp mutant of Xac but others features related to cell adhesion and motility that are important in the virulence process were altered in the mutant bacteria, suggesting a possible role of Tfp of Xac in citrus canker.

6.10. BtaE and BtaF: two members of the trimeric autotransporter family involved in adhesion of *Brucella suis* to host cells

[POSTER]

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Species belonging to the *Brucella* genera are intracellular pathogens responsible of an endemic disease called brucellosis that causes important economic losses. Even though the intracellular traffic of *Brucella* was well characterized, the adhesion of *Brucella* to the host cell was little explored. By phage display we have previously identified a protein from the monomeric autotransporter family (BmaC) that is involved in the attachment of *Brucella suis* to epithelial cells, probably by interaction with fibronectin. By bioinformatical analysis of the *B. suis* genome we identified two putative adhesins belonging to the trimeric autotransporter family: BtaE and BtaF. By inmunofluorescence we confirmed that both proteins are exposed on the bacterial surface, as expected for adhesins.
We generated clean deletion mutants in the *btaE* and *btaF* genes of *B. suis* 1330. Both mutants showed a marked loss in its ability to attach to HeLa cells. Interestingly, the *ΔbtaE* mutant had a reduced ability to bind to immobilized hyaluronic acid, while the *ΔbtaF* showed a reduced attachment not only to hyaluronic acid but to other components of the extracellular matrix (ECM), such as fibronectin and collagen.
By heterologous expression of *btaE* or *btaF* in a non adherent-non invasive *E. coli* strain we showed that BtaE confers to *E. coli* the ability to interact with hialuronic acid while BtaF confers the ability to interact with hyaluronic, collagen and fibronectin. These observations are consistent with the adhesive phenotypes of *ΔbtaE* and *ΔbtaF* mutants. Finally, the strain of *E. coli* expressing *btaF* showed an enhanced ability to attach to plastic (hydrophobic), but not to glass (hydrophilic).
These results suggest that both BtaE and BtaF are involved in the adhesion to eukaryotic cells by a process that could involve the interaction with components of the ECM.
Taken together, our observations indicate that adhesion of *B. suis* to host cells would be mediated by multiple adhesins. Alternatively, different bacterial adhesins may contribute to a differential tissue tropism.

6.11. *Bacillus licheniformis* INCREASES GROWTH AND INDUCES SYNTHESIS OF DEFENSE-RELATED TERPENES IN GRAPE PLANTS

[POSTER]

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Plant growth promoting bacteria (PGPR) are bacteria that improve growth and yield in plants, but also help to protect the host against several abiotic and biotic stresses. The mechanisms by which bacteria produce such a benefit for the plant include production of phytohormones, which act as chemical signals that may induce metabolic changes in the host. We have previously isolated and characterized by 16S rRNA gene sequences analysis several bacterial strains from roots and adjacent soil of grape plants, among them *Bacillus licheniformes*. We also found that some of the isolates produce abscisic acid (ABA), indole acetic acid (IAA) and gibberellins (GAs) in chemically-defined medium. The aim of this study was to evaluate growth differences and defense-related terpenes in grape plants inoculated or not with this indigenous bacterium. Roots of 15 days *in vitro* grown grape plants were inoculated with 100 µl of *B. licheniformes* culture ($2x10^7$ $CFUml^{-1}$). After 45 days the plants were removed, growth parameters were measured, and the leaves were extracted for ABA, GAs, IAA and terpene profile determination by capillary gas chromatography coupled with electron impact mass spectrometry (GC-EIMS). Inoculation with *B. licheniformis* increased stem and root length, and enhanced the defense-related terpenes; e.g. the monoterpenes pinene, terpinolene and cineole, as well as the

sesquiterpenes farnesol, trans-α bergamotene, α-farnesene and nerolidol. In leaves of control plants only pinene and nerolidol were detected but in trace amounts. The results suggest that *B. licheniformes* promotes the plant growth by increasing ABA, IAA and GA levels and induces the synthesis of defense-related compounds against potential pathogens.

6.12. Effect of rhizobacterial volatile organic compounds (VOCs) in the production of essential oil of *Mentha piperita L*.

[POSTER]

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Inoculation of plant with PGPR (promoting-growth plant rizobacteria) bacteria is a useful tool to increase biomass. These microorganisms can cause their effect in the plant by producing soluble molecules and/or volatile compounds, (which diffuse in aqueous or aerial media, respectively).
Mentha piperita L was subject to volatile organic compounds (VOCs) produced by different PGPRs such as *Pseudomonas fluorescens* WCS417r, *Bacillus subtilis* GB03 and *Azospirillum brasilense* SP7. The aim of this work was to determinate if the action of VOCs affect the production of essential oil (EO) in *Mentha piperita L*.
Plant growth parameters such as shoot fresh weight, number and area of leaves, were measured. The plants in contact with VOCs of *P. fluorescens* and *B. subtilis* showed a 2-fold increase in shoot fresh weight and area of leaves relative to control plants not exposed to VOCs, without changes in number of leaves. No significant variations in the parameters analyzed were observed in plant exposed to VOCs of *A. brasilense*. The content of essential oil was also analyzed. Gas chromatography was used to determinate qualitatively and quantitatively the composition of monoterpenes. A 2-fold increase in total EO was observed with VOCs of *P. fluorescens*, relative to control. In this plants were also detected a 2,5-fold increase in content of pulegone and a 10-fold increase in content of menthone. The plants in contact with VOCs of *A. brasilense* showed a comparable increased in content of menthone, but was observed a 5-fold decrease of the content or menthol and to a 6-fold decrease of the content of menthofurane relative to control and the others bacterial VOCs analyzed. No significant variation was detected in relative percentage of majority monoterpenes; although plants exposed to VOCs of *P. fluorescens* displayed a major number of compounds in chromatographic profile relative to control and the others bacteria. Plants in contact with VOCs of *B. subtilis* not showed remarkable increase of monoterpenes in spite of the increment presented in biomass.
Even when knowledge of plant promoting-growth activity of VOCs and its mechanism of action are very poor, we consider that bacterias like *P. fluorescens* are able to improve the production of essential oils in aromatic plants such as *Mentha piperita L*.

6.13. Use of *Azospirillum brasilense* co-inoculated with rhizobia: alternative to enhance peanut yields

[POSTER]

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Bacteria of the family Rhizobiaceae interact with leguminous plants in a host-specific manner and form N_2-fixing root nodules. On the other hand, bacteria of the genus *Azospirillum* are free-living, surface colonizing, sometimes endophytic diazotroph and plant growth promoting rhizobacteria (PGPR). They are capable of increasing the yield of important crops growing in various soils and climatic regions.
Arachis hypogaea L. (peanut) is one of the most important legume crops cultivated in the central area of Argentina. Inoculation of peanut is a controversial practice because nodulation by native bacteria is usually assumed to be sufficient. The experiments carried out in this work are in the context of a national project whose main goal is to optimize the symbiotic association between peanut/rhizobia using different co-inoculation methods. In this framework, it has to be provided that there are no problems of mutual exclusion, displacement, or competence between the inoculant strains.
The aim of this research was to study the effect of co-inoculation of recommended and indigenous bradyrhizobia and a PGPR (*Azospirillum brasilense* Cd) on nodulation and symbiotic performance of peanut. There were inoculated at the time of transferring the pregerminated seeds with *A. brasilense* Cd at $1x10^6$ CFU ml^{-1} and rhizobia strains $1x10^5$ CFU ml^{-1}.
All values of root dry weight of inoculated peanut did not show significant changes with respect to the control conditions. A general positive effect on nodulation patterns number of nodules and growth was observed in the presence *Azospirillum*-PC34 strain co-inoculation.
Co-inoculation with recommended bradyrhizobia (SEMIA6144 and TAL1000) and *A. brasilense* Cd caused a significant increase (80 % and 50 % respectively) in the total number of nodules per plant by comparison with the number for plants treated with SEMIA6144 or TAL1000 alone.
Possible mechanisms involved in the influence of *A. brasilense* on this symbiotic system will be discussed.

Sección 7 - Biotecnología y Fermentaciones

7.1. SODIUM AZIDE BINDING TO GOAT PROBIOTIC

[POSTER]

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In a previous paper the effect of probiotic supplementation on the diminution of mutagenic compounds in gut goat were determined *in vivo* (Apas et al 2010). In addition, the diminution of putrescine, a gut cancer marked was observed. In order to study if the antimutagenic effect could be due to the absorption of mutagen by probiotic strains, we study the probiotic binding.
The strains used in the probiotic mix, *Enterococcus faecium* DDE39, *Lactobacillus alimentarius* DDL48, *Lactobacillus reuteri* DDL 19, were challenger in the presence of sodium azide (15µg/mL) during two hours at 37°C. After this time, using the AMES test and the strains *Salmonella typhimurium* TA 100 and *Salmonella typhimurium* TA 98, as biological mutagen indicator, the binding was evaluated.
The results indicated that the binding of *Lactobacillus reuteri* DDL 19, *Enterococcus faecium* DDE39 and *Lactobacillus alimentarius* DDL48 was of 74%, 69% and 59%, respectively using as bioindicator the strain *Salmonella typhimurium* TA 100 and 72, 72 and 44% using as indicator *Salmonella typhimurium* TA 98
The goat probiotic strains used in this study have shown high antimutagenicity and binding properties

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7.2. Identification and isolation of activated sludge bacteria that deflocculate after thermal and phenol-induced stresses and correlation to their auto-aggregation properties

[ORAL]

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The efficient solid-liquid separation is a critical step in activated sludge wastewater treatment. The formation and stability of the biological floc depend on the aggregation of multiple species of bacteria within an extracellular matrix. Wastewater treatment plants, especially those treating industrial wastewater, are exposed to various sources of perturbations that affect the stability of the floc, resulting in increased solids loss and an overall reduction of effluent quality. We hypothesized that thermal and chemical stresses induce deflocculation by affecting the aggregation properties of key members of the activated sludge floc community. To test this proposition we identified, isolated and examined the physiological properties of bacteria that displayed a distinctive detachment from the flocs, following thermal and phenol shocks.
Four lab-scale bioreactors were operated using synthetic sewage, at a controlled temperature of 25°C in a sequential batch mode, without biomass wasting. After ten days of operation, two reactors were exposed transiently to high temperature (50°C) and the other two reactors were subjected to a shock of phenol (1.25 g/L of phenol). In all cases, shock treatments resulted in an immediate increase in turbidity of the supernatants. At the microscopic level, partial deflocculation was confirmed by a marked decreased in the average size of the flocs. The shifts in the bacterial communities were evaluated by denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rRNA genes. Two bands, whose intensity in the supernatant increased significantly after both thermal and phenol shocks, were excised, reamplified and sequenced. Based on the phylogenetic analysis, the two bacteria were classified as *Thauera*, a Betaproteobacteria, and *Sediminibacterium* (phylum Bacteroidetes). Signature portions of the sequenced fragments were targeted with newly designed primers and used in Southern blot DGGE to confirm the response of both bacteria to the deflocculating shocks.
Isolation was attempted by direct culturing in a variety of agar media. Screening was performed by PCR, using the novel specific primers. Several strains that were positive for the *Thauera*-specific primers were isolated, but none of them matched the correct DGGE mobility. PCR-positive colonies of *Sediminibacterium* were found in standard medium (e.g. R2A) supplemented with autoclaved, particle-free activated sludge extract. Growth of the *Sediminibacterium sp.* takes several days and requires several vitamins and oligoelements in the growth medium. Auto-aggregation properties depend dramatically on the composition of the medium. We conclude that *Sediminibacterium sp.* is a floc-forming bacteria that may have an important role in the formation and stability of the activated sludge floc.

7.3. Biosorption of molasses dyes by viable and non-viable microbial biomass

[POSTER]

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Molasses, a by-product of sugar extraction process, is a common raw material used in several industrial fermentations. However, molasses contains colored substances, some of them with inhibitory activities. Thus, innovative technologies, such as biosorption, are needed as alternatives to conventional methods to find inexpensive ways of removing dyes. With respect to dye biosorption, microbial biomass (bacteria, fungi, microalgae, etc.) outperformed macroscopic materials (sea-weeds, crab shell, etc.). The reason for this discrepancy is due to the nature of the cell wall constituents and functional groups involved in dye binding. In this connection, the

major objective of this study was to investigate the potential of viable and non-viable microbial biomasses as biosorbent for the removal of dyes from sugar cane molasses. Materials and methods: Both viable and inactivated by autoclaving biomasses of *Aspergillus niger* ATCC MYA 135, *Saccharomyces cerevisiae* and *Brevibacillus agri* MIR E12 were used. Microorganisms were grown in. potato glucose, LB and YEPD both, solid and liquid for *A. niger*, *B. agri* and *S. cereviciae*, respectively. Decolorization experiments were conducted at 30 °C by shaking molasses solution at 300 rpm during 20 min in the presence of 1.5 or 3.0 % of microbial biomass (wet weight/v). The molasses pH was adjusted at 3, 7 or 9 being its initial reducing sugar concentration 10 g/l. Decolorization capacity was determined by monitoring the absorbance at 475 nm. Results and conclusions: The initial molasses pH value was an important factor for the biosorption process. In the primary screening step, the inactivated biomass of *B. agri* grown in liquid medium showed the highest decolorization capacity (47 %) when a molasses at pH 3 was used. Interestingly, the total amount of reducing sugar did not significantly change. In addition, the production of an extracellular lipase from *B. agri* was significantly increased when the treated molasses was used as carbon source. On the other hand, the best decolorization capacity obtained with either *A. niger* or *S. cerevisiae* was detected with viable biomass growing on agarized medium and using molasses at initial pH 9. These results show the ability of microbial biomass to remove colored substances from sugar cane molasses.
This work was supported by grands PIP-CONICET 297 and CIUNT 26/D409.

7.4. A rapid-BOD bioassay based in lyophilized *Klebsiella pneumoniae*

Se sugiere presentación POSTER

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A microbial amperometric bioassay where *Klebsiella pneumoniae* strain employs ferricyanide as the last electron acceptor while degrading organic compounds has been developed in order to replace the established BOD_5 determination method when active interventions for environmental monitoring and control process are needed.
The ferricyanide-mediated approach has been proposed years ago to overcome O_2 low solubility and long incubation times. Higher solubility of ferricyanide enables the increase of microbial load reducing determination times from days to hours.
The traditional BOD_5 test correlates biodegradable amounts of organic matter with dissolved oxygen consumed by microorganisms in samples after 5 days of incubation. Instead, our bioassay correlates easy biodegradable amounts organic matter with ferrocyanide concentrations, ferricyanide oxidized form, employing electrochemical techniques as amperometry.
The used strain has been isolated from a commercial non-pathogenic consortium and identified by 16S rDNA sequencing process and BLAST sequence alignment tool as *Klebsiella pneumoniae* strain K30 or *Klebsiella pneumoniae* strain K8, both originally isolated from rhizosphere.
Issues as replacement of centrifugation by the use of formaldehyde and avoidance of N_2 sparging of the samples, procedures usually made in ferricyanide-mediated approaches have already been successfully reached using freshly harvested cultures and a Pt electrode in order to develop a field dispositive to determine BOD values in-situ. This bioassay has given accurate $BOD_{K.\ pneumoniae}$ values of real municipal wastewater samples compared with the BOD_5 method. Now, the last improvements to the final dispositive is the employment of a disposable vibratory screen-printed Au electrode using freeze-dried bacteria suitable for a commercial bioassay mass production.
Several lyophilization methods have been assayed. Trehalose 100 mM used as lyoprotectant drove to a bioassay as sensitive as freshly harvested cultures (the slope of a GGA calibration curve was similar in both cases 10.4 and 9.6 -nA L mg^{-1} respectively) even when the growth

rate decreased to 96%.
These results enable the design of a disposable microbial bioassay for rapid BOD determination in wastewaters, treatment waters and water natural sources samples. Even when our *Klebsiella pneumoniae* is an environmental strain, these were the last assays done using it given the existence of pathogenic, antibiotic resistant strains of this species.

7.5. Determining biomass in solid state fermentation cultures of *Aspergillus terreus* during lovastatin production

[POSTER]

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Lovastatin, a class of secondary metabolite typically produced by *Aspergillus terreus* strains, has become the focus of great attention due to its ability to block the *de novo* synthesis of endogenous cholesterol, which is nearly 2/3 of human total body cholesterol. Additionally, statins present potential applications for the treatment cancer, Parkinson and Alzheimer diseases, as well as viral and fungal infections, because of their capability to inhibit mevalonate derivatives biosynthesis. High lovastatin production yields may be microbiologically obtained by implementing culture systems in solid state. Since biomass levels are closely related to lovastatin productivity, appropriate methods for the determination of fungal growth are usually required. However, one of the main drawbacks is that the direct analysis of biomass by dry weight determination is not possible in solid state fermentation systems. Therefore, this work was aimed at comparing two indirect methods for biomass determination: one based on the N-acetylglucosamine content, a component of the fungal cell wall, and the other one, based on the ergosterol content, a fungal cell membrane steroid. N- acetylglucosamine was assessed by the colorimetric method with MBTH reagent, after sample acid hydrolysis. Extracted ergosterol, previous sample saponification, was analyzed by RP-HPLC with a photodiode array detection (PDA) system. A high correlation coefficient (r^2: 0.98) was found between fungal biomass dry weight and the ergosterol content as determined by HPLC, confirming the validity and reliability of this standardized technique. Additionally, the ergosterol method showed to be highly reproducible and time-efficient. On the other hand, the obtained results according to the N-acetylglucosamine method denoted that this technique would not be applicable to any solid culture system due to the false positive reactions (interference) observed for abiotic controls in complex systems with certain solid substrates.

7.6. Development of bioprocess using immobilized lactic acid bacteria

[ORAL]

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Biocatalysis is an interesting alternative to replace traditional multistep chemical methods because it can develop simple reactions without environmental impact and satisfactory stereo and regiospecificity.

Enzyme immobilization is the most important route that can provide enzyme features under industrial conditions. Immobilized biocatalysts show high chemical and mechanical stability and can be separated from medium for their reuse in consequence reaction. Additionally, these biocatalysts allow reactors' design with easy handling and control.
In general, enzymatic synthesis of nucleosides is catalyzed by nucleosides phosphorilases (NPs). In lactic acid bacteria (LABs), this reaction could be carried out for constitutive nucleoside 2-deoxyribosyltransferase (NDTs, 2.4.2.6) that catalyze the exchange between the purine or pyrimidine base of 2-deoxyribonucleosides and free pyrimidine or purine bases. This reaction proceeds by a ping-pong bi-bi mechanism with formation of a covalent deoxyribosyl-enzyme intermediate. NDT provides an alternative to NPs because catalyze the same process in one-pot reaction.
In this work, we did a screening with different strains of LAB and we selected the best strain to develop biocatalytic systems using immobilized whole cells and protein extract. These immobilized biocatalysts showed high hydrolytic activity, near of 100% in 3 h; and stability, with negligible loss of microorganisms (10%) after 50 h of use. Moreover, we used the biocatalytic systems to obtain halogenated nucleosides. These compounds are frequently used as very powerful chemotherapeutic agent.
The aim of this study was to develop immobilized biocatalysts to obtain compounds with potential antitumoral activity using a nucleoside 2-deoxyribosyltransferase (NDT). This biotransformation is a promising process because the reaction takes place under very mild conditions and offers a smoother, cheaper and environmentally friendly methodology.

7.7. DESIGN AND CONSTRUCTION OF GOLD-SELECTIVE BACTERIAL BIOSENSORS

[ORAL]

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Gold (Au) is continuously demanded both as a valuable commodity and by modern industry because of its unique physicochemical properties that makes it appropriate for many applications. Most analytical methods used to evaluate Au content in economically suitable deposits require sophisticated instrumentation and trained personnel. The use of whole-cell bacterial biosensors has emerged as a simple and cost-effective alternative to conventional detection procedures for several metals, including copper (Cu). However, these bioreporters are usually not selective enough and detect a group of chemically related metals. In this study, we developed whole-cell bacterial sensors that selectively detect Au ions. These sensors are based on the regulatory unit controlling resistance to this toxic metal in *Salmonella enterica* serovar Typhimurium. In the biosensor, expression of the *gfp* reporter gene is directed by the *Salmonella golB* promoter which is transcriptionally controlled by GolS. The sensor/regulatory protein distinguishes Au(I) from either Cu(I) or Ag(I) ions, which differentiates it from other monovalent metal sensors such as CueR that binds the three metals with similar affinity. In order to be used as a biotechnological tool, we introduced the *Salmonella gol* locus in the chromosome of a non-pathogenic *Escherichia coli* strain. The designed bacterial sensor exhibits low background fluorescence, high signal-to-noise ratio, and improved sensitivity for detecting Au ions in a wide range of concentrations (from 5 to 470 nM) with an estimated detection limit of ~33 nM, equivalent to ~ 6 µg l^{-1} (or parts per billion) Au(I). Due to the intrinsic characteristics of the regulatory protein, the fluorescent Au-biosensor exhibited also minimal interference by chemically related metals such as Cu or Ag that are commonly found associated with Au in deposits. These highly specific and sensitive Au detectors will allow the development of rapid and robust screening tools to improve discovery and extraction procedures for this precious metal.

7.8. Recycling of organic waste generated in gastronomic activity

[POSTER]

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The solid waste generated in gastronomic activity, in our country, represents, a health problem. The solution could begin with a differential collection of these wastes and their recycling for specific purposes. The aim of this study was to design a culture medium for bacteria and fungi, whose nutrients came from organic waste generated in restaurants. The average percentage composition was: Crude protein 0.77 ± 0.18, dry matter: 1.53 ± 0.05, carbohydrates: 1.00 ± 0.03 and ash: 0.95 ± 0.14. This medium was used for biomass (bacteria and yeasts) and no significant differences ($p > 0.05$) were found in relation to commercial culture media, when *Lactobacillus plantarum* ATCC7469, *Lactobacillus rhamnosus* O236, *Lactobacillus* sp L46, *Enterococcus* sp C999, *Staphylococcus aureus* E2, *Shigella sonnei* S3, *Salmonella enteritidis* S4, *Saccharomyces cerevisiae* L1 and *Schizosaccharomyces* sp L2 were cultivated. The only organism included in this study, unable to develop in the culture media designed was *Streptococcus* Pa E1. The *Streptococcus* genus requires, for an optimal performance, complex media enriched with blood. Since the purpose of our study was to consider a minimal medium, blood addition was not considered, even knowing the nutritional requirements of this genus of bacteria.
Our results allowed infer the conversion of organic waste in an alternative culture medium with a good yield in biomass. In addition, this medium showed some characteristics as accessibility (in any region of the country can be elaborated), low cost because, for processing, wastes generated in quantities and often highly were required; versatility (allows the growth of Gram-negative and Gram positive bacteria and also yeast), The single cell proteins have an important nutritional value due to high content of vitamins and lipids. Moreover these proteins present, in general, a whole set of essential amino acids. For this reason, yeast cells are usually used in animal feeding. The studies about technological process for elaborating the designed medium are in progress.

7.9. Immobilized extremophile microorganisms for using in Green Chemistry Processes

[ORAL]

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Bioprocesses have become widely used in several fields of biotechnological industry, offering several advantages over conventional chemical methods of production. These biocatalytic processes use renewable resources as raw materials and greater product quantities can be produced with less energy consumption.
Extremophile microorganisms have been extensively used in biocatalysis and their technological interest is linked to their potential use in extreme conditions of pH, temperature and saline concentration.
The use of whole cells in biocatalysis can be improved by an immobilization process. This

allows viability retention of the cells and their activity as catalysts, protecting them from the shear forces of the process. Also, the reclusion of microorganisms in a porous matrix allows the use of continuous process, improving the final product purification methodology.
In this work, different strains of *Geobacillus* were tested to obtain several antiviral compounds using green chemistry processes. High yields (near to 90%) were obtained in short times of reaction and without sub-products. These compounds and their derivatives can be used as prodrugs or antiviral agents for treatment of HCV, SARS, HBV and HIV because their ability to inhibit viral replication.
The best strains were immobilized using different hydrogels (acrilamide and agarose). Some parameters as thermal stability, mechanic resistance, cell retention, storage and reusability were evaluated. The yields were similar to those obtained with free cells. The immobilization technique allows reusing the biocatalyst for more than 60 times without significant loss of catalytic activity.
We have been able to develop immobilized extremophile biocatalysts for potential in Green Chemistry application.

7.10. Development of an anti-inflammatory fermented milk product using IL-10 producing *Lactococcus lactis*

Se sugiere presentacion POSTER

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Inflammatory bowel diseases (IBD) have become one of the major gastroenterologic problems in the Western world. Although the exact etiology of these disorders has not yet been elucidated, it is known that a cytokine imbalance is involved. Interleukin-10 (IL-10), an important anti-inflammatory cytokine, is thus essential in preventing IBD.
Oral treatment with IL-10 is difficult because of its sensitivity to the harsh conditions in the gastrointestinal tract (GIT), and systemic treatments are related to undesirable side effects. On the other hand, intragastric administration of genetically modified *Lactococcus (L.) lactis* producing IL-10 in situ in the intestine was shown to be effective in the treatment of IBD; however its use is hindered by the sensitivity of these bacteria to freeze-drying and their poor survival in the GIT.
The aim of this study was to develop a fermented milk product using a strain of *L. lactis* that produces IL-10 under the control of the xylose inducible expression system (XIES) and evaluate its anti-inflammatory effect using a murine colitis model.
Two genetically engineered strains of *L. lactis* NCDO2118 (hereafter referred as the wild-type (Wt) strain) harboring the XIES to target rodent IL-10 to the cytoplasm (Cyt strain) or to the extracellular medium (Sec strain) were used.
Reconstituted sterile nonfat milk containing 1% xylose was inoculated with one of these strains (Wt, Cyt or Sec) at a concentration of 1% (v/v) and incubated statically for 16 h at 30°C. IL-10 concentration in the fermented milks was determined by ELISA.
The anti-inflammatory potential of the fermented milk products was evaluated using a chemically induced trinitrobenze sulfonic acid (TNBS) colitis murine model.
Animals inoculated with TNBS were sub-divided into 4 experimental groups: 1) Inflammation group (TNBS group) without special feeding; 2) TNBS-Wt group, where mice received milk fermented with the Wt strain; 3) TNBS-Cyt group, where mice received milk fermented with the Cyt strain and 4) TNBS-Sec group, where mice received milk fermented with the Sec strain.
Microbial translocation to liver, macroscopic and histological damage of the intestines and cytokine production profiles in fluids and tissues were evaluated in each group.
Mice that received milks fermented by *L. lactis* strains producing IL-10 in the cytoplasm (Cyt strain) or secreted to the product (Sec strain) possessed anti-inflammatory properties as shown by lower damage scores in their large intestines, decreased IFN-γ levels in their intestinal fluids

and lower translocation to liver compared to mice receiving milk fermented by the Wt strain or those not receiving any treatment.
The use of fermented milks as a new form of administration of IL-10 producing *L. lactis* was effective in the prevention of IBD in a murine model. These results could lead to the development of novel functional or therapeutical foods.

7.11. Bioprospection of Argentinean native microalgae with selective traits for next generation biofuels

[ORAL]

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Research and development of alternative renewable sources of energy has increased in recent years mainly due to the depletion of fossil fuels and global warming, presumably as a consequence of carbon dioxide accumulation in the atmosphere. Biofuels are carriers of solar energy collected in the form of biomass. Microalgae biomass is one of the most promising feedstocks for third generation biofuels because of their relative high rate of oil-rich biomass accumulation per unit of space and time and the possibility of using marginal lands for large scale cultivation.
Demonstration-scale projects in developed countries have confirmed the technical feasibility of the approach. However, most of the studies have agreed that the strategy is still not economically competitive using the available microlgae strains and technology according to the current prize of oil and laws for environmental protection.
Pioneering projects aimed at exploring native microalgae for desirable properties for biofuels production have started in the most developed countries and have regain enthusiasm during the last years spreading towards as much territory as practical.
As a contribution to such a major task, we have started a program for the bioprospection of oleaginous microalgae starting from southeastern Buenos Aires. At present we have isolated more than thirty strains in monoalgal culture, while more than fifteen of them are maintained as axenic isolates. Most of them have been identified by classical taxonomy (morphological characters) what has allowed a preliminary identification of strains at the level of genus as *Chlorella* spp., *Scenedesmus* spp., *Desmodesmus* spp., *Chlamydomonas* spp., *Ankistrodesmus* spp., *Haematococcus* spp., *Pseudokirchneriella* sp. Additionally, identification has been complemented by sequencing of a ribosomal intergenic region. This approach showed that while some of the strains are identical or very close relatives to strains described in other places, others displayed differences compatible with the isolation of novels strains, what is currently under confirmation. We have used a protocol for a relative high-throughput analysis of neutral lipids accumulation based on the fluorescence of the dye Nile Red what has allowed the identification of the up-till-now most promising strains from our collection. These strains accumulate lipids at more that 30 % (w/w) of dry biomass under high light intensity and nitrogen deficiency as confirmed by gravimetric determination of total and neutral lipids.
Some of the selected strains have low to very low doubling times of 5 to 9 h under laboratory conditions, using a diluted mineral medium, what highlights its potential for oil-rich inexpensive-biomass production for next generation biofuels and carbon dioxide sequestration. These results stimulate further bioprospection of the national natural resources searching for novel strains potentially useful for biofuels or other applications of inexpensive biomass.

7.12. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF PARASTREPHIA LUCIDA (ASTERACEAE), A PLANT SPECIE FROM THE ARGENTINE PUNA.

[POSTER]

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Parastrephia lucida (Meyen) Cabrera is a plant species that grow in the Argentine Puna, a place where extreme conditions like high altitude (3000-4200 meters above sea level), high exposure to UV radiation, low concentration of oxygen, temperature variations (30°C/day) induce particular adaptations on organisms that allow them to survive under these conditions. In the folklore medicine it is used as an antimicrobial, antipyretic and anti-inflammatory plant, also for bone fractures and hematomas. The antibacterial activity of ethanolic extracts on pathogenic human bacteria was previously demonstrated. The aim of this work was to isolate of fractions with antibacterial and antifungal activities from the aerial parts of them. Methanolic extract (ET); aqueous (Aq) and dichlormethanic (DCM) subextracts were obtained. The Minimal Inhibitory Concentration and Minimal Bactericidal Concentration (MIC/MBC) against methycillin resistant *Staphylococcus aureus*, methycillin resistant Staphylococcus coagulase negative, *St. aureus* ATCC29213, *Enterococcus faecalis*, *E. faecalis* ATCC29212, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *E. coli* ATCC35218, *Candida albicans*, *C. tropicalis*, *C. parasilopsis*, *C. glabrata* and *C. guilleirmondii* were determined.
DCM and Aq subextracts showed antimicrobial activity against all Gram positive bacteria and fungi strains with MIC values between 100 to 400 ug/ml. DCM subextract showed the lowest MIC values for almost all the strains tested.
According to these results, the DCM subextract was chosed for the isolation of the compound/s responsible of the antimicrobial activity. A chromatography column with Sephadex LH-20 on the DCM subextract was made and seven fractions (A-G) were obtained. The fractions C, D and E showed MIC values g/ml against Gram positive bacteria. Fractions F and G were between 100 and 400 active not only against Gram positive bacteria, with MIC values of 100 ug/ml, but also against Gram negative bacteria with MIC values between 200 and 400 ug/ml. For all strains of Candida we found MIC and MBC values between 100 and 400 ug/ml.
These results show that as we advance in the isolation of the subextract, the active compounds are more concentrated and show higher activity than the phytocomplex.

7.13. KILLER ACTIVITY OF COMBINED TOXINS FROM DIFFERENT *Saccharomyces cerevisiae* STRAINS

[POSTER]

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Some yeast strains are capable to produce proteinaceous or glicoproteinaceous toxins that can kill or inhibit the growth of other microorganisms. In winemaking, these yeasts are being used increasingly as fermentation starters, because of their possible predominance during the process. This could ensure the obtaining of products with controlled quality. We evaluated the killer activity of different *Saccharomyces cerevisiae* supernatants, obtained from cocultures or mixing monocultures (ratio 1:1) of autochthonous killer strains (Cf5, Cf8, Cf13, Cf19, Cf21, M12) and a reference killer yeast (*S. cerevisiae* type K1), against the sensitive strain *S. cerevisiae*

P351. In all combinations *S. cerevisiae* Cf8 was utilized. This strain was selected in a previous studies as the best killer toxin producer. Supernatants were prepared from yeasts inoculated into YPD-MB broth (pH 4.2) during 96 h at 18 ºC. The culture supernatants were sterilized by filtration and enriched with sterile 10X YPD-MB broth (final 1x). The filtrates were inoculated with the strain P351 and incubated at 18 ºC, and their killer effects were evaluated by OD_{600} at 0, 18 and 36 h. Supernatants from monocultures were used as killer activity control, and non fermented medium as growth control. Killer activity was expressed as *% reduction of absorbance* measured against growth control. The highest killer activities were observed with the mix of the M12 and Cf8 monoculture supernatants, which showed an increase of 19.14 and 55.58% compared with the M12 and Cf8 controls, respectively. Also the supernatant from the coculture Cf19-Cf8 showed an increase of 28.94 and 55.74% with regard to the Cf19 and Cf8 controls, respectively. The other assayed strains did not exhibit significant differences with the controls. This study evidenced that the killer activity of *S. cerevisiae* Cf8 strain could be improved by combining different toxin-producing strains, being this effect strain-dependent as well as culture-condition dependent.

7.14. Manipulation of the global regulator CreC to increase the synthesis of bioproducts in *Escherichia coli*

[POSTER]

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E. coli CreBC is a two-component system involved in the regulation of carbohydrates catabolism. Previous studies in our laboratory found that mutants having constitutive allele *creC510* (Const), and a deletion involving the global regulator *arcA*, express a particular phenotype in which high levels of reduced equivalents and increased oxygen uptake are two outstanding characteristics. ArcA controls redox balance in the cells, affecting many metabolic steps. To have a deeper knowledge of the role of CreC on carbon metabolism, and to study its potential use for metabolic manipulations, we decided to study different mutations in *creC* in an *arcA*$^+$ (wild type for redox regulators) background.
For this purpose, we constructed two strains from K1060 (wild type for both *arcA* and *creC* genes), K1060C and DC1060, carrying *creC510* (Const) and *ΔcreC* mutations respectively. DC1060 was obtained by the Datsenko technique, while K1060C was constructed by combining this technique with P1 mediated transduction, so as to minimize unwanted modifications in the *creABCD* operon.
Having this set of three strains, we performed metabolic assays in shaked flasks. The cells were grown in M9 supplemented with glucose and glycerol. Aeration was also manipulated to create low or high oxygen availability. Basic biomass parameters were measured, including cell dry weight (CDW) in 24-h coulters, growth curves and μmax in the four different growth conditions obtained combining each carbon source with each aeration level. There was a small but significant increase in μmax in the CreC deletion mutant, DC1060, compared to the other two strains, when grown in low oxygen availability, independently of the carbon source. In contrast, when the aeration was maximal, DC1060 had a lower μmax than the other strains. K1060 and the *creC* constitutive mutant, K1060C had, in all these conditions, a similar maximal growth rate.
To make a metabolic profile of all three strains, metabolic products in 24-h culture supernatants were analyzed by HPLC, allowing the identification and quantification of many organic acids secreted by the cells. DC1060 produced, both with glycerol and glucose, more succinate and formate but less lactate, when the cultures were grown under low aeration conditions. Citrate synthesis was also higher for DC1060 but just when the carbon source was glycerol. In high oxygen availability, the only significant difference was seen in acetate production, where K1060 and K1060C produced the same levels of this metabolite, but more than DC1060.
These results strongly suggest that CreC has an important role in carbon metabolism, independently of other mutations, such as those in the redox regulator ArcA, making it an

excellent candidate for genetic manipulations to increase the production of metabolites with industrial applications, such as succinate.

7.15. BIOSPECKLE AS A NEW TOOL TO DISCRIMINATE BETWEEN FUNGI AND BACTERIA

[POSTER]

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Chemotaxis is the movement of microorganisms towards or away chemical gradients. This phenomenon has a relevant importance in bioremediation, plant physiology, veterinary, biotechnology and other disciplines.
In this work, we present a further development of dynamic speckle methods for the study of biological activity in bacteria under swarming assays. We attempt to determine biological activity differences between bacteria and other microorganisms. Although there are different optical methods to detect bacteria accumulations, none of them can differentiate between degrees of motility in the clusters of one image.
Experiments using the bacteria *Pseudomonas aeruginosa*, *Pseudmonas putida* F1 CheA and the fungi *Phanerochaete chrysosporium* RP-78, have demonstrated that the biospeckle can differentiate fungi from bacteria, and different sort of bacteria in one in situ surface screening. This was achieved for the biospeckle discrimination of different degrees of motility of bacteria and between the movement of bacteria and fungi. The biospeckle method offers the advantage of being fast, non invasive, inexpensive and accurate.

7.16. Discrimination of Gram-Positive and Gram-Negative Bacteria In Milk by Means of Voltammetry Analysis

[POSTER]

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Milk represents the main dairy product, and it is consumed all around the world, playing a fundamental roll in international commerce; this gives milk a great economic importance. Electrochemical techniques have various applications and have been widely used in food analysis as wine, coffee, fruit juices or milk examination. Voltammetry is an electrochemical technique that seems to have several advantages such as high sensitive, versatility, simplicity and robustness. The current registered as a result of the potential applied depends on the composition of the sample and the presence of electro-active molecules. This electrochemical method has been used for classification of Gram-negative and Gram-positive intestinal bacteria and in complex media like milk for monitoring milk freshness. In this work, two planar working

electrodes (WEs) were done and tested. The primary objective of our investigation is to develop a method to detect udder colonizing bacteria and contribute to veterinary diagnosis. Milk samples were made with rehydrated ($100gL^{-1}$) powdered skimmed milk (La Serenisima, Arg.). Determination of bacteria in order to find a practical method to be used at dairy farms, where rapid identification could be an important advantage over traditional (culture) microbiological methods, were done. As a probe concept, two bacteria were used: *Escherichia coli* K12 (Gram-negative) and *Bacillus cereus* (Gram-positive). Microorganisms were growth in LB medium at 37 ºC. After centrifugation, the pellet was resuspended in rehydrated skimmed milk in order to simulate contaminated milk samples with an OD of 4.5 (± 0.1). Electrochemical experiments were performed with a standard three electrode systems using Au and a Pt WE. A saturated Ag/AgCl was used as reference electrode, and a stainless steel helicoidally electrode was used as counter electrode. Cyclic voltammetries were performed with a potentiostat (Gamry 300) under control of its own software. Measuring conditions were a scan rate of 10 mV/seg and potential window from -0.5 to 1 V. Direct observation of cyclic voltammetries shows mayor complexity (number of peaks) when Au WE was used. The anodic peak at ca. 680 mV is more important at Gram-positive bacteria. Cathodic peaks at ca. 0 mV shows more complexity is Gram-positive voltammogram. The anodic peak at ca 300 mV looks similar at both bacteria. When the data was analyzed by principal component both bacteria and milk control samples were separated in different groups. These preliminary results demonstrate that practical use of this method could be possible. More experiments with increasing number of Gram-positive and -negative strains will be accomplished to demonstrate and validate the suggested method.

7.17. ANTIOXIDANT ACTIVITY OF SCLEROGLUCANS FROM *Sclerotium rolfsii* ATCC 201126

[POSTER]

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Carbohydrate polymers have been reported to modulate in vitro and in vivo inflammatory responses. β-D-(1,3)-glucans, such as scleroglucan, may possess free radical scavenging activity. If glucans are free radical scavengers then, it might partly explain the ability of these ligands to modulate inflammatory responses. In the present work, the free radical scavenging activity of lab-fermenter scale produced scleroglucans from the filamentous fungus *S. rolfsii* ATCC 201126 (EPS I, EPS II and EPSi) and a commercial scleroglucan (LSCL) was examined. The study involved the use of the phycoerythrin/AAPH fluorescence assay based on the method of Glazer. The antioxidant properties of both triple and single helix scleroglucan conformations were also compared. The oxygen radical absorbance ability of these carbohydrate polymers in aqueous medium was compared and contrasted with commercial antioxidant agents (PDTC and Trolox). As a general rule, single helix conformation showed greater antioxidant ability than triple helix. With the exception of LSCL, all tested scleroglucans when treated with 0.2 N NaOH (corresponding to the single helix conformation) exhibited a variable degree of free radical scavenging activity (EPS I > EPSi > EPS II), and the antioxidant effect was concentration-dependent (optimal at 0.25 µg/mL). The lower values of EC_{50} (the dose that corresponds to a 50% antioxidant ability) exhibited by alkali-treated samples allowed to confirm their marked antioxidant activity (EC_{50}= 121-194 µg/mL). EPS I single helix conformation showed an antioxidant activity comparable to PDTC (equivalent to ~84%) and superior to Trolox (> ~160%). Meanwhile, the EC_{50} values obtained for native samples (EC_{50}= 805-5920 µg/mL) denoted the weak free radical scavenging activity exhibited by triple helix. Polysaccharide antioxidant effects have been already correlated with the monosaccharide composition. However, polymers are significantly better free radical scavengers than either of the monosaccharides. This fact would indicate that the polymeric structure confers additional free radical scavenging ability. According to our results, the antioxidant activity herein demonstrated for scleroglucans from *S. rolfsii* could be not only a consequence of their glycosidic composition but also a property associated to the

conformational state of the polysaccharide. The demonstrated antioxidant ability would represent a further contribution to the great biological potential of the produced fungal polysaccharides.

7.18. Study of folate production by wild-type strains of lactic acid bacteria for the elaboration of novel bio-enriched foods

[POSTER]

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Folate, an essential B-group vitamin, is involved in many metabolic pathways such as energy usage and DNA and RNA biosynthesis. Human beings cannot synthesize folate so an exogenous supply of this vitamin is necessary to prevent nutritional deficiency. Extensive researches have shown that many health benefits are associated with increased folic acid intakes. However, numerous studies have put into evidence that high intakes of this synthetic form of folate can cause adverse effects in some individuals, such as the masking of hematological alterations of vitamin B_{12} deficiency. This does not occur with natural folates present in foods or produced by microorganisms. Currently, many researchers are evaluating novel strategies to increase concentrations of naturally occurring folate in foods. The proper selection and use of folate-producing lactic acid bacteria (LAB) is an interesting alternative to increase “natural” folate levels in foods. Aim: To find and select wild-type LAB able to produce folate and to study its production. Experimental: Screening of 43 strains of *Lactobacillus (Lb.) bulgaricus* and 52 *Streptococcus (St.) thermophilus* was performed and different parameters were evaluated such as: a) growth in absence and presence of folate in a vitamin-deficient medium, b) total concentration of folate, c) concentration of secreted and intracellular folate, d) pH of the folate-free medium, e) absorbance at 580 nm, and f) UFC/ml of the selected folate-producing strains. Folate concentrations were estimated by using a *Lb. rhamnosus* NCIMB 10463 microbiological assay. Results: from the 95 analyzed LAB only 37 strains (5 *Lb. bulgaricus* and 32 *St. thermophilus*) produced varying amounts of folate (between 19.3 ± 0.1 to 144.4 ± 0.1 µg/l) when growing in absence of folates. From these 37 strains, only 2 *Lb. bulgaricus* secreted significant amounts of folates (>80.0 ± 0.1 µg/l) and 11 *St. thermophilus* showed the highest intracellular vitamin concentration (>15.0 ± 0.1µg/l). From these results, four LAB (2 *Lb. bulgaricus* and 2 *St. thermophilus*) were selected for future studies. Conclusion: native strains of LAB were able to produce significant amounts of folate and it was shown that the vitamin production was not directly associated to microbial growth. The selected strains could be used in the elaboration of novel bio-enriched fermented products.

7.19. OXIDATIVE TREATMENT INHIBITS THE GROWTH OF *Penicillium expansum*

[POSTER]

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Argentina is the main apple producer in the Southern hemisphere. Blue mold rot caused by *Penicillium expansum* is the most important postharvest disease of apples, causing major economic losses. Synthetic fungicides are used to control this disease, but their effectiveness

can be seriously compromised by the appearance of certain resistant biotypes of *P. expansum*. Therefore, alternative methods to the conventional fungicides would be desirable to control blue mold rot. In our laboratory, we have standardized a sequential oxidative treatment (SOT), which is able to inhibit the growth of *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum*, causal agents of citrus postharvest diseases. SOT consists in a first incubation with NaClO followed by a second incubation with H_2O_2 and $CuSO_4$. The combination of these compounds in the SOT generated a synergistic effect. The effects of the oxidizing compounds on *P. expansum* growth and conidia germination were subsequently analyzed. The Minimal Inhibitory Concentration (MIC) for each SOT compound was determined and conditions for an oxidative treatment against this fungal pathogen were established. Results showed that *P. expansum* was more sensitive than *P. digitatum* and *P. italicum*, since in vitro growth was completely inhibited by a brief incubation of 2 min with 5 ppm NaClO, 6 mM $CuSO_4$ and 100 mM H_2O_2. Moreover, under lethal and sublethal conditions, conidial germination inhibition showed a direct correlation with oxidative conditions. Thus, we demonstrated that this oxidative treatment could be used as an alternative method to inhibit *P. expansum* conidial growth.

7.20. EVALUATION OF LACTOBACILLI RESISTANCE TO FREEZE DRYING FOR THEIR POTENTIAL USE AS PROBIOTIC ADDITIVES FOR NEWBORN CALVES

[POSTER]

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Probiotic additives in animals feed are a new alternative to replace antibiotics as growth factors. The design of a new probiotic product to be used in newborn calves was the main objective of our group. After the selection of the bacterial strains based on their beneficial properties, some technological preservation methods were evaluated, being freeze-drying one of the most frequently used processes for the survival and storage of microorganisms for a market product. Nevertheless, the method involves critical injuries to bacteria that could affect their viability. The aim of this work was to freeze dry six different strains of lactobacilli (with beneficial characteristics) with different cryoprotectants (skimmed milk, milk whey and lactose), to evaluate their survival to the process and their viability during six months of storage. The microorganisms were evaluated both individually and in mixtures, based in their beneficial properties. Milk-lyophilized product was added with,minerals, vitamins and inulin to advance in the design of the final product. *Lactobacillus johnsonii* CRL1693, *L. murinus* CRL1695, *L. mucosae* CRL1696, *L. salivarius* CRL1702, *L. amylovorus* CRL1697 and *Enterococcus faecium* CRL1703 were freeze dried with the different lyoprotectors and later stored at 4ºC for six months. Before and after the process, the number of viable cells was determined in selective media based on the microbial sensitivity to antibiotics. The efficiency of the process was calculated for all the combinations assayed. Results showed that the resistance of each strain to the freeze drying process depended on the intrinsic characteristics of each particular strain, the conditions of the process and the cryoprotector employed. *E. faecium* CRL1703 was the most resistant strain to the freeze drying process and conservation, whilst *L. mucosae* CRL1696 and *L. amylovorus* CRL1697 were more resistant to lyophilization than the other assayed lactobacilli. *L. johnsonii* CRL1693 and *L. amylovorus* CRL1697 were highly affected during storage. No viable cells were recovered from *L. johnsonii* CRL1693 at the end of the assay. Among the cryoprotectors, skimmed milk was the most efficient, not only during the process, but also during storage for most of microorganisms. The addition of lactose did not surpass the protection of milk and similar results were obtained with milk whey. The survival of freeze-dried mixed strains was lower in all the experiments. The viability was also affected during storage: only the combination of *L. salivarius* CRL1702 and *L. mucosae* CRL1696 with 10% inulin

survived after six months of storage. The results obtained showed that milk would be the optimal cryoprotective agent for lactobacillus lyophilization, which must be individually freeze-dried and later combined for the development of a probiotic product for calves.

7.21. Production of 1,3 propanediol in recombinant *Escherichia coli*

[POSTER]

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1,3- propanediol (1,3-PD) is an industrial chemical with numerous applications in cosmetics, foods, lubricants, medicines, and that is used as a monomer for polycondensations in order to produce polyesters, polyethers, and polyurethanes. 1,3-PD is primarily produced through chemical synthesis from petroleum derivatives, with high production costs, but it can also be obtained by microbial fermentation, an environmentally friendly process with lower costs. In the last years there has been a growing interest in using glycerol as substrate for bacterial fermentations, because it is generated in large volumes as a by-product in biodiesel production. Besides, given the highly reduced state of carbon in glycerol, its conversion to fuels or reduced products could result in higher yields than those obtained with the use of common sugars. Species from the genera *Citrobacter, Clostridium, Enterobacter, Klebsiella* and *Lactobacillus* are able to produce 1,3-PD from glycerol, but these microorganisms are not suitable for industrial production because of their particular culture conditions, and in the case of pathogens, because special safety precautions are required . The conversion of glycerol to 1,3-PD by recombinant *Escherichia coli* eliminates these problems. The abundant available information on the genetics and metabolism of this strain, added to the ease to manipulate it, make it an ideal model to study the effects of different mutations in genes involved in metabolism and global regulators, in order to improve the synthesis of this bioproduct. *Escherichia coli* strain K1060, a strain previously used for production of other bioproducts such as polyhydroxybutyrate (PHB) and ethanol, was transformed with plasmids that overexpress genes dhaB1B2, from *Clostridium butyricum* and yqhD from *Escherichia coli*. The gene dhaB1 encodes a vitamin B12-independent glycerol dehydratase, that enables the conversion of glycerol to 3-hydroxypropionaldehyde (3-HPA) and dhaB2, encodes an activating factor for this enzyme. To complete the pathway for the synthesis of 1,3-PD, an endogenous NADH-dependent oxidoreductase encoded by yqhD, transfers a reducing equivalent from NADH to 3-HPA, yielding 1,3-PD. The recombinant strain constructed was confirmed to produce 1,3-PD by fermentation of glycerol. The 1,3-PD production and growth rate of the constructed recombinant strain was tested in aerobiosis and microaerobiosis in flask cultures. The production was also studied in batch fermentations in a bioreactor. Production of 1,3-PD was determined by gas chromatography (GC). Because the synthesis of 1,3-PD requires reducing power, the effect of mutations in global regulators involved in the redox state, such as *arcA*, will be further analized in order to improve 1,3-PD production.

7.22. First steps of carbon metabolism pathway in continuous cultures of *Gluconacetobacter diazotrophicus* under different nutritional status

[POSTER]

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An acid tolerant N_2-fixing bacterium, *Gluconacetobacter diazotrophicus*, was reported to be associated with sugarcane. It is thought to provide significant amounts of N to this and other crops. This bacterium is able to grow on high sucrose concentration (10%) but this sugar cannot be transported or respired by *G. diazotrophicus*. This organism secrets an extracellular enzyme, levansucrase (LsdA), that hidrolyses sucrose into fructose and glucose. Glucose is the main carbon and energy source for *G. diazotrophicus*. The first spteps of its metabolism involve a periplasmic glucose oxidation mediated by a pyrrolo-quinoline-quinone (PQQ)- linked glucose dehydrogenase (PQQ-GDH).
In this work we have studied the influence of diverse growth conditions on the regulation of the oxidative pathway of sucrose metabolism in *G. diazotrophicus*. Chemostat cultures of *G. diazotrophicus* PAL 5 were grown using the modified LGIM medium at 30ºC. A 2-l fermentation unit with a working volume of 1.0 l was used and dilution rate was adjusted at 0.05 h^{-1}. The pH was automatically maintained at 6.0 using either 1N NaOH or 1N H_2SO_4. Dissolved oxygen was continuously measured and maintained at the desired level of air saturation by varying the agitation speed. Different nutritional conditions were tested; i.e. limited carbon source (sucrose 20 g/l) and excess of carbon source (sucrose 100 g/l); BNF (N_2) under microaerophilic conditions (dissolved $O_2 \leq 2\%$) and NoBNF (3 g/l $(NH_4)_2SO_4$). Under steady-state conditions biomass, CO_2 production, O_2 consumption and enzymatic activity were measured.
Activity of LsdA increased with sucrose concentration in the culture medium. Surprisingly PQQ-GDH, although considered the main route for glucose catabolism in this organism, was not detected in cultures with 20 g/l of sucrose, but significant activities were found with sucrose 100 g/l. The same was observed for GaDH. These results suggest that, in the presence of sucrose, *G. diazotrophicus* seems to express a different metabolic pathway for C-metabolism in relation to cultures performed with glucose. It seems that PQQ-GDH was expressed only when significant glucose concentration could be detected in culture supernatants (as found in cultures with 100 g/l of sucrose) but not under conditions where free glucose was undetectable (as in cultures with 20 g/l sucrose). Moreover, gluconic acid was only detected in cultures performed with sucrose in excess.

7.23. Fructans production by cell-free sistems from *Gluconacetobacter diazotrophicus* culture supernatants

[POSTER]

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The main commercial production of fructans (levans and fructooligosaccharides (FOS)) comes from enzymatic transformation of sucrose by bacterial or fungal enzymes. The development of more efficient enzymes, with high activity and stability, is required and this has attracted the interest of biotechnologists and microbiologists. Fructans have received particular attention because of their excellent biological and functional properties for the use as prebiotic compounds. They are used as components of functional foods and are generally recognized as safe by the FDA (Food and Drug Administration – U.S). Likewise, fructans are calory-free and non cariogenic sweeteners, stimulate the growth of bifidobacteria, and have been claimed to contribute towards the prevention of colon cancer and to reduce cholesterol, phospholipid and triglyceride levels in serum. *Gluconacetobacter diazotrophicus* secrets a constitutive levansucrase (LsdA). This extracellular LsdA hidrolyzes sucrose to produce free glucose and fructans (which contain β-(2→6) linked fructosyl units) of low (FOS) and high (levans) molecular weight.
In this work we approach and discuss the ability of *G. diazotrophicus* LsdA secreted to culture

supernatants to produce fructans. *G. diazotrophicus* PAL5 was grown at 30ºC in LGI medium supplemented with 1.5 g/l yeast extract and 1.5 g/l triptone. Ten-day-old cultures were centrifuged at 16000 × *g* 30 min. Supernatants were added to solutions of sucrose dissolved in a 0.1 M sodium acetate buffer pH 5,2 and sterilized by filtration. These mixtures were incubated in order to follow fructans production. Different conditions were tested: 1- enzyme:substrate ratios; 2- incubation temperature (30ºC and 40ºC); 3- sucrose concentration in the incubation mixture (100, 300 and 700 g/l). Fructan production was followed by sampling the mixtures during 7 days. Levan production was assayed by polysaccharide precipitation after addying 2 ethanol volumes to the mixture, recovering the precipitates by centrifugation, drying and weighing. FOS production was tested by Thin Layer Chromatography (TLC). Remanent glucose in the mixture was assayed using an enzymatic kit.
Culture supernatants from sucrose containing media, although expressing a lower LsdA activity than supernatans from cultures performed with glycerol, gave a higher fructan production. Levan production was observed in all the conditions tested, with concentrations increasing with time until about 96 h of incubation. Higher levan production was obtained by incubating at 40ºC than at 30ºC. FOS could be detected after 12 h of incubation (mainly 1-kestose) in all the mixtures assayed. Higher concentrations of sucrose seemed to favour production of low molecular weight fructans, whilst low sucrose concentrations in the mixture showed a high rate of sucrose hydrolysis but most of it remained free in the mixture.

7.24. LACTIC ACID BACTERIA ON FREEZE-DRIED FORM FOR THEIR APPLICATION IN RANICULTURE

[POSTER]

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Lactic acid bacteria (LAB) are used as probiotics in aquaculture, being raniculture an emerging area where there are no available probiotic products containing indigenous strains. In previous work, LAB strains from *Lithobates catesbeianus* hatcheries were isolated and selected by their beneficial characteristics to prevent red-leg syndrome (RLS). The protective effect of probiotics depends on the administered concentration and the preservation method. The freeze-drying process has commonly been used to maintain the viability and functional properties of bacteria. In this work, the effect of different concentrations of cryoprotective supports [skim milk, whey protein concentrate (WPC), sucrose, lactose] individually added or combined was evaluated on the survival of beneficial LAB strains: *L. lactis* CRL 1584 and 1827; *L. garvieae* CRL 1828 and *Lb. plantarum* CRL 1606, during freeze-drying. Beneficial LAB properties included production of inhibitory metabolites (lactic acid, hydrogen peroxide or bacteriocin), and/or hydrophobicity or autoaggregation characteristics. The strains were grown in MRS broth for 24 h at 37°C, washed twice, resuspended in the cryoprotective media and in water, and the number of CFU was determined (pre-lyophilized). Then, the samples were frozen at -70°C and desiccated under vacuum for 12 h at 0.3 mbar (post-lyophilized). A full two-factor ANOVA test, considering drying-media and strain, of the decrease in viability during the freeze-drying process was applied. Results showed that the number of viable cell was significantly higher for all the LAB strains ($p \leq 0.005$) when dried in the presence of all the cryoprotective media compared with water as control, being *L. lactis* CRL 1584 the most sensitive strain to the drying-process. The significance of the interactions between all the studied factors supports that there is not an unique optimal drying condition for all the strains evaluated. Thus, the best conditions for each LAB strain were selected considering the cost of the cryoprotective media, mainly WPC and sucrose, which represent the main products of milk and sugar cane industries in Argentina. The cryoprotectors chosen were 10% WPC for *L. lactis* CRL 1584, 10% sucrose for *L. lactis* CRL 1827 and *Lb. plantarum* CRL 1606, whilst a mixture of 5% WPC and 5% sacarose was required for *L. garvieae* CRL 1828. Also, the freeze-drying process did not affect the expression of the

beneficial properties (production of inhibitory metabolites, hydrophobicity and autoaggregation) of the LAB strains under study.
These studies represent the first approach on the resistance to the freeze-drying process of LAB to be used in raniculture and the maintenance of their beneficial properties. Further assays are being performed to evaluate the viability of LAB and their beneficial properties during storage.

7.25. COMPARATIVE STUDY OF FLOCCULENT AND NON-FLOCCULENT YEAST STRAINS FOR ETHANOL PRODUCTION

[POSTER]

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The gradual depletion of crude oil and the biological environmental deterioration resulted from both the fuels over consumption and petroleum-derived transportation have gained attention again, making urgent to develop renewable and environmentally friendly alternatives. Ethanol is an important industrial chemical with emerging potential as a biofuel to replace vanishing fossil fuels, whose utilization could improve energy security and decrease urban air pollution.
The entry into force of Law 26,093 of biofuels in Argentina from 2010 will mean an opportunity for the sugar sector to expand ethanol production in order to supply 5% of this alcohol to all the naphthas.
Our work proposes a microbiological approach to use fermentative microorganisms with high tolerance to alcohol in order to increase the currently obtained ethanol concentration (11%) by the alcoholic fermentation of molasses and compare the results when using flocculent or non-flocculent yeast strains for fermentation. To take up this, isolation and identification of ethanol hyper-producing yeasts strains from sugar cane molasses was carried out. Samples of molasses were taken from different mills of Tucuman and used to inoculate YPS (w/sucrose), YPD (w/dextrose) and molasses media with antibiotics. YPS medium with 50g/L sucrose was used for the propagation of microorganisms by incubating in a thermostatically controlled bath at 30ºC with agitation. Fermentations of selected isolates were performed in duplicate in flasks with 200 ml of YPS medium with 250 g/L sucrose and incubated at 30ºC without aeration. Every 8 h, Total Reducing Sugars (TRS), Direct Reducing Sugars (DRS), biomass dry weight and ethanol concentration were determined.
Three yeast isolates showing high ethanol production and named as A2, A10 and A11, which produced 11.74, 12.81 and 13.20% ethanol, respectively, were selected. A10 and A11 were flocculent yeast strains and A2 a non-flocculent yeast strain. These isolates were identified by molecular taxonomy tools according to the sequence analysis of their rDNA intergenic spacers, which allowed assigning identities of 99 and 100% to that of *Saccharomyces cerevisiae*.
Fermentations were carried out with a massive inoculum, which allowed to reduce the time of fermentation, and in the case of the A2 strain an ethanol concentration of 11.95% could be reached after 10 h of incubation, a higher value than the one currently achieved in industry, which is 10%.
It was determined that the strain A2 showed an homogeneous growth in liquid media, a feature that is compatible with the technology used in industry. For this reason, the A2 strain could be used in ethanol industrial production without the need for technology investments that would be indispensable for the recycling of flocculent strains. However, the A10 and A11 flocculent yeasts showed a very important potential for ethanol production, and these promising results justify further studies leading to an optimization in the production of bioethanol.

7.26. Optimization of the growth of lactic acid bacteria in different concentrations of pectin extracted from lemon peel

Se sugiere presentación POSTER

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Probiotics are microorganisms (e.g., bifidobacteria and lactobacilli) that can reach the end of the digestive tract remaining viable and have positive effects on consumer health, whether human or animals.
Pectin is a polysaccharide composed of galacturonic acid monomer units with different degrees of esterification and neutralization. Its applications include elaboration of jams, jellies, candies, essential oils, mayonnaise and cosmetics, among others. It is a protector and regulator of the gastrointestinal system, used in the treatment of hypercholesterolemic individuals.
The aim of this study was to evaluate the growth of probiotic lactic acid bacteria in a culture medium supplemented with different concentrations of pectin.
The studied lactic bacteria, *Enterococcus faecium* (strain RRC 14) and *Enterococcus faecium* (strain RRC 38), were provided by the Department of Public Health, School of Biochemistry, Chemistry and Pharmacy of the UNT. Bacteria were grown in LAPTG liquid culture medium, containing yeast extract (1.0 g), peptone (1.5 g), tryptone (1.0 g), glucose (1.0 g), distilled water (100 ml), pH = 7. They were incubated 24 h at 37°C. After that, serial dilutions were performed and 0.5 ml were inoculated in liquid LAPTG medium supplemented with different pectin concentrations (1, 0.1, 0.5%). They were incubated at 37ºC for 24 h and aliquots were withdrawn at different intervals (from 0 to 24 h) to measure the OD_{640} in a spectrophotometer. At the same time, aliquots taken at different times were plated on solid LAPTG medium, supplemented with different pectin concentrations, as before. Plates were incubated for 72 h at 37ºC for viable cell count.
The results showed good growth in pectin concentrations of 0.5 and 0.1%, with the advantage to the latter concentration where the lag phase was reduced by 75% for strain RRC38 and 50% for strain RRC14. The viable counting when using 0.1% pectin was 1.03×10^{10} CFU/ml for strain RRC14, and 0.74×10^{11} CFU/ml for strain RRC 38. With 0.5% pectin, 0.82×10^{10} CFU/ml for strain RRC14 and 0.80×10^{11} CFU/ml for strain RRC38 were found. Values were lower when using 1% pectin in the grown medium. These results showed the ability of the assessed lactic bacteria to grow at low concentrations of lemon´s pectin with an adequate number of viable cells and in a short period of incubation, thus giving the possibility for using them in the formulation of a symbiotic diet.

7.27. CHARACTERIZATION OF ANTIBIOTIC PHARMACEUTICAL FORMULATION CONTAINING BACCHARIS EXTRACTS

[ORAL]

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Baccharis incarum (Wedd.) Perkins (syn.: *Baccharis tola* Phil.), known under the popular names of lejia, tola and baila buena, is common in the arid Andean at 3,800 meters above sea level in Antofagasta de la Sierra, Catamarca, Argentina. Previous phytochemical studies on the leaves and top parts (aerial parts) of *B. incarum* described the isolation of chlorogenic acid and

flavones. The anti-inflammatory, antioxidant and anti-xanthine oxidase effects as well as the antibacterial activity against antibiotic-resistant Gram positive and Gram negative bacteria were demonstrated in *B. incarum* ethanolic extract. The aim of the present work was to obtain an antibiotic pharmaceutical formulation containing *B. incarum* ethanolic extract. The tincture was incorporated in a topical pharmaceutical formulation (hydrogel). The hydrogel containing *Baccharis* extract showed microbiological, physical and functional stability during storage for 90 days. The preparation was active against antibiotic-multi-resistant Gram negative and Gram positive bacteria and was not genotoxic in the *Salmonella typhimurium* assays. Studies which measure the drug release as determination of bioavailability were also carried out using a Franz diffusion cell. according to HPLC analysis, two compounds (chlorogenic acid and 4', 5-dihydroxy-3', 3, 6, 7, 8-pentamethoxyflavone) were identified in the tincture and in the receptor solution of the Franz diffusion cell. The flavone also showed antimicrobial activity. The results obtained demonstrated that some natural compounds with antibacterial activities from *B. incarum* are released from the pharmaceutical formulation and that this preparation might be potentially used as a topic antibiotic.

7.28. Antibiotic effectiveness of *Rosmarinus officinalis* bioactive compounds against multidrug-resistant bacteria of difficult clinical treatment

[ORAL]

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Infectious diseases have represented a major cause of mortality in humans, specially in immunodeficient individuals, at the end of life and in developing countries. In the past two decades, the increasing resistance to antibiotics of clinical use became an issue of great concern not only because of the costs generated by infectious diseases but also for the mortality rates. The last decade witnessed the proliferation of antibiotic-resistant bacteria such as those belonging to *Enterobacteriaceae* family and non-fermenting resistant to third generation cephalosporins and carbapenems, resistant enterococci and staphylococci (particularly *Staphylococcus aureus*) resistant to methicillin (SAMR). The medical field demands urgent solutions to meet the growing resistance developed by bacteria to antibiotics, particularly known as a result of irrational use. This search has focused special attention on natural compounds that have significant antimicrobial activity. The procedure followed involved the *in vitro* determination of the minimum inhibitory concentrations of natural compounds on antibiotic-resistant bacteria by the broth microdilution method. We evaluated the sensitivity of multidrug resistant bacteria isolated in the field of the Garrahan Pediatric Hospital to different bioactive compounds and extracts of *Rosmarinus officinalis* (rosemary), including essential oils, non-volatile ethanol extracts and isolated compounds of these extracts such as α-pinene, 1,8-cineole and the diterpene carnosic acid. Results showed that both, the essential oil rich in α-pinene such as pure α-pinene and carnosic acid, inhibited 100% the growth of strains of SAMR and *S. aureus* resistant to vancomycin (VISA), as well as multiresistant strains of *Enterococcus faecium* (EN902 and EN892). In addition to these compounds, 1,8-cineole also showed significant activity against multi-resistant strains of *Enterococcus faecium* (EN902). Experiments indicate that there is a clear association between the chemical composition of the essential oils, determined by GC-FID-MS, and its antibiotic activity (potency and efficacy) against specific multi-resistant bacterial strains. It was also noted that the antibiotic effectiveness of plant compounds is dependent on the type of bacterial resistance even in the case of the same

species. Therefore, this work has a potential impact on areas of health, since it will allow new alternatives in the treatment of multidrug resistant bacteria, either as monodrugs or working in synergy with other compounds. In view of these results, future evaluations of the functional efficacy of the investigated compounds using *in vivo* experimental models will be carried out for their possible incorporation into therapeutic procedures directed against nosocomial bacterial infections or those who have already won the community.

7.29. ELIMINATION OF FOODBORNE BACTERIA BY AN OXIDATIVE TREATMENT

[POSTER]

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Most food daily consumed is exposed to both Gram-positive and Gram-negative pathogen bacteria. Contamination can occur during growing, harvesting, processing, storing, and shipping of fresh or minimally processed products. For those reasons, it is important to study disinfectants that are able to eliminate a wide range of undesirable microorganisms. Oxidizing biocides such as hypochlorite and peroxides are widely used in food sanitization because of their antimicrobial effects, availability and low cost. In our laboratory, we have previously standardized a sequential oxidative treatment (SOT) for the elimination of fungal phytopathogens and *Xanthomonas axonodopodis* pv *citri*, consisting in two sequential incubations, first with NaClO, and then with H_2O_2 in presence of $CuSO_4$. The combination of these compounds in the SOT generated a synergistic effect. Here, we tested the *in vitro* biocidal capacity of the mentioned oxidizing compounds over several microorganisms. The test bacteria used were *Escherichia coli* MC4100, *Listeria innocua*, *Salmonella typhymurium*, and *Klebsiella pneumoniae*, as pathogenic and surrogate foodborne bacteria. First, the MIC for each SOT compound was determined in individual form and then, we established the optimal combination of them for each tested microorganism. The most sensitive foodborne bacterium was *K. pneumoniae* and the most resistant one was *L. innocua*. We could eliminate all tested bacteria when an oxidative treatment consisting on a brief incubation (2 min) with 50 ppm NaClO, 0.1 mM $CuSO_4$ and 200 mM H_2O_2 was applied. This effective treatment would be easy to apply an involves short times of contact between the oxidant and bacteria. Thus, it may be applied in the disinfection of food contact surfaces and materials used in the manufacturing of food products.

7.30. Advances in the design of a novelty veterinarian product containing *Lactobacillus* and natural substances to improve the reproductive performance in cattle

[POSTER]

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Metritis in cows increases herd health costs, reduces the reproductive efficiency, the conception rate (extra inseminations are required) and the feed intake, and also causes a reduction in milk

production. The probiotic lactobacilli therapy provides a valid alternative to the antibiotic application for the prevention or treatment of these infections.
Four autochthonous strains, i.e. *Lactobacillus gasseri* CRL1412, *Lactobacillus gasseri* CRL1421, *Lactobacillus gasseri* CRL1460 and *Lactobacillus delbrueckii* subsp. *delbrueckii* CRL1461 were selected by the adhesion to bovine vaginal cells, the inhibition of metritis-causing pathogens and by their technological properties (resistance to freeze-drying process stress conditions and shelf-life period in two pharmaceutical forms). In this work, the effects of natural substances with active action on the impaired epithelium and its resident microbiota, were evaluated on the growth kinetic of these microorganisms to be included in the design of a pharmaceutical product.
A complete factorial experimental design was applied to determine the effect of three natural substances at different concentrations: ascorbic acid (30, 20 and 10 mg/mL), inulin (15, 10 and 5 mg/mL) and *Matricaria chamomilla* extract (10 and 5 mg/mL) on the growth parameters of all the tested strains. Growth was evaluated in modified MRS medium at 37°C. The O.D. at 540 nm was determined at 30 min intervals during 14 h, and the increase in biomass, the maximal growth rates and the length of lag and stationary phases were calculated. The experimental procedure was repeated three times.
The maximal growth rate was significantly ($p \leq 0.005$) affected by the substance added to the culture medium, the effect being different in each strain (interaction between strain x substance, $p \leq 0.005$). Inulin significantly increased the growth rate, mainly in the CRL 1412 strain. The increase in biomass was affected by the type of substance, but the response depended on the concentration and on the microorganisms (interaction strains x substance and substance x concentration, $p \leq 0{,}005$). The main effect was observed on the growth of CRL 1421. The time of lag phase was specific for each microorganism and it was independent of the other factors. The length of the stationary phase was significantly ($p \leq 0{,}05$) shortened with inulin, being CRL 1421 and CRL 1461 faster than other strains.
The efficacy of the probiotic treatment depends on the viability of the microorganisms in the pharmaceutical formula during the shelf life and up the arrival to the target site in the animal. These results will allow the adequate design of a probiotic product by the combination of beneficial microorganisms with natural substances to be applied in the bovine reproductive tract. This could be used in organic farmers or other safe production systems.

7.31. TYPE IV PILI ARE REQUIRED FOR BIOFILM FORMATION BY *Moraxella bovis*

[POSTER]

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Infectious bovine keratoconjunctivitis (IBK) is a highly contagious ocular disease of cattle caused by *Moraxella bovis*. The economic impact of IBK in the cattle industry and the lack of effective strategies to its control, highlight the need to better understand the infectious process of *M. bovis* and its pathogenesis. We have recently begun to explore the biofilm-forming ability of this pathogen hypothesizing that this surface-attached, community-based mode of growth is adopted by *M. bovis* as a strategy to colonize bovine ocular surface. Here we demonstrate that *M. bovis* forms biofilm on multiple abiotic surfaces. In addition we present evidences that the type IV pili (TFP) of *M. bovis* play an important role for biofilm development. The addition of $MgCl_2$ to culture medium was shown to prevent biofilm formation by a wild type *M. bovis* strain on polypropylene and glass surfaces. The inhibitory action of $MgCl_2$ in biofilm formation was found to correlate with the disruption and release of TFP from cell surfaces. Examination of biofilm growth on abiotic surfaces of a phenotypically non-piliated *M. bovis* strain, which does not produce TFP, revealed severe defects in biofilm biomass accumulation. This strain showed

at an early time-point to exhibit similar defects to grow on corneal epithelial cells. The absence of TFP was further demonstrated to impair microcolonies formation, as well as the development of structured biofilm. Our studies support the idea that TFP contribute to these events by governing both cell-substrate and cell-cell interactions. We propose that TFP-mediated biofilm formation in corneal epithelia may allow *M. bovis* to establish persistent ocular infections in cattle.

7.32. Purification and partial characterization of the thermophilic lipase produced by *Bacillus licheniformis*

[POSTER]

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Lipases (triacylglycerol acylhydrolases; EC 3.1.1.3) constitute a group of enzymes defined as carboxylesterases that catalyze the hydrolysis (and synthesis) of long chain acylglycerols at the lipid-water interface. In recent years, lipases have received considerable attention with regard to industrial application, since they have a number of unique characteristics such as substrate specificity, regio-specificity, and chiral selectivity.
Given that such reactions are sometimes most efficiently performed at elevated temperatures and in organic solvents, converging attempts have been made to find thermostable lipases which would have advantages over labile enzymes in such applications. In recent years, a number of thermophilic microorganisms producing thermoactive lipases and esterases have been purified and characterized.
In this study, we describe the purification and characterization of a thermophilic lipase produced by the thermophilic strain *Bacillus licheniformis*, isolated from a hot spring.
Screened thermophilic *Bacillus* strains showed activity bands in native-PAGE indicating the production of extracellular lipase and/or esterase. The partial 16S rDNA sequencing of the selected *Bacillus* strain showed the highest similarity (99%) with *Bacillus licheniformis*. This was chosen for lipase purification and characterization since it produced the highest lipase activity. Purification was performed with an electroelution technique. Lipase was eluted from the polyacrylamide gel slices and the enzyme-containing solution was concentrated under vacuum. Purity was confirmed by the migration of a single band. This enzyme showed residual activities equal or above 100% after treatment for 1 h at: temperatures from 45 to 60°C, alkaline pH from 8 to 10 and non-polar solvent (DMSO and n-hexane).
Higher thermostability, higher activity at elevated temperatures, and more resistance to chemical denaturation are desirable properties of thermophilic lipases which make them ideal tools in industrial and chemical processes where relatively high reaction temperatures and/or organic solvents are used.

7.33. Operational parameters for biopolymers production from inexpensive sugar substrates

Se sugiere presentación ORAL. Por favor comunicar aceptación a la Secretaría (secretaria_samige@leloir.org.ar)

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Biodegradable polymers have recently attracted much public and industrial interest, being the polyhydroxyalkanoates (PHA) and their derivatives the most widely synthesized microbial bioplastics. PHA are a family of biopolymers produced by many bacterial species as energy and carbon storage materials and represent a good alternative to conventional oil-based plastics because of their biodegradability and capability of being produced from renewable resources. Unfortunately, the cost of PHA production is still an obstacle for large-scale commercial exploitation. It is estimated that 40-48% of the total production costs are ascribed to the raw materials, where the carbon source could account for 70-80% of the total expense. For this reason, many researchers are seeking for inexpensive substrates and alternative methodologies that contribute to the process optimization and costs reduction for PHA production.

This research investigated the biomass and PHA production and the reducing sugar consumption by *Bacillus megaterium*, at different fermentation times, using glucose, sugar cane molasses, fique juice and an unpublished substrate as the sole carbon source. This strain was previously isolated and characterized using different microbiological and molecular techniques and in this work it was confirmed its potential as a PHA producing bacteria. Additionally, the effect of pH, temperature and substrate concentration in biomass and PHA production was investigated using the response surface methodology.

It was concluded that all the substrates, except fique juice, can be successfully used as the sole carbon source for poly (3-hydroxybutyrate) (PHB) production. PHB is the most common and comercializated type of PHA. The higest PHB production was obtained with glucose (1446,6 mg/L), but this is an expensive reactive that was used just as a positive control. Sugar cane molasses and the unpublished substrate showed similar results (66 mg/L and 67 mg/L, respectively). The first source is recommended in regions where this is an abundant and cheap substrate. Fique juice did not show a good behavior as a carbon source for this process (< 0.002 mg/L). The best fermentation time for all substrates was observed in the stationary phase, after 36h.

The results obtained with the response surface methodology suggest that the three parameters have a strong influence on PHB synthesis, being pH and substrate concentration the two most influential of them for the explored region. Maximum biomass production was obtained at pH 6 and 25ºC. These are the most similar conditions to the natural environment of the isolated bacteria. Nevertheless, maximum PHB production was found at both higher temperature and pH values.

Further studies are needed to improve the PHA production process. We strongly recommend large-scale studies of this process and the continuous exploration of new potential carbon sources.