25 y 26 de setiembre 2008, Rosario. Argentina
Sede de Gobierno. Universidad Nacional de Rosario

V CONGRESO ARGENTINO DE MICROBIOLOGÍA GENERAL

SAMIGE Sociedad Argentina de Microbiología General

ÁREAS TEMÁTICAS

1. Biodiversidad / Biodiversity
2. Biorremediación y Biocontrol / Bioremediation and Biocontrol
3. Fisiología y Metabolismo / Physiology and Metabolism
4. Microbiología Molecular / Molecular Microbiology
5. Microbiología Ambiental / Environmental Microbiology
6. Interacción procariote-eucariote / Procaryote-eucaryote Interaction
7. Biotecnología / Biotechnology

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Jueves 25 de setiembre / Thursday September 25th

08:00 INSCRIPCIÓN / Registration

09:15 BIENVENIDA / Welcome

09:30 CONFERENCIA / Lecture
Marc Mussmann

10:30 PAUSA CON CAFÉ / Coffee Break

11:00 COMUNICACIONES ORALES / Oral Communications
FIS 4 / FIS 8 / FIS 22

12:00 ALMUERZO / Lunch

14:00 COMUNICACIONES ORALES Oral Communications
AMB 5 / AMB 14 / AMB 16
TEC 14 / TEC 22

15:40 PAUSA / Break

16:00 SESION de POSTERS / Posters Session

17:30 PAUSA CON CAFÉ / Coffee break

18:00 CONFERENCIA / Lecture
Hugo Gramajo

19:30 PAUSA / Break

20:30 BRINDIS de APERTURA / Opening Cocktail

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09:00 COMUNICACIONES ORALES / Oral Communications
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10:50 CONFERENCIA / Lecture
Cyril-Léopold Kurz

11:50 PAUSA / Break

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13:00 ALMUERZO Lunch

15:00 MINI CONFERENCIA / Junior Conference
Leonardo Curatti

15:30 COMUNICACIONES ORALES Oral Communications
MOL 19 / MOL 20 / MOL 22 / MOL 23

17:00 SESION de POSTERS CON CAFÉ / Poster Session with coffee

18:30 CONFERENCIA / Lecture
Javier Pozueta-Romero

19:30 ASAMBLEA / SAMIGE Assembly

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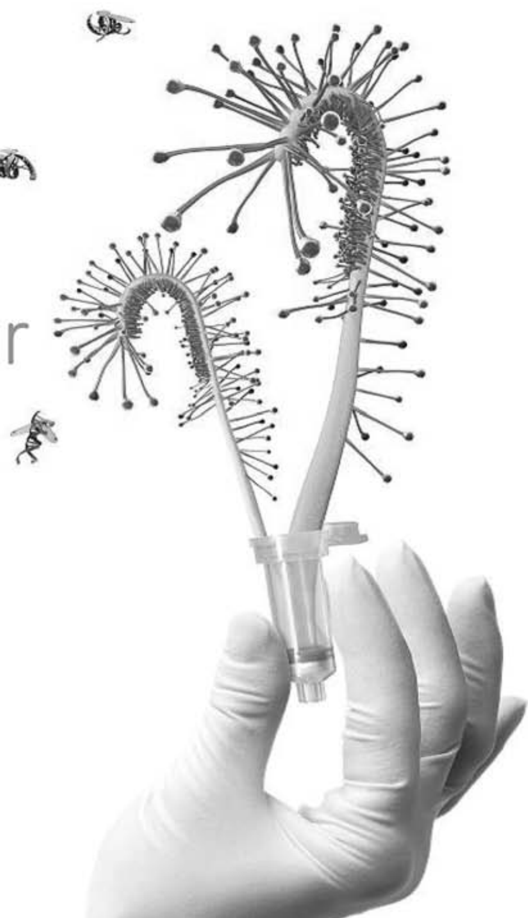

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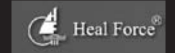

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Biodynamics

Jueves 25 de setiembre / Thursday September 25th

08:00 INSCRIPCIÓN / Registration

09:15 BIENVENIDA / Welcome

09:30 CONFERENCIA / Lecture

Marc Mussmann, University of Vienna, Ecology Centre, Vienna, Austria
"The microbial ecology of sulfur cycling in marine coastal sediments"

10:30 PAUSA CON CAFÉ / Coffee Break

11:00 COMUNICACIONES ORALES / Oral Communications

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FIS8: Andrea M. Dallangol, Graciela Font de Valdes, Graciela Rollán
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Characterization of bacteria isolated from pristine High Altitude Andean Wetlands resistant to extreme environmental conditions

MOL11: María M. Ibáñez, Susana K. Checa, Fernando C. Soncini
Specific amino acid residues in GolS metal-binding loop determines the metal selectivity

TEC14: Micaela Pescuma, Elvira M. Hébert, Michèle Dalgalarrondo, Jean C. Gaudin, Fernanda Mozzi, Jean M. Chobert, Graciela Font de Valdez
β-lactoglobulin hydrolysis by *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL 656 reduces its allergenicity

TEC22: Oscar A. Sosa, E. N. Santillán, M. C. Manca de Nadra, Marta. E. Farías
Evidence for lectinic factor involved in self-flocculation induction of *K. apiculata* mc1 by glucose.

15:40 PAUSA / Break

16:00 SESION de POSTERS / SESION de POSTERS

17:30 PAUSA CON CAFÉ / Coffee break

18:00 CONFERENCIA / Lecture

Hugo Gramajo, IBR, Instituto de Biología Molecular y Celular de Rosario (CONICET & Universidad Nacional de Rosario) Rosario, Argentina"
"Acyl-CoA Carboxylases in Actinomycetes: from polyketide biosynthesis to structure-based inhibitor design"

19:30 PAUSA / Break

20:30 BRINDIS de APERTURA / Opening Cocktail

Viernes 26 de setiembre / Friday September 26th

09:00 COMUNICACIONES ORALES / Oral Communications

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Structural and functional characterization of DesK, the membrane fluidity sensor of *B. subtilis.*

MOL7: María I. Giménez, Mecky Pohlschroder
The unique features of a Twin-arginine translocation component are essential for survival in haloarchaea.

MOL13: Lucas B. Pontel, Fernando C. Soncini
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MOL14: Cecilia Quiroga, Paula Scalzo, María Paula Quiroga, Daniela Centrón
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10:20 PAUSA CON CAFÉ / Coffee Break

10:50 CONFERENCIA / Lecture
Cyril-Léopold Kurz, Centre d'Immunologie Marseille-Luminy, Francia
"Infections and Immunity in *Caenorhabditis elegans*"

11:50 PAUSA / Break

12:00 COMUNICACIONES ORALES / Oral Communications

REM18: Natalia C. Maldonado, Clara V. Silva de Ruiz, Maria Elena F. Nader-Macías
Isolation of *Bacillus* from newborn calves. Preliminary evaluation of their properties for their inclusion in probiotic products.

INT10: Fernando A. Martín, Diana M. Posadas, Verónica Ruiz, Mariela C. Carrica, Silvio Cravero, David O'Callaghan, Angeles Zorreguieta
Transcriptional regulation of RND drug efflux pumps in *Brucella suis*.

MOL18: Mariela I. Sciara, Eleonora García Véscovi
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15:00 MINI CONFERENCIA / Junior Conference
Leonardo Curatti, University of Californa at Berkeley & Centro de Estudios de Biodiversidad y Biotecnología de Mar del Plata
"*In vitro* Reconstitution of the Biosynthesis of the Iron-Molybdenum Cofactor of Nitrogenase"

15:30 COMUNICACIONES ORALES Oral Communications

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MOL20: Alfonso J. Soler Bistué, Hongphuc Ha, Doreen E. Carpio, Jonathan Joaquín, Angeles Zorreguieta, Marcelo E. Tolmasky
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Some insights of the mechanism of action of salivaricin CRL 1328 against pathogens

MOL23: Karina Herrera Seitz, Victoria Shingler
GGDEF/EAL domains and motility in *P. putida*: a novel protein involved in motility regulation

17:00 SESION de POSTERS CON CAFÉ / Poster Session with coffee

18:30 CONFERENCIA / Lecture
Javier Pozueta-Romero, Instituto de Agrobiotecnología, CSIC/UPNA/Gobierno de Navarra, España.
30 years of bacterial glycogen metabolism revisited

19:30 ASAMBLEA / SAMIGE Assembly

Conferencias / Lectures

C1

The microbial ecology of sulfur cycling in marine coastal sediments

Marc Mussmann

Department of Microbial Ecology,
University of Vienna, Vienna, Austria.

In coastal sediments microbial sulfate respiration is a key process in sulfur and carbon cycling. The re-oxidation of toxic sulfide produced by sulfate respiring prokaryotes (SRP) is essential to sustain benthic and planktonic life. However, the microorganisms involved in both processes are largely unknown or are not cultured yet. To get insights into their ecophysiology we apply different strategies to retrieve genomic sequences from environmentally abundant populations of sulfur oxidizing and sulfate respiring microorganisms. Two individual filaments of large, sulfur oxidizing *Beggiatoa* sp. were directly recovered from the sediment surface. Their genomic DNA was amplified and sequenced. The genome encodes physiological adaptations closely linked to the unique life style of *Beggiatoa*. In particular, we propose a mechanism how *Beggiatoa* stores large amounts of nitrate of up to 1 M in their vacuole. In addition, strategies for accessing genomes of populations inhabiting sand biofilms are presented. The synopsis of fosmid libraries from bulk DNA, fluorescence in situ hybridization and functional gene analyses revealed globally occurring proteobacteria likely involved in sulfur oxidation. Furthermore, we analyzed genomic fragments of *in situ* abundant SRP. Pathways for utilization of oxygen and organic compounds are encoded that are unusual among SRP. Alternatively, fluorescence activated cell sorting and subsequent genomic analyses allow more detailed insights into the biology of specific populations. The recovered findings may facilitate the cultivation of organisms and will contribute to a better understanding of the different pathways in the marine sulfur cycle. Nevertheless, the generated hypotheses require further lab experiments.

C2

Acyl-CoA Carboxylases in Actinomycetes: from polyketide biosynthesis to structure-based inhibitor design

Hugo Gramajo

IBR (CONICET)
Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR

The acetyl-CoA carboxylase (ACC) catalyzes the first committed step in the biosynthesis of long-chain fatty acids in all animals, plants and bacteria. The reaction catalyzed by this enzyme involves two half-reactions and three components. In the first reaction the biotin carboxylase (BC) transfers the carboxyl group from carboxyphosphate to the biotin that is attached to the biotin carboxyl carrier protein (BCCP). In the second reaction, catalyzed by carboxyltransferase (CT), the carboxyl group is transferred from biotin to acetyl-CoA to form malonyl-CoA, the building block of fatty acid biosynthesis. The function, organization and stoichiometry of ACC are highly diverse among different organisms. In actinomycetes, for example, the system is different than in most bacteria, because they do not have a canonical acetyl-CoA carboxylase, instead they posses enzyme complexes commonly named as acyl-CoA carboxylases (ACCase), due to their relaxed substrate specificity, e.g. they can carboxylate other substrates besides acetyl-CoA like propionyl- and butyryl-CoA to yield methyl- and ethylmalonyl-CoA.

We became interested in these enzymes complexes in the context of two different genera of actinomycetes, *Streptomyces* and *Mycobacterium*, for completely different reasons. In Streptomyces, because the ACCases provide the building blocks (malonyl-CoA, methylmalonyl-CoA, ethylmalonyl-CoA) for the synthesis of a vast array of secondary metabolites (polyketides) with important biological properties. In *Mycobacterium* because these enzymes provide the substrate for the synthesis of all the exquisite variety of complex lipids present in these bacteria, like the mycolic acids and multy-methyl branched fatty acids, that are key constituents of the cell envelope and are essential for the survival and the pathogenicity of these microorganisms.

In this talk I will present the biochemical and structural characterization of two ACCase complexes from *Streptomyces* coelicolor and *Mycobacterium smegmatis* and the molecular basis of their substrate specificity. I will also present genetic and physiological experiments that confirm which is the dedicated acetyl-CoA carboxylase in both micro-organisms. Finally, I will address how we used the knowledge gained by studying these enzymes for the synthesis of secondary metabolites (polyketides) in heterologous host like *E. coli* and for the structure-based identification of ACCase inhibitors and its use as putative anti-mycobacterial agents.

C3

Infections and Immunity in *Caenorhabditis elegans*

Cyril-Léopold Kurz

Centre d'Immunologie Marseille-Luminy, Francia.

Studies using the genetically tractable organism *Caenorhabditis elegans* have greatly contributed to advances in our understanding of biological processes such as development, cell death and RNA interference. Over the past ten years, this animal has been increasingly used as an alternative model to dissect host-pathogen interactions. Virulence mechanisms used by fungi, Gram-negative and Gram-postive bacteria, including important human pathogens, have been studied using this nematode. Significantly, many of the identified pathogenicity traits identified using *C. elegans* as a host are required for infection in mammals. Innovative *in vivo* approaches aiming at the discovery of molecules with antimicrobial activity using *C. elegans* have also been developed. In addition, the establishment of these infection systems has permitted the characterization of a complex host innate immune response that shows some interesting similarities with mammalian innate defense mechanisms.

C4

30 years of bacterial glycogen metabolism revisited

Javier Pozueta-Romero[1], Manuel Montero[1], Gustavo Eydallin[1], Francisco José Muñoz[1], María Teresa Sesma[1], Goizeder Almagro[1], Edurne Baroja-Fernández[1] and Alejandro M. Viale[1,2]

[1] Instituto de Agrobiotecnología (CSIC, UPNA, Gobierno de Navarra). Mutiloako etorbidea zenbaki gabe, 31192 Mutiloabeti, Nafarroa, Spain.

[2] Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET), Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, U.N.R., Suipacha 531, 2000 Rosario, Argentina.

Bacterial glycogen is a branched homopolysaccharide of α-1,4-linked glucose subunits with about 5% α-1,6-linked glucose at the branching points that provides a source of energy and carbon surplus for the organisms in unfavorable environmental conditions. In some bacteria, glycogen metabolism is required for colonization and virulence (1-3). Synthesized by glycogen synthase (GlgA) using ADP-glucose (ADPG) as the sugar donor nucleotide, accumulation of this polysaccharide in *Escherichia coli* occurs when cellular carbon source and energy are in excess under conditions of limiting growth such as nutrient deficiency. Regulation of glycogen biosynthesis in *E. coli* involves a complex assemblage of factors that are adjusted to the physiological and energy status of the cell (4). At the level of enzyme activity for instance, the glycogen biosynthetic process is subjected to the allosteric regulation of GlgC, a protein that catalyzes the production of ADPG (5). At the level of gene expression, it has been suggested that the process depends on the regulation of *glgBX* (encompassing the genes coding for glycogen branching (GlgB) and debranching (GlgX) enzymes), *glgS* and *glgCAP* operons (6), the latter encoding the GlgC and GlgA anabolic enzymes as well as the catabolic glycogen phosphorylase (GlgP). Glycogen accumulation is negatively affected by the central carbon storage regulator CsrA, and positively affected by the guanosine 5´-diphosphate 3´-diphosphate (ppGpp) stringent response regulator, the general stress factor RpoS, and the c AMP/cAMP receptor protein complex (6).

Using a systematic and comprehensive gene-disrupted mutant collection of *E. coli* (the Keio collection) (7) we have recently carried out a genome-wide screening of the genes affecting glycogen metabolism (8). This and other studies carried out in our lab showed that ppGpp, RpoS, protein breakdown, end-turnover of tRNA, intracellular AMP levels, extracellular carbon and Mg^{2+} sensing, transport and metabolism of iron, carbon and sulfur, and less well-defined systems likely sensing the energy status through the activity of the electron transport chain are major determinants of glycogen levels in *E. coli.* In addition, we have provided strong evidences about the occurrence of important sources, other than GlgC, of ADPG linked to glycogen biosynthesis (9,10). With the overall data accumulated until now we conclude that synthesis and utilization of glycogen is under complex and intricate control linked to the intracellular energy state, and to the environmental nutritional status. In this meeting we will propose an integrated metabolic model wherein glycogen is highly interconnected with various cellular processes and metabolic pathways.

References: (1) McMeechan, A., Lovell, M.A., Cogan, T.A., Marston, K.L., Humphrey, T.J. and Barrow, P.A. (2005) Microbiol. 151, 3969-3977

MC

In vitro Reconstitution of the Biosynthesis of the Iron-Molybdenum Cofactor of Nitrogenase

Leonardo Curatti[2,1], Jose A. Hernandez[1], Zhao Dehua[1], Ludden W. Paul[1], Rubio M. Luis[1]

[1] Department of Plant and Microbial Biology. University of Californa at Berkeley. [2] Centro de Estudios de Biodiversidad y Biotecnología de Mar del Plata (lcuratti@fiba.org.ar)

The biological conversion of atmospheric N2 to NH3 takes place according to the following reaction: N_2 + 8e- + 16 MgATP + 2 NH_3 + H_2 + 16MgADP + 16 Pi and is an essential step of the→8 H^+ biogeochemical cycle of nitrogen that supports life on Earth. The major part of biological nitrogen fixation is catalyzed by the molybdenum nitrogenase enzyme, which is composed of two distinct proteins: dinitrogenase (NifDK) and dinitrogenase reductase (NifH). Dinitrogenase carries at its active site one of the most complex biological metalloclusters known to date, the iron-molybdenum cofactor (FeMo-co), composed of seven Fe, nine S, one Mo, one R-homocitrate, and one light unidentified atom.
Genetic and biochemical analysis of nitrogen fixation (nif) genes and proteins, mainly in *Azotobacter vinelandii* and *Klebsiella pneumoniae*, led to the identification of at least 11 genes (nifUS-BQ-ENX-V-H-Y and nafY) proposed to be involved in the biosynthesis and insertion of FeMo-co into apo-dinitrogenase. We have accomplished the purification of the most critical proteins for the biosynthesis of FeMo-co, which has provided an unprecedented opportunity to obtain direct biochemical evidence to support and extend the current working model for FeMo-co biosynthesis and apo-NifDK activation. We show the in vitro conversion of apo-NifDK into catalytically competent holo-NifDK in a composition-defined reaction mixture containing pure NifB, NifEN, , NifH, and apo-NifDK as protein factors, along with Na2MoO4, R-homocitrate, $(NH_4)_2Fe(SO_4)_2$, Na_2S, SAM, Mg-ATP, and DTH. These results gave strong and definitive support to the current model of FeMo-co biosynthesis in which NifB, NifEN and NifH constitute a catalytic core comprising all the critical steps for the chemical reactions for the assembly of the cofactor from Fe, S, Na_2MoO_4, R-homocitrate. Nevertheless, non-essential Nif proteins for *in vitro* synthesis of FeMoCo play critical roles, especially *in vivo*, for the assembly of the cofactor and may function as the physiological donors of the substrates of the pathway. To this regard, we have provided evidence for the participation of NifS and NifU in the biosynthesis of the iron-sulfur cluster core of FeMo-co (NifB-co) and for NifQ as a specific molybdenum donor.

Índice

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7. Biotecnología / Biotechnology

1. Biodiversidad / Biodiversity

DIV1

A deterministic view of biological floc development

Joaquín M. Ayarza[1], Leonardo Erijman[1]

[1] INGEBI-CONICET (ayarza@dna.uba.ar)

In this study we aimed at gaining insight into the assembly of bacterial communities during floc formation in lab-scale bioreactors. Sludge from the aeration basin of a full-scale domestic wastewater treatment plant (WWTP) was vortexed in order to break the floc and incubated at 20 ºC with daily addition of synthetic sewage (SS), without sludge wasting. The biomass suspension was diluted four-fold with SS and used to seed four lab-scale reactors. Reactors A (two replicates) were operated as chemostats, with the hydraulic retention time (HRT) equals the solid retention time (SRT) of 2 days. Reactors B (two replicates) were operated in a sequential batch mode with mass retention, including settling and decanting phases. In this case HRT was 2 days and SRT was 6 days. The diversity and community structure the sludge were analyzed using denaturing gradient gel electrophoresis (DGGE) of RT-PCR amplified as well as PCR amplified rRNA 16S genes. Application of the Raup and Crick probability-based similarity index (Src) for the comparison of RNA based fingerprinting patterns within reactors indicated that bacterial communities within reactors were not significantly similar between day 1 and 4 (Src < 0.7) and became significantly dissimilar between day 4 y 11 (Src < 0.05), indicative of a highly dynamic process. However, significant similarity was observed among all 4 reactors at days 1 and 4 (Src > 0.99) and between each pair of replicate reactors at day 11 (Src > 0.99). Bacterial communities between reactors subjected to different treatments were not significantly similar at day 11 (Src < 0.85). Identical results were obtained for the analysis based on rDNA profiles. The fact that the patterns between replicates were more reproducible than expected by chance suggests that floc development displays a strong deterministic character.

DIV2

***Isolation of lactic acid bacteria* (LAB) from grapes and musts from the Northwestern Patagonia.**

Bárbara M. Bravo Ferrada[1], Danay Valdés La Hens[1], Yolanda Curilén[2], Adriana Caballero[2], Horacio Bibiloni[3], Liliana C. Semorile[1]

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Lactic acid bacteria (LAB) play dual roles in wine fermentation: as agents of wine spoilage and as the main effector of a secondary, or malolactic fermentation (MLF). Most LAB found in wines include members of *Oenococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc*. MLF is essential in some highly acidic red wines, as well as in white wines with aging potential, as is the case in the oenological region of Northwestern Patagonia. However, MLF sometimes fails, due to very harsh environmental conditions in the wine for bacterial survival and growth. A more efficient MLF could be achieved by the inoculation with suitable indigenous LAB. So far there are no studies on biodiversity of BAL in grapes and musts of the Patagonian region. Due to the important role of LAB in winemaking, the isolation and identification of these microorganisms is of great scientific and practical interest.
To date we have made progress in the isolation of LAB from healthy grapes and musts from one vineyard in the Northwestern Patagonia area. Two red wine varieties were monitored: Merlot and Pinot noir collected, respectively, in consecutive years (2007 and 2008 vintages). The fermentation conditions of these varieties were: a- spontaneous from the activity of yeasts and bacteria naturally present in musts, and b- a combination of spontaneous activity plus the addition of a commercial inoculum of yeasts. Samples from grapes and six different stages of alcoholic fermentation (AF) and MLF were collected, processed and cultured in different conditions.
The Merlot samples were cultured at 30 ºC, in aerobiosis, and using two media: MRS and MRS + tomato. Of a total isolates obtained only the 15% were Gram positive and catalase negative bacteria exhibiting the morphologies of bacilli and cocci in different percentages. The hetero and homofermentative analysis of the isolates showed that the 55.1% of the bacilli were heterofermentative and 44.9% were homofermentative, while all cocci were heterofermentative.
The Pinot noir samples were cultured at 30 ºC, in anaerobic conditions, and using five different media: MRS, MRS-tomato, GJ-LAB pH 4.8, E-LAB pH 4.8 and MRS-ethanol 5% pH 3.5. Of a total isolates obtained a 37,0% were characterized as LAB being the 86,0% of the bacilli heterofermentative and 14,0% homofermentative. The difference found between the percentage of LAB recovered in the Merlot and Pinot noir varieties showed that culture conditions selected in the second case were better than those selected in the first case for the isolation of this group of bacteria.

DIV3

Bacterioplankton assemblage structure and incidence of artificial UV-B radiation on the composition of bacterial communities from four Andean wetlands (over 4,400 m) with different saline conditions

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Bacterioplankton communities from 4 high Andean wetlands (HAW), Laguna Negra (L. Negra; 4,400 m), L. Verde (4,460 m), L. Vilama (4,600 m) and L. Chiro (4,500 m), were exposed to artificial UV radiation for 24 h. Modifications were described using denaturing gradient gel electrophoresis (DGGE), Fluorescent in situ Hybridization (FISH) and accumulation of cyclobutane pyrimidine dimers (CPDs). The 4 shallow lakes showed contrasting trophic conditions and covered a relatively wide range of limnological characteristics mainly regarding salinity. Gel analyses generated a total of 52 sequences, present in different positions. From the total of bands registered in the gels, none band were shared among the wetlands. Among these sequences the bacterial group best represented in all lakes was related to the Gamma-Proteobacteria group with 38 %, while Alpha proteobacteria represented 17 %, Beta proteobacteria 6 %, Bacteroidetes 23 % and uncultured bacteria 6 % of the bands sequenced. Interestingly, most of the sequences showed high similarity to GenBank-stored data of taxa found in halophilic habitats elsewhere in the world. These results suggest that the ubiquity of microorganisms may be probably due to their high dispersal rates, but their success in a particular habitat could be subject to environmental selection, generating bacterial assemblages well adapted to cold habitats.
Little difference in DGGE profiles was detected in all four exposed wetlands. A larger band diversity was found in the more saline wetlands L. Negra and L. Vilama than in L. Verde and L. Chiro with more oligotrophic conditions. DGGE profiles of the four wetlands showed no decrease in the number of bands after UV exposure. In fact, an increase in their number was observed. CPD accumulation in all four wetlands was clearly lower than in biodosimeters and all wetlands presented almost similar damage that remained stable throughout exposure.
From these results it can be concluded that bacterial communities in HAW were well adapted to high exposure of UV-B radiation, and in many cases UV-B even stimulated their growth and helped select different diversities. Environmental conditions did not seem to be connected with UV resistance since all communities assayed showed resistance regardless of salinity, oligotrophy, arsenic content, etc.

DIV4

A simple method to determine microbial diversity measuring DNA reassociation in a thermal cycler with fluorescence detection

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The diversity of communities is important in microbial ecology, but unfortunately very difficult to determine. In 1990 Torsvik et al. developed a method to estimate diversity based on DNA heterogeneity. DNA heterogeneity can be determined by thermal denaturation and reassociation. Renaturation of homologous single-stranded DNA follows second-order reaction kinetics. The fraction of renatured DNA is usually expressed as a function of the product C0t of DNA nucleotide concentration (C0) and the reaction time (t). Under defined conditions C0t for a half-completed reaction (C0t1/2) is proportional to the genome size or the complexity of DNA. Therefore, genetic diversity of a mixed bacterial community can be estimated from the measured C0t1/2 value. The advantage of this method is that allows the estimation of diversity without bias, as opposed to molecular methods based on PCR or phenotypic characterization of isolated strains. The measurement of thermal denaturation and reassociation requires the use of a UV-spectrophotometer equipped with a thermoelectric cell holder, a temperature controller, and a temperature programmer. This type of instrument is not readily available in most laboratories. We propose the alternative use of a standard real time PCR instrument, based on the measurement of fluorescence emitted by the dye SYBR Green I bound to dsDNA.
Total DNA extracted from pure cultures of Escherichia coli and Pseudomonas putida and PCR amplification products were used to set up the optimal experimental conditions for the technique. Tested variables were DNA concentration, average DNA fragment size and method for shearing (French press or sonication), buffer (SSC 1x, SSC 6x, TE and HEN), concentration of DMSO and annealing temperature. DNA size range was not too critical; 400 bp and 600 bp fragment, obtained after shearing with either French press (20000 psi) or tip sonication (120 to 150 sec), yielded similar results. On the other hand, uniformity in the size and purity of the DNA fragment were extremely important in order to obtain clear reassociation patterns. To achieve this goal, sheared DNA was further purified from an agarose gel. Reassociation was adequately observed using buffers SSC 1x plus 30% DMSO or HEN plus 30% DMSO. Concentrations up to 100 mg/L of dsDNA were linearly proportional to the intensity of fluorescence. Our long term goal is to apply this technique for natural and engineered complex environments. We are currently applying these conditions for the comparison of the biodiversity of several wastewater treatment plants.

DIV5

Biodiversity analyses of Laguna Catal, an Andean High Altitude wetland (4,000 masl)

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Laguna (L.) Catal is a wetland of Salar del Hombre Muerto. It is placed at 4,000 masl in the Argentinean north western andean Puna desert (22 ° 35 ' S and 66 ° 55 ' W). This wetland is characterized by extreme environmental conditions as high salinity (160 ppm), sharp aridity, high ultraviolet radiation, significant extreme temperature daily, clear sky and constant winds. Thus, planktonic communities that live in this wetland are able to resist such severe environmental conditions.

Aim:
The aim of this work was to study the planktonic organisms biodiversity of water samples from L. Catal by molecular and microbiological techniques.

Material and Methods:
Salinity, water temperature, deep and ultraviolet-b radiation irradiance were measured *in situ*. Samples waters were fixed with formaldehyde 4 % for DAPI bacterial count and zooplankton and phytoplankton microscopy analyses. Surface water samples were collected in sterile polyethylene bottles, after pre-rinsing the containers with wetland water. They were stored at 4 ° C until further processing in the laboratory (within approximately 24 h after collection).
Bacterioplankton community was analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) and Fluorescents In Situ Hybridization (FISH). Isolated bacteria were identified by sequencing of 16S rDNA.

Results:
Phytoplankton counts showed an abundance of between 100 and 220 (cells/ml) characterized by Bacillariophyceae (Diatoms), Cyanophyceae and Chlorophyceae. Zooplankton was represented by copepods with different distribution along the wetland. Bacteria diversity was related mainly to Gammaproteobacterias group, Bacteroidetes and Halobacteria (ie, *Marinobacter* sp. *Halomonas* sp. and *Halobacillus* sp.). However, low similarity with bacteria previously described suggests that this environment has an abundance of new microorganisms for phylogeny.

DIV6

Influence of moisture and organic matter in soil microorganisms abundance of the Médanos Grandes de Caucete, San Juan

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Soil microorganisms play a fundamental role in organic matter decomposition and nutrients recycle because several stages of the nutrient cycle are mediated exclusively by microorganisms, and some of them can participate in one or more biogeochemical cycles. In arid soils microorganisms' abundance and activity can be restricted by climatic conditions, water availability mainly. This is reflected in organic matter decomposition and nitrogen fixation. The objective of this work was to evaluate the soil microbial biomass which corresponds to functional groups and their relation with organic material and moisture amounts in Médanos Grandes, Caucete, San Juan province, seasonally. Samples of soil (0-20 cm depth) at two well differentiated environments (sand dune and interdune) were taken in autumn and winter. It was determined soil moisture and organic matter content. The abundance of ammonifiers, nitrifiers, and cellulolytic functional groups of microorganisms was determined by MPN method. The abundance of free living N fixing was analyzed by plating count. Total biomass is the accumulate abundance of studied functional groups. Results: in the entire studied place, microorganisms' abundance show significant differences ($p \leq 0.05$) between seasons (winter: 18.14 log CFU g-1; autumn: 14.59 log CFU g-1). Organic matter and moisture did not show significant differences. Seasonal analyses of parameters from each environment show significant differences in accumulated biomass (autumn: 14.42 log CFU g-1, winter: 18.16 log CFU g-1) and moisture (autumn: 0.52%; winter: 0.94%) of sand dune. In autumn the accumulate abundance of microorganisms was related to the moisture and organic matter in both environments studied: Sand dune (Biomass/Humidity: R2= 0.42; Biomass/OM: R2= 0.94); Interdune (Biomass/Humidity: R2= 0.38; Biomass/OM: R2= 0.52). On the other hand, was found correlation between parameters only in interdune (Biomass/Humidity: R2= 0.66; Biomass/OM: R2 = 0.42) in winter. Conclusion: seasonally changes in edaphic microorganisms' abundance can be related to changes of water availability. The characteristics of sand dune can influence water retention so; this fact can be better reflected in this place. In these environments water availability depends on rainfall pulses. The use of the recourse when the pulse occurs can be responsible for the variation on edaphic microorganisms' abundance. The organic material is not a limiting factor.

DIV7

Variation of the abundance of soil microorganisms under and around canopy of *Bulnesia retama* and *Larrea divaricata* (ZYGOPHYLLACEAE) shrubs of Médanos Grandes, San Juan.

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Soil microbial diversity shows complexity and variability of biological organization. Microbial communities play a key role in soil stability, organic matter formation and litter decomposition. Microorganisms abundance varies spatial and temporally. This fact is related to nutrients amount and quality variations such as ammonium, organic matter content, soil moisture and organic carbon availability. Soil moisture constitutes one of the limiting factors that influence primary activity, microorganisms' abundance and activity of desert ecosystems. Discontinuous and scarce rainfalls provoke soil microbial biomass fluctuations. The objective of this work was to evaluate microorganisms' abundance fluctuations in *B. retama* and *L. divaricata* patches and interspaces; and physicochemical characteristics in sand dune and interdune soils. Sampling was performed in April and August, 2007. Soil samples were taken under the canopy of both species and interspaces with a sterile device. Counting plate method was used to determine microorganisms' abundance using specific media for each microbial group. Results: First sampling: *B. retama* and *L. divaricata* soils and interspaces did not show significant differences in microbial content (bacteria, yeasts and filamentous fungi abundance) in sand dune environment. Bacteria abundance associated with *L. divaricata* was significantly different from *B. retama* and interspaces (p=0.0032) in interdune. Second sampling: Soil microbial abundance did not show significant differences from patches and interspaces. Physicochemical characteristics analyses showed that interspaces pH was significantly different from patches in both environments (p=0.0028). Rainfalls were 33.65 mm in first sampling and 0.325 mm in second one.*L. divaricata* patch showed that moisture content was significantly different from the other microsites (p=0.0488) in interdune environment from first sampling. In second sampling there were no significant differences among the three microsites. Abundance results showed that bacteria were the most important microbial group. On the other hand, data obtained suggest that patches of vegetation structure did not determine the microbial groups' distribution in the studied site.

DIV8

Abundance of microorganisms in soil and canopy of *Bulnesia retama* and *Larrea divaricata* in Médanos Grandes of Caucete, San Juan Province

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In Arid Ecosystems, vegetal cover is generally constituted by discontinuous perennial shrubs species. It is distributed in "vegetation patches" dispersed in an almost bare soil matrix. Herbs species can occasionally appear in soil between patches, especially in zones that are among sand dunes (interdunes). Shrubs that form patches can provoke positive effects, increase water infiltration rate and decrease water evaporation rate so, improve moisture balance. Many microorganisms associated to patches are important to plant growth. Nutrients cycle and uptaken are favored. The objective of this work was to determine and compare in soil and canopy of *Bulnesia retama* and *Larrea divaricata* the abundance of bacteria, yeasts and filamentous fungi, in interdunes. Samples were taken from soil and canopy of patches shrubs. Total biomass was determined by plating count method with specific culture media for each microbial group. The results showed significant differences in bacteria and yeasts abundances respect to filamentous fungi in *L. divaricata* (p=0.0041) and *B. retama* (p=0.0051) soil patches. Filamentous fungi abundance was significantly greater than yeasts in canopy of *L. divaricata* (p=0.0026). In canopy of *B. retama*, bacteria abundance was significantly greater than yeasts and filamentous fungi (p=0.0229). Statistical analysis of *L. divaricata* showed that bacteria abundance of soil was significant different from canopy (p=0.0022). Soil yeasts abundance was significantly greater than canopy (p=0.0022). Filamentous fungi abundance in soil and canopy did not show significantly differences. Statistical analysis of soil and canopy of *B.retama* determined that only filamentous fungi showed significant differences (p=0.0022). Bacteria and yeasts did not show significant differences. Conclusion: in soil and canopy of *B. retama* and *L. divaricata* interdune environment was not observed a clear distribution pattern of microorganisms' abundance (bacteria, yeasts and filamentous fungi).

2. Biorremediación y Biocontrol / Bioremediation and Biocontrol

REM1

Cu(II), Cd(II), Zn(II) and Cr(VI) influence on bacterial chemotaxis

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Several studies have shown the relevance of bacterial motility in bioremediation processes. Motility, biofilm development and pollutant bioavailability are variables to be considered for the proper design of a biological treatment. Chemotaxis towards biodegradable compounds is a microbial mechanism which is the key for the treatment success. Metals like copper, cadmium, zinc and chromium are ordinary components of industrial wastes, so their influence on bacterial motility and chemotaxis defines the efficiency of a bioremediation process. The aim of our work is to optimize an easy and rapid technique for microbial chemotaxis testing in presence of different substrates, including metals of environmental relevance. Preliminary, Escherichia coli chemotaxis was studied following a modified version of the "chemical in plug" assay, using a drop of PYG broth (casein peptone 2.5 g/L, yeast extract 1.25 g/L, glucose 0.5 g/L) in agarose 0.5% as attractant with different Cu(II), Cd(II), Zn(II) and Cr(VI) concentrations, surrounded by a bacterial suspension (in exponential growth) in buffer HEPES or K2HPO4/KH2PO4 (pH=7) and agarose 0.35%. Chemotaxis results were observed after an incubation of 2 hs. at 32ºC. The effects of Cr(VI) and Cu(II) on E. coli chemotaxis have been previously described by other authors, so these metals were used as control experiments in this improved assay. We could confirm that Cr(VI) and Cu(II) behaved like poor repellents for this microorganism, and put in evidence that Cd(II) and Zn(II) produced a negative effect on chemotaxis when these two metals were present with the chemoattractant. Pseudomonas veronii 2E, Delftia acidovorans AR and Ralstonia taiwanensis M2 are autochthonous strains, isolated from contaminated environments and are involved in the development of industrial waste biotreatments because their ability to retain Cu(II), Cd(II) and Zn(II) and biotransform Cr(VI) (Vullo et al., 2008, Bioresource Technology 99: 5574-5581). Effects of metals on swimming and swarming of these microorganisms have been detected. This chemotaxis test applied on these bacteria supplied the required information for a complete evaluation of metal influence on cell motility.

REM2

Cadmium accumulation and tolerance in Bradyrhizobium spp. (peanut microsymbionts).

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Cadmium (Cd) is incorporated into agricultural soils through phosphate fertilizers, sewage sludge, and atmospheric fallout from industrial and urban activities. Because of its high toxicity, at low concentrations, Cd imposes a serious threat to organisms and the food transfer of metals ions has become a major public concern. Mechanisms of Cd tolerance in microorganisms are diverse, such as immobilization within the cell wall and biochemical transformation of the metal which involves the participation of the glutathione (GSH). We have recently informed that GSH plays a crucial role in the survival of *Bradyrhizobium* sp. SEMIA 6144 against the toxic effects of acid, saline and oxidative stresses using a GSH-deficient mutant (*Bradyrhizobium* SEMIA 6144-S7Z) obtained by disruption of *gsh*A gene which encodes the enzyme γ-glutamylcysteine synthetase (ÁECS). The objectives of this work were 1) to investigate Cd accumulation and its effect on growth of two strains recommended as peanut inoculants (*Bradyrhizobium* sp. SEMIA 6144 and *Bradyrhizobium* sp. C145) and two native peanut isolates (NLH25 and NOD31) obtained from Córdoba soils; 2) to elucidate the role of GSH in the tolerance to Cd. The microorganisms were grown in YEM medium supplemented with CdCl2 concentrations ranging from 0 to 100 μM. B. sp. SEMIA 6144 and B. sp. C145 strains grew up to 15 μM Cd meanwhile *B*. sp. 6144-S7Z mutant strain was unable to grow at this concentration. NLH25 and NOD31 isolates grew up to 50 μM Cd. The Cd uptake by cells growing in presence of 15 μM Cd was assayed by inductively coupled plasma-atomic emission spectrometry (ICP-AES). In *B*. sp. SEMIA 6144 was found the highest Cd accumulation in relation to other strains. At 15 μM Cd, the intracellular GSH content in *B*. sp. C145 and NLH25 showed a significant increase in comparison to B. sp. SEMIA 6144 and NOD31 whose content was not modified. In conclusion, the differences in Cd tolerance among *Bradyrhizobium* sp. genotypes suggests that beyond the basic metabolic machinery of this species, there are also variations that allow strains to display distinct responses. Thus, the native peanut isolates tolerated higher Cd concentration than the reference strains, possibly the GSH level found could act as a detoxifying agent. This approach is currently being investigated.

Sopported by SECyT-UNRC

REM3

Isolation of actinomycete strains and screening of heavy metals resistance

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Heavy metals (HM) constitute a potential hazard for waters, soils and sediments. It has been shown that HM at certain concentrations can have long-term toxic effects within ecosystems. One of the biggest problems of contaminated soil and/or sediments in our province (Tucumán) as well as that of other regions is the co-contamination with various metals. Actinomycetes constitute a significant component of the microbial population in most soils. Their metabolic diversity and particular growth characteristics, mycelial form and relatively rapid colonization of selective substrates, indicate them as well suited agents for bioremediation of metal and organic compounds.
Microorganisms have developed changes in its genome that have enabled them to the acquisition of resistance to various heavy metals. By sharing complex habitat, such as soil, such resistance can be expressed in a cooperative manner and carry out successfully the bioremediation of environments contaminated with heavy metals.
The aim of this work was to determine the presence of multimetal resistance in new isolated actinomycetes.
Thirty-six isolated actinomycetes were used to carry out a qualitative screening of chromium (VI), copper (II) and cadmium (II) resistance. Twenty-four were isolated from the Pocitos Channel, four from Amaicha, five from Guillermina Channel, two from common soil of Tucumán city and one from heavy metal contaminated soil.
Primary qualitative screening assays were carried out in plates containing Minimal Medium supplemented with Cr(VI) (100, 300 and 500 ppm), copper (II) (200, 500 and 1000 ppm) and cadmium (II) (10, 50 and 100 ppm). In this case, rectangular wells were made in the center of Petri dishes by aseptically removing strips of agar which were filled with the heavy metal solutions. Isolates were inoculated by streaking spore suspension perpendicularly to the troughs.
Thirty-eight percent of isolated strains were resistant up to 500 ppm of chromium, thirteen percent were resistant up to 1000 ppm of copper and thirty-eight percent were resistant up to 100 ppm of cadmium. Only two isolates were resistant to the highest concentrations of the three metals, and twelve isolates were resistant up to the middle concentration of each metal.
This study showed the potential ability of actinomycetes as tools for bioremediation of co-contaminated soil with Cr(VI), Cu and Cd.

REM4

Microbiological process applied to olive mill wastewaters treatment

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Olive industry generates large amounts of olive mill wastewaters (OMW). The wastewaters are highly polluting and potentially producing environmental problems, since they have characteristics such as acidity, high chemical oxygen demand, high level of polyphenols and volatiles compounds and dark color due to tannic substances.
Generally the OMW is discharge into sewerage systems, spreading on land or deposited in open ponds near the plants. These procedures generate many environmental problems caused by the phenolic compounds characteristics as phytotoxic and antimicrobial (mainly antibacterial) properties.
The aim of this study was to investigate the feasibility of implementing a microbiological treatment of aerobic OMW, which does not require prior application of chemical processes, sterilization, dilution, or nutrients addition to make it feasible for industrial application, using a strain isolated from a edible fungus basidiomycete, gender *Pleurotus* strain PSC01.
The biological process evolution was followed by measuring the concentration of polyphenols and total COD in the substrate and the concentration of biomass as a measure volatile suspended solids. The results point to poliphenols degradation reached up to 48% and a COD reduction of 60%. The decolourization of the liquid medium does not result appreciable during the 21 days of treatment.

REM5

Study of *Pseudomonas sp.* 14-3 capability to accumulate polyhydroxybutyrate (PHB) from different hydrocarbon sources.

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Pseudomonas sp. 14-3 strain was isolated from the Antarctic environment. It's able to grow at low temperatures and has a high stress resistance. In conditions of Carbon-Nitrogen unbalance, this strain produces a large amount of PHB (70 to 80% PHB / dry weight). Due to its characteristics, this strain could be a good candidate for the remediation of environments contaminated with hydrocarbons, which have a high C/N ratio. We challenged this strain with several hydrocarbons without success. For that reason, *Pseudomonas sp. 14-3* and its mutant, *Pseudomonas sp. 14-3* Δ*phb*, were transformed with pGEc47 plasmid, in a way to provide the necessary genes for alkane degradation (alkBAC-alkR). *Pseudomonas sp. 14-3* Δ*phb* is a *phaC* mutant (PHB synthase) and is unable to synthesize PHB, but it is able to synthesize PHA. To analyze if the strains were able to grow in the presence of hydrocarbons, the cultures were grown at 32°C in 100 ml E2 minimal medium supplemented with 10% w/w of n-octane, gasoline, kerosene and diesel as the only carbon source. To avoid solvent evaporation, the cultures were done in 500 ml capped bottles in strong agitation (300 rpm). The growing rate was analyzed by OD measurement during 10 days. Alternatively, the strains were grown in Petri dishes with E2 agar supplemented with the hydrocarbons cited above.
To analyze PHB or PHA accumulation, sodium octanoate was utilized as sole carbon souse as positive control and a sample was taken even 48 hr. PHB accumulation was detected by staining with Nile Blue dye and observed in a fluorescence microscope.
Our results shown that:
- *Pseudomonas sp. 14-3* and its mutant, *Pseudomonas sp. 14-3* Δ*phb*, were able to grow in E2 minimal media supplemented with n-octane, kerosene and diesel as the only carbon source
- Neither the wild type nor the mutant were able to grow in a liquid media supplemented with gasoline as sole carbon source, but in E2 gasoline Petri dishes, colonies appeared after six days, perhaps due the presence of bacterial growth inhibiting substances in the gasoline.
- For all tested hydrocarbons, the wild type strain always showed a higher OD than the PHB- mutant.
- The staining with Nile blue revealed that the wild type strain accumulated PHB after the forth day of culture when hydrocarbons were used as the only carbon sources, while in the presence of sodium octanoate, the PHB accumulation was observed after 16 hours.
- Neither PHB nor PHA accumulation was observed in *Pseudomonas sp.14-3*ΔΔ*phb* strain.

These preliminary studies pretend to be the first step to analyze the effect of the capability to accumulate and degrade PHB into the hydrocarbon bioremediation.

REM6

Efect of biostimulation strategies on the dynamics of the soil microbial community in a chronically hydrocarbon polluted soil.

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Microorganisms play an important role in the biogeochemical cycles of organic matter in terrestrial and aquatic ecosystems and microbial degradation is one of the main processes involved in the natural decontamination of hydrocarbons and other environmental pollutants. Enhancement of this biological activity represents the basis of bioremediation technologies.
The biostimulation is the strategy developed to promote microbial degradation by addition of different components and/or improving the oxygen and water availability of the soil.
In this study we analyzed the effect of biostimulation with organic and inorganic compounds, either in the presence or absence of a commercial surfactant (Brij 700®), with the aim to promote the soil microbial activity in a weathered hydrocarbon contaminated soil. We prepared soil microcosms containing a mix of two soils from two places of the petrochemical pole La Plata-Ensenada. The effect of the fish flour (HP), inorganic salts (IS) and commercial fertilizer (NPK), with or without surfactant, were monitored by heterotrophic (HB) and hydrocarbon degrading (HDB) bacterial counts, dehydrogenase activity, PCR-denaturing gradient gel electrophoresis (DGGE) profiles and hydrocarbon concentration in soil during 44 days.
A significant increase in bacterial counts and dehydrogenase activity, with or without Brij 700®, was observed in the presence of HP during the experiment. Bioestimulation with IS or NPK did not cause significant effects on HB and dehydrogenase activity, compared with the control. In addition both, inorganic biostimulated and control soil microcosms showed a sharp decrease in HDB during the first five days remained constant for the rest of the assay.
The control DGGE profiles evidenced a stable bacterial community during all the experiment and a similar behavior was observed with inorganic salts. On the contrary, the bioestimulation with HP (with/without Brij 700®), caused a time-variable DGGE profile, with the appearance of some bands and disappearance of others. These changes were in accordance with the increase in microbial activity observed in the organic biostimulated microcosms.
Considering that two different contaminated soils were mixed to prepare the microcosms, some compounds from one soil could be harmful for the microbial community from the other and reciprocally. This harmful effect could have produced a decrease in HDB density of the new microcosm determining a new and different bacterial community. Under that condition, the biostimulation with salts would not be able to promote a significant increase of the bacterial populations. Instead, microcosms amended with organic nutrients (HP with/without Brij 700®), would allow an efficient recovery, as it was evidenced by the higher HDB compared with the control soil at day 44.
It was not possible to detect any significant reduction in soil hydrocarbon concentration between microcosms, probably due to the short time-course of experiment.

REM7

Aerobic degradation of m-nitrophenol by an indigenous bacterial community

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Nitrophenols are widely used as intermediates in the production of pharmaceuticals, dyes, plastics, pesticides and fungicidal agents, explosives and industrial solvents. These compounds are toxic and usually found as contaminants in aquatic environments, because of industrial, agricultural and hospital effluent discharges. Moreover, nitrophenols can also be found in soils as a result of the hydrolysis of several pesticides. In Argentina, the National Act on Hazardous Wastes (Act 24051/92) recommends levels of 0.2 μg/L of nitrophenols in order to protect aquatic wildlife. The aims of this study were: a) to select m-nitrophenol degrading microorganisms from natural sources, b) to study the aerobic biodegradation of m-nitrophenol and c) to asses detoxification using a toxicity test. Aerobic degradation assays were performed using a 2-litre microfermentor at 28 ºC with agitation (200 rpm). Biodegradation was evaluated by spectrophotometry, high performance liquid chromatography and microbial growth. The efficiency of the process was evaluated by a chemical oxygen demand (COD) test. Detoxification was assessed by a *Vibrio fischeri* toxicity test. We selected an indigenous bacterial community that can use m-nitrophenol as the sole carbon, nitrogen and energy source under aerobic conditions. The community degrades 0.36 mM and 0.72 mM within 24 hours and 40 hours, respectively. The process efficiency was 89% expressed as COD removal. Toxicity was not detected after the aerobic biodegradation of m-nitrophenol. We hope that the use of this indigenous bacterial community will become a valuable strategic tool to bioremediate m-nitrofenol polluted environments as well as m-nitrophenol contaminated effluents.

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REM8

Interaction of different biocontrol agents and PGPR bacteria with other beneficial microorganisms in the development of leguminous plants.

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Side effects cause by the introduction of biocontrol agents to the soil was evaluated. Compatibility with other beneficial microorganisms and possible synergistic effects on the development of soybean (Glycine max L Merril) and common bean (Phaseolis vulgaris L.) were evaluated. Co-inoculation with biocontrol agents (BA), arbuscular mycorrhizal fungi (AMF) and nitrogen fixing bacteria (NFB) was carried out.
A total of five strains were used in this study: Bacillus amyloliquefaciens BNM122, Pseudomonas fluorescens BNM296, Bacillus cereus BNM343, Burkholderia cepacia BNM345 (Banco Nacional de Microorganismos, IBYF-CONICET, Argentina), and Burkholderia brasiliensis M130-BR11340 (Agrobiología collection, Embrapa, Brasil). Assays were carried out in pots filled with tyndalized soil. Treatments consisted on individual inoculation of pre-germinated seeds (root:1.5 cm) with each one of the five selected strains. At sowing time, inocula of AMF (Gigaspora margarita plus Glomus clarum), plant specific NFB (Rhizobium etli BR322-BR520 for bean or Bradyrhizobium japonicum BR29-BR96 for soybean) were added to all pots. Plants were grown for 60 days in a greenhouse under controlled conditions. The registered and analyzed parameters were: fresh and dry weigh of aerial biomass, plant height at 30 and 60 days after sowing, mycorrhizal colonization, mycorrhizal spore number (MSN) and the number and weigh of nodules.
In bean plants the treatment with strain BNM345 showed an increase of 53 and 66 % in fresh and dry weigh of plants compared with the non-inoculated control. No differences were found in the rest of treatments and parameters measured. In soybean plants despite no differences in aerial biomass were registered, the inoculation with BNM345 increased the MSN and also the number and weigh of nodules. Also, strain BNM343 increased the MSN although a reduction in the fresh weigh of nodules was observed as occurred in the rest of the treatments.
Strain BNM343 deserves further studies in order to verify its positive effect on mycorrhization, a well known PGPR mechanism. Inoculation with strain BNM345 showed a beneficial effect for the two leguminous plants, suggesting a direct stimulation in both AM and NFB colonization. Inoculation with the other bacterial strains did not report any beneficial effect on none of the two leguminous plants and in addition, the soybean nodulation was reduced.

REM9

Isolation and characterization of PAH-degrading bacteria from polluted soil in Patagonia

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The oil industry is the main economic activity in East Patagonia (Argentina). Following extraction, transportation and storage of crude oil, some places in this region are frequently contaminated with hydrocarbons. A major fraction of petroleum mixtures, polycyclic aromatic hydrocarbons (PAH), have toxic, mutagenic or carcinogenic effects. In the semiarid soils of Patagonia several factors, such as inorganic nutrients, water, pH and temperature, limit the degradative activity of the autochthonous microbial community. Seeding by introducing microorganisms has been considered a valuable tool for increasing the rate and extent of biodegradation of PAH. In the context of an agreement between CONICET and Oil M&S S.A. company, we started an inter-institutional study for increasing the efficiency of bioremediation processes of contaminated soils in semiarid Patagonia, Argentina. The aim of this work was to isolate PAH degrading-bacteria from Patagonia soil samples chronically contaminated with PAH, and to study their physiological properties that could suggest the presence of mechanisms of adaptation to the typical environmental local conditions. To obtain bacteria with distinct abilities to promote bioavailability of PAH different isolation strategies were used: 1) enrichment cultures in liquid mineral medium with PAH crystals or PAH sorbed to hydrophobic membranes, 2) direct isolation on solid mineral medium supplied with PAH by spread of soil suspension or soil granules.
In total 7 PAH-degrading Gram (-) strains were isolated, of which 3 were obtained from enrichment cultures with PAH crystals and others from direct isolation from soil suspension, indicated that the isolated strains would be able to grow well in suspension. In spite of the fact that the PCR-DGGE analysis and sequence of 16S rRNA gene of the different strain showed that the all strain are very closely related and would belong to Sphingomonadaceae family, the physiological characterization (the ability to grow using different PAH as the sole carbon and energy, extension of PAH degradation and the C-starvation resistance) and the DNA fingerprint obtained by RAPD-PCR showed clear differences. A better understanding of the physiology of the isolated bacterial strains and their interactions with contaminants and environment will hopefully permit successful large-scale application of these inocula for bioremediation of semiarid soils in Patagonia.

REM10

Characterization of the enzymatic chromate reductase activity of *Streptomyces* sp. MC1

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In natural water and subsurface soils, chromium occurs in two major oxidation states: III and VI. Cr(VI) is approximately 1,000-fold more cytotoxic and mutagenic than Cr(III). As the application of Cr is extensive in several industries, chromium-associated pollution is an increasing problem.
With advances in biotechnology, bioremediation has become one of the most rapidly developing fields in environmental restoration, utilizing microorganisms to reduce the concentration and toxicity of heavy metals. In recent years, more and more attentions are put on the remediation of Cr(VI) contamination with chromate resistant microorganisms. Actinomycetes are the dominant bacteria population in soils; also their metabolic diversity and particular growth characteristics indicate them as potential agents for bioremediation.
Considering that Cr(VI) is more toxic than Cr(III), the treatment strategy could include the reduction of Cr(VI) to Cr(III). There are microorganisms that are able to grow with Cr(VI) and reduce it to Cr(III). This biological reduction may provide a less costly and more environmentally friendly approach to remediation. Previously, we isolated a Cr(VI) resistant actinomycete, *Streptomyces* sp. MC1. This strain showed ability to reduce enzymatically Cr(VI) in minimal medium. There is not reports on characterization of chromate reductase enzyme in *Streptomyces* strains.
The aim of this work was to characterize the chromate reductase enzyme of Streptomyces sp. MC1, a chromate reducing strain.
Streptomyces sp. MC1 was grew in minimal medium without and with Cr(VI) 1 mM. After four days of growth, cells were harvested, crushed and resuspended in water. Enzyme activity was determinate in supernatant of culture(S), cell wall (CW), cytoplasmatic fraction (CF) and whole cell (WC), using sodium phosphate or citrate buffer to obtain pH between 5 at 8, NADH or NADPH as electron source, and Cr(VI) as substrate. Reactions were incubated from 19 to 41 ºC.
The highest activity was observed in CF. Cr(VI) reduction was better in presence of NADH than NAD(P)H; also the optimum activity was found at pH 7 and 30 ºC. This activity was constitutive, however it was enhanced 200% when *Streptomyces* sp. MC1 was cultivated in presence of Cr(VI). This is the first time that chromate reductase enzyme was characterized in a *Streptomyces* strain. These results are the first step for studying the possibility to use *Streptomyces* sp. MC1 in Cr(VI) bioremediation process.

REM11

Fluorescent seed and root exudates from soybean and its antifungal effect against *Macrophomina phaseolina*

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The capacity of the seed and root exudates to excrete fluorescent compounds have been demonstrated in very few species, in addition the mechanisms that regulate the liberation of these compounds and whether they have some beneficial effect for the plant, for example in the defense against pathogens, are not known. The identification of these fluorescent compounds in soybean has not been reported. Nevertheless, it is known that certain phenolic compounds such as caffeic, ferulic, p-coumaric acids which are present in leaves of some plants emit fluorescence to a wavelength near 440nm. We have found similar fluorescence spectra, from exudates of seed and root of soybean. The aim of this study was to partially isolate fluorescent exudate components and correlate them with root or seed anti-fungal activity.
Four methods of phenolic compound extraction were evaluated, having used organic solvent, with three of these methods we separated a fluorescent extracts and a non-fluorescent extracts. Furthermore, the fluorescent extracts were dialyzed using bags with a cut-off of 2000Kd. Dialysis was performed in order to discard antifungal activity due to small peptides and enzyme.
Samples of the crude extract and all the subsequent separation phases were tested on agar plates against *Macrophomina phaseolina*, a pathogen responsible for the charcoal rot of soybean.
Microscopic examination of *M. phaseolina* in the presence of seed and root crude extracts revealed clear signs of stress and deletion, such as increases in numbers of sclerotia, vacuolation, granulation of cytoplasm, hyphal squeezing and lysis of mycelia fragments. After extractions, these signs only accompanied the fluorescent fractions and that one with compounds smaller than 2000Kb.
These results indicate that small molecular weigh fluorescent components of seed and root exudates have negative effect towards growth of pathogen fungus; being a competitive advantage for non-pathogenic microorganisms to colonize seeds.
The practical impact of this study is the possible use of natural compounds and their derivatives that could potentially being used as effective alternatives to conventional antifungal agents.

REM12

INHIBITION OF STAPHYLOCOCCUS AUREUS BY ROSMARINIC ACID AND CARNOSIC ACID, TWO MAIN POLYPHENOLS ISOLATED FROM ROSEMARY

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Rosmarinus officinalis L, belongs to the Lamiaceae family, is a rich source of phenolic phytochemicals with antioxidant and antimicrobial potential. This plant produces phenolics which are an important sub-group of secondary metabolites with diverse functional and medicinal potential. Lamiaceae plants have long been used in traditional medicine and have potential for control of pathogenic bacteria. Earlier, we reported the antioxidant and antimicrobial properties of non-volatile compounds of rosemary and our results showed that carnosic acid and rosmarinic acid may be the main bioactive antimicrobial compounds present in rosemary (Free Radical Research, 40 (2) 223-231, 2006). Here, we study the anti-*S. aureus* activity of rosmarinic acid and carnosic acid by the broth dilution method. *S. aureus*, a Gram-positive organism, is a human pathogen of significant importance. It is responsible for numerous infections and syndromes ranging in severity from skin and soft tissue infections to endocarditis and septic arthritis. As is the case for many other infectious diseases, *S. aureus* can develop resistance to antibiotics and it is one of major causes of treatment failure. The prevalence of resistant isolates, their virulence and increasing resistance indicates the need for alternative therapy to treat these infections with effective antibacterial agents with new modes of action. The objective of this research was to evaluate the efficiency of carnosic acid and rosmarinic acid alone and in combination with other compounds to inhibit this human pathogen.
Comparison using pure forms of other phenolic compounds found in a range of herbs species belonging to Lamiaceae as gallic acid and cafeic acid were also evaluated. In addition, combinatory assays with common antibiotics were performed. The final objective is to explore the potential for managing *S. aureus* infection in conjunction with current treatment options. Results showed that the anti-S. aureus activity of all compounds improved with increased dosage. Bactericide as well as bacteriostatic actions of the phenolic compounds was observed. A different mode of action for rosmarinic acid and carnosic acid are suggested. The antibacterial activities of these natural products make them interesting as antibacterial agents with potential new targets.
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REM13

GROWTH CONTROL OF LISTERIA MONOCYTOGENES IN TEMPERATURE ABUSED HAMBURGERS STORED AT OF 5ºC

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For more than two decades, *Listeria monocytogenes* continues to raise food safety concerns especially in ready-to-eat frozen stored products. Apart from the frequent isolation of the pathogen from meat and meat products, various reports have confirmed the implication of such products in listeriosis outbreaks. Bacteriocins or bacteriocin-producing starters were often suggested to be part of the "hurdle technology" to enhance the safety and keeping quality of meat products. Numerous lactic acid bacteria have been described to inhibit *L. monocytogenes* in vitro and in meat systems. In this work the inhibition effectiveness of *Lactobacillus curvatus* CRL705 and *Lactococus lactis* CRL1109 used as bioprotective culture and their bacteriocins, lactocin AL705 (12.800 UA ml^{-1}) and nisin (6.400 UA ml^{-1}) against *Listeria monocytogenes* (initial inocula 10^2 CFU g^{-1}) in temperature abused hamburgers stored at 5ºC, was evaluated. The addition of 106 CFU g^{-1} of each bacteriocinogenic strain to meat did not allow the growth of *L. monocytogenes*, showing a bacteriostatic effect during 72 h of aerobic storage at 5ºC. No synergistic effect was observed when both bacteriocin-producing strains were used together. However, after the addition of lyophilized bacteriocins to the hamburguers an immediate inhibition of the pathogenic organism in the was observed, while in the control samples *L. monocytogenes* grew, its number increasing by 1 log cycles. Bacteriocin activity in samples inoculated with the bioprotective cultures as well as with the bacteriocins was detected during all of storage period. These results demonstrated that the added bacteriocins were more effective when compared to the bioprotective cultures inhibiting *L. monocytogenes* in hamburgers stored at 5ºC during 72 h. The application of bacteriocins produced by *L. curvatus* CRL705 and L. Lactis CRL1109 as protective agent would provide and additional hurdle to enhance the control of *L. monocytogenes* in hamburgers stored at abuse temperature of 5ºC.

REM14

Effect of pH and temperature on antimicrobial activity of compounds produced by regional goat milk Lactic Acid Bacteria

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Lactic Acid Bacteria (LAB) have been used as starters of food and beverage fermentation and contribute to the development of taste and flavors and retardation of food spoilage. LAB protective role is due not only to their capability to decrease the pH in the medium but to bacteriostatic and bactericidal substances secreted by them. These substances include: hydrogen peroxide, ethanol, carbon dioxide, bacteriocins or other similar compounds. In this work, our goal was to determine the effect of different pHs and temperatures on the antimicrobial activity against *Listeria monocytogenes* of cells-free supernatants obtained from regional LAB strains. For this study, we used three LAB strains isolated from regional raw goat milk and typified as *Lactococcus lactis ssp lactis* (L1), *Lactococcus lactis ssp lactis 2* (L2) and *Lactobacillus paracasei ssp paracasei 1* (L3), using API CH 50 commercial kit. The strains were grown in MRS broth during 48 h at 30ºC in anaerobiosis and showed inhibitory activity against *L. monocytogenes* 74902. Cells-free supernatants were obtained by centrifugation at 45000 rpm during 20 min at 20ºC and filtered through 0.2 μm pore filter. Aliquots of supernatants were subjected to different treatments: a) they were heated at 30, 60 and 100ºC during 10, 30 and 90 min each; b) their pH was adjusted to 2, 5, 9 and 12 using HCl (10N) or NaOH (1N) as needed, during 2 h. After that, aliquots pHs were re-adjusted to 6.5 as in controls. The antimicrobial activity against *L. monocytogenes* 70902 was determined in the temperature- and pH-treated supernatants using the agar diffusion assay. Briefly, 20 ml of TSA plated in 10cm Petri dishes were inoculated with a *L. monocytogenes* suspension corresponding to 0.5 of Mc Farland. Wells of 5 mm diameter were cut and 30 ul of each treated supernatant were added to them and allowed to diffuse during 48 h at 30 ºC in aerobiosis. Macroscopic visualization of *L. monocytogenes* growing-inhibition zones showed 8.5, 8 and 7 mm diameters for the untreated supernatants from L1, L2 and L3, respectively. Interestingly, antimicrobial inhibitory activity was completely lost in the three cells-free supernatants subjected to the assayed pHs or heated at 60 and 100 ºC during any time, in comparison to controls. Additionally, L2 showed a decrease in the inhibitory activity when supernatant was heated at 30 ºC during 30 min. The results showed here would allow us to conclude that antimicrobial substances are susceptible to extreme pHs and temperatures treatments. These observations would contribute to the biochemical characterization of antimicrobial compounds secreted by L1, L2 and L3 strains which could have a potencial application as biopreservants or fermentation starters in food industry.

REM15

ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM COOKED MEAT PRODUCTS

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Lactic acid bacteria (LAB) are considered as food grade organisms that are safe to consume and have a long history of use in food. They have the potential to be used in biopreservation due their hability to produce several antimicrobial substances including lactic acid, diacetyl, hydrogen peroxide and bacteriocins. The use of bacteriocins, the organism which produces them or both, is attractive to the food industry because it is facing both increasing consumer demand for natural products and increasing concern about foodborne disease. Recent approaches in the preservation of cooked meats and minimally processed refrigerated foods are increasingly directed towards biocontrol using a protective microflora, usually LAB, to inhibit growth of Listeria monocytogenes, *Staphylococcus aureus* and others. The objective of the present work was to isolate LAB from cooked meat products manufactured in the province of Chaco, evaluate their antimicrobial activity against L. Innocua (as a model of L. Monocytogenes) and S. aureus and characterize the nature of the inhibition.
The products screened for antimicrobial activity from LAB comprised Vienna-type cooked sausage, “mortadela”, “Cracovia” and cooked ring sausage. All the samples were purchased at different markets throughout the province and stored at 7ºC. 10 g sample of the product was taken aseptically and a decimal serial dillution in peptone solution (1 g/l) was prepared to spreadplate the sample on MRS agar to allow isolation of LAB. Then, 234 colonies of LAB were randomly picked and mantained on MRS broth + glycerol (20 % v/v). An agar well diffusion assay was used for detection of antimicrobial activity. Indicator microorganisms were L. innocua and S. aureus. Inhibition was recorded as negative if no zone was observed around the agar well. Antagonistic activity was related to the area (mm2) of the inhibition zone displayed. Addition of NaOH and/or catalase to the cell-free supernant were used to rule out acid inhibition and hydrogen peroxide inhibition. The inhibitory subtance was tested for susceptibility to proteases.
A total of 22 isolates were shown to produce inhibition zones against L. innocua but only 8 of these isolates showed inhibition against S. aureus. These LAB isolates were characterized for their antibacterial compounds finding that they were bacteriocin-like after testing their proteinaceous nature and ruling out acid and peroxide inhibition.
LAB isolates adapted to meat substrates have a good potential to be used for the biopreservation of cooked meat products. Further research will be done in order to confirm whether these strains do improve safety of cooked meat products due to an inhibitory effect towards pathogen microorganisms.

REM16

NISIN SENSITIVITY OF COOKED MEAT PRODUCTS SPOILAGE BACTERIA

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Lactic acid bacteria (LAB) were identified as the major spoilage population of vacuum-packaged emulsion-type sausages and other processed meats stored at refrigeration temperatures. *Brochothrix thermosphacta* is also found to be a numerical significant component of the microflora of meat and meat products stored under these conditions. LAB and *B. thermosphacta* significantly influence the quality of meat and meat products, both being associated with spoilage in these products. Under anaerobic conditions LAB may cause souring, slimy, swelling of the pack and/or greening, while B. thermosphacta produce mainly lactic acid, ethanol and only small amounts of short chain fatty acids causing off-odours. One approach to extending the storage and shelf life of meat products is to introduce antimicrobials, preferably naturally occurring antimicrobials, to the products. Nisin, produced by *Lactococcus lactis* subsp. lactis, is a small, heat-stable protein, classified as a lantibiotic. Its spectrum of activity is limited and includes many gram-positive bacteria and their spores. Nisin is generally recognized as safe (GRAS) for use as a food additive in many countries. The aim of this work was to evaluate nisin sensitivity on spoilage microflora associated to cooked meat products manufactured in the province of Chaco. Vienna-type cooked sausage, “mortadela”, “Cracovia” and cooked ring sausage were prepared in local meat processing plants by traditional techniques. The products were vaccum-packaged in low oxygen permeability films and stored at 8ºC until signs of deterioration were observed.
From these products several strains were isolated, selected and purified followed by characterization using Gram stain, cell morphology and catalase and oxidase reaction. Agar well difussion assay was used to determine microorganism sensitivity towards nisin. Minimun inhibitory concentration (MIC) of nisin for each strain was performed by the critical dilution method; being 78 ppm the lowest concentration tested. Plates were incubated in the optimal conditions for each isolate and then inhibition zones were examined.
Thirty-seven strains were selected. According to the characterization, four of them were Brochothix spp. and the rest corresponded to LAB. The MIC could only be determined on seven LAB isolates since the rest of them showed sensitivity even at the lowest concentration of nisin. In further assay, dilution series will be extended in order to determine the MIC of the total isolates.
The high sensitivity to nisin displayed by all the strains isolated from deteriorated cooked meat products suggests its promising use as a biopreservative in the local meat industry. In the future, the combined utilization of nisin with lysozyme and EDTA will also be investigated in order to achieve a broader spectrum of inhibition which comprises Gram (-) bacteria.

REM17

Inhibitory effects of Lactobacillus from goat milk and artisanal goat´s cheese

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In Santiago del Estero, Argentina, the indigenous goat population is very high. The caprine livestock is concentrated in arid areas. These goats are very adaptable to the climatic conditions (yearly highest average temperature is between 40-45°C) and ensure the production of meat, milk and hide. The milk must be cooled immediately to avoid the proliferation of pathogenic and spoilage microorganisms. Novel strategies such as biopreservation systems have gained increasing attention as a means of naturally controlling the growth of unwanted microflora.

The aim of the present work is to select lactic acid bacteria from indigenous goat milk products that produce inhibitory substances, especially bacteriocins, in order to use them during the prematuration of raw goat milk, at chilling temperatures. The wild lactobacilli strains used throughout this work were isolated from 12 distinct samples of raw goat milk, natural whey starters and artisanal goat raw milk cheeses made without the addition of starters at 4 different locations of Santiago del Estero. The herds were composed of indigenous goats. 120 strains were isolated. The antagonistic effect of the neutralized cell-free culture supernatant fluids treated with catalase (CTCFS) of the 120 potential producer strains on various Gram-positive and Gram-negative organisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus lúteus, Listeria monocytogenes, Bacillus subtilis, Escherichia coli, Enterobacter sp. y Salmonella enteritidis*) was tested by the well diffusion assay. As bacteriocine-producer reference strain it was used L. casei CRL 705. 8 strains produced inhibition halos against the indicator species (L. plantaum CRL 691) and they were phenotypically classified as L. fermentum. The substance excreted by L. fermentum UNSE 212 was active against *Listeria monocytogenes, Pseudomonas aeruginosa, E.* coli and a wide range of lactobacilli. The activity of the antibacterial compound from L. fermentum UNSE 212 was destroyed by protease IV, trypsin III, a chimotrypsin, pronase E, trypsin and by heating 60°C 5 min. but was resistant to protease XV and catalase. The substance was stable within a range of pH from 3 to 7 at 35 °C. The formation of the best inhibition halo was achieved by precipitating the inhibitory substance with ammonium sulfate at 60 %. By inoculating at 10% a mixed culture composed of L. fermentum UNSE 212 (107 CFU/ml) and Listeria monocytogenes (102 CFU/ml) in raw goat milk at 12°C, at 10 hours a reduction of 1-log cycle was obtained by the bactericidal agent. Consequently, no changes in goat milk quality, determined by the titrable acidity, was observed when the inhibitory substance producer strain was present.

L. fermentum UNSE 212 is important from a technological point of view, since the addition of this culture to raw goat milk could be used to prevent pathogen growth during chilling at 12°C.

REM18-ORAL

ISOLATION OF Bacillus FROM NEWBORN CALVES. PRELIMINARY EVALUATION OF THEIR PROPERTIES FOR THEIR INCLUSION IN PROBIOTIC PRODUCTS

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Probiotics are used as viable microorganisms for animal feed, improving the intestinal microbial balance and preventing diarrheal diseases. Although bacteria from the genus *Bacillus* are used for technological applications, a few years ago has been proposed as an alternative for probiotic use, mainly by their spore forming characteristic. Probiotic products containing spores offer advantages over probiotics with vegetative cells by the longer storage time and their survival to the Gastro Intestinal Tract (GIT) conditions. The aim of this work was to isolate and select microorganisms of the genus *Bacillus* from new born calves, for their inclusion in a potential novel probiotic product that could prevent diarrheic processes by improving the establishment of the indigenous microbiota in the GIT. The samples were obtained from calves ranged from 0 to 4 months old. Nine *Bacillus* strains were isolated from faeces and 1 from the oral cavity. The strains were identified by phenotypic characteristics as catalase activity, type of haemolysis, lecitinase activity, anaerobic growth, acid production from glucose, nitrate production, gelatine and casein hydrolysis and growth in 7% NaCl. Two of the strains were identified as *Bacillus subtilis,* 1 as *Bacillus cereus* and the others as *Bacillus sp.* The *Bacillus subtilis* strains were selected for further studies. The surface properties were determined in spore and comparatively with vegetative cells. The autoaggregation degree was studied after two hours incubation. The hydrophobic nature of the cell surface was studied through the MATH (Microbial Adhesion to Hexadecane) technique. Neither the vegetative cells nor the spores assayed showed autoaggregative patterns. In contrast, the spores showed to be more hydrophobic than the vegetative cells in all the strain studied. The peroxide production was assayed by the plate technique with TMB (tetramethyl-benzidine). None of the strains were able to produce peroxide by this plate method. The production of inhibitory substances was assayed against *Y enterocolitica, E coli, S Dublin, S infantis, S enteritidis, S thyphimurim, Klebsilella sp, B cereus, S areus, S epidermidis and St uberis* by the plate diffusion method. All the *Bacillus* supernatants hardly inhibited the growth of the Gram positive strains assayed. The biosurfactant production was studied by the drop collapse technique and the emulsification capacity by the Cooper technique by using hexadecane for the disperse phase. Only one of the assayed strains shared these properties.

The strains selected will be further studied to evaluate their beneficial characteristics for their potential inclusion in a probiotic product for the prevention of newborn calves diarrhea. Also, the combination of bacteria including spore-forming bacilli and lactobacilli could be applied in the design of a new product to be use in animal nutrition for dairy farms.

REM19

Superficial properties of Lactic Bacteria (BL) isolated from bovine milk in dairy herds of Córdoba

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Mastitis, defined as the inflammation of mammary gland, is included among the prevalent diseases of the dairy cattle. The traditional strategies to prevent the disease are often costly and with adverse effects. The use of BL with beneficial properties for intramammary administration in cows during the dried period, would constitute an alternative method for the control of the disease together with the application of preventive measures. On the other hand, the specificity of species of the indigenous microbiota is an important characteristic to promote the settling of an ecological specific niche, like bovine mammary gland. The aim of the present work was to realize the isolation, phenotypical identification and the determination of superficial properties of BL isolated from bovine mammary gland, for potential incorporation in a probitic product of local application.
A total of 51 milk samples were collected from cows in lactation belonging to 8 dairy herds from the south of the province of Córdoba. The herds were visited once during August - October, 2007. The 17.6 % and 43.1 % of the samples were coming from cows with clinical and subclinical mastitis respectively, whereas 39.3 % was obtained of healthy animals. The samples were place in selective culture medium for BL's isolation, of which there 117 BL were isolated and identified by phenotypical tests. Most of the strains (47.87 %) were isolated from healthy quarters, while 38.46 % of quarters with subclinical mastitis and the rest from quarters with clinical signs of mastitis. There were studied the superficial properties of BL isolated by the determination of hidrofobicity (MATH with hexadecan) and of auto aggregation, which allow to predict its capacity of adhesion to epithelia. The majority of the strains isolated independently of the condition of the quarters showed low hidrofobicity (94 %) and lower capacity of auto aggregation (100 %). Nevertheless strains with median and high hidrofobicity (7) were isolated from healthy quarters (5), subclinical mastitis quarters (2) and none of them from clinical mastitis quarters. There was not correlation between both properties analyzed (Coefficient of Pearson's correlation: 0.136). The low hidrofobicity and the capacity of auto aggregation would be a characteristic of the BL isolated from this particular ecological niche. We are working on the determination of mastitis pathogens inhibition to complete the report of the beneficial properties. These results will allow advance in the selection of microorganisms that would be included in a probiotic veterinary product of local application for the prevention of bovine mastitis.

REM20

EFFECT OF THE ADMINISTRATION OF PROBIOTIC STRAINS ON THE INTESTINAL MICROBIOTA AND GROWTH PERFORMANCE PARAMETERS OF WEANED PIGLETS.

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The efficiency of animal digestion depends on the endogenous intestinal microbiota. Antibiotics are used to improve the health of animals and the extensive production in poultry and piglet industries. However, the use of these growth-promoting substances has led to an imbalance of the beneficial intestinal flora and the development of antibiotic-resistant bacteria.
In recent years, there has been a considerable interest in using probiotic microorganisms as an alternative to the use of antibiotics. Probiotics are viable microorganisms that once ingested produce beneficial effects to the host. Many lactic acid bacteria (LAB) are able to resist the gastrointestinal environment and to produce antimicrobial substances so they are usually used as probiotics.
In the present work we evaluated the enterobacteria population and growth performance parameters of weaned piglets after the administration of probiotic LAB strains.
On the 35th day of age, a total of 20 piglets were distributed into 2 groups: the non-treated control group and a probiotic supplemented fed group. Each group was housed separately in individual cages. Each experimental group was fed *ad libitum on* a commercial pelleted diet with free access to tap water for 35 days. In the probiotic supplemented fed group, 3 ml of a 108CFU/ml mixed probiotic culture was daily delivered to animals.
Body weight (BW), feed intake (FI), feed conversion efficiency (FCE: indicates the ratio between food consumed and body weight gain) and enterobacteria population counts in faecal samples were measured before and throughout experimental trial (7, 14, 21, 28 and 35 days).
The results obtained showed that the group receiving probiotics bacteria exhibited lower FI and FCE values than the control group *(P=0.05)* but the mean final BW values were not significantly different. Significant changes *(P=0.05)* in the enterobacteria population were found between control and probiotic supplemented fed group during the experimental period. There were no significant differences in lactobacillus counts in both groups. This fact would be probably due to the intestinal colonization of probiotic LAB administrated to the treated group.
These results suggest that the probiotic bacteria used in this study could be used as suitable strains to use widespread as a way to improve the well-being of animals and their growth performance parameters.

3. Fisiología y Metabolismo de Microorganismos / Physiology and Metabolism

FIS1

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF PSEUDOALTEROMONAS SP. P8 A COLD-ACTIVE XYLANASES PRODUCER

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Xylanolitic enzymes are involved in a variety of industrial reactions. Several studies have reported that enzymes produced by psychrophiles organisms have high specific activity at low and moderate temperature, and an elevate thermosensibility. Those properties are very useful to perform biological transformations at low temperatures. The aims of this work were: 1) Qualitative and quantitative determinations of xylanolitic activity from the *Pseudoalteromonas* sp. P8 strain. 2) Biochemical and physiological assays to characterize the microorganism.
P8 strain was grown in minimum medium added with xylan and hemicellulose. Enzymatic activities were detected at 4 and 20 ºC by the appearance of clarification halos after Congo red staining. Xylanase activity was determined at several temperatures (15, 25, 30 and 37 ºC) by the 3,5 dinitrosalicylic acid (DNS) method. The highest specific activity was registered at 25 ºC. Biochemical and physiological characterization were carried out using the API 20 NE (BioMerieux) test, and by growth curves at 4 and 20ºC. The obtained results demonstrate that *Pseudoalteromonas* sp. P8 is a promissory strain for cold- active xylanase expression.

FIS2

Increase of antihypertensive peptides from nitrogenous macromolecular fraction of argentinean red wine by Oenococcus oeni exoprotease.

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Hypertension is a risk factor for cardiovascular disease and stroke. Angiotensin-converting enzyme (ACE) hydrolyzes the inactive Angiotensin I and release Angiotensin II, which exercises a powerful vasoconstrictive action, with the consequent increase in artery pressure. Different peptides with ACE-inhibitory (I-ACE) activity have been isolated from different food sources, released after hydrolytic or fermentation processes. Its in situ production, can confer to food product an additional beneficial property.
The nitrogenous macromolecular fraction (NMF) of wines is comprised by, amino acids, peptides and proteins. The few studies carried out in wine, demonstrated that the low molecular weight peptides have I-ACE activity. *Oenococcus oeni* X2L, isolated from argentinean red wine, release an exoprotease to medium that can modify the peptidic fraction and their biological activity. The objective of this research was determine the I-ACE activity in the NMF of wine, and evaluate the increase of this activity by the presence of the *O. oeni* X2L exoprotease.
NMF was isolated from a commercial red table wine. Wine was dialyzed 48 h against tap water using a 5000 Da pore size membrane. The retentate was vacuum concentrated and lyophilized. *O. oeni* X2L was grown in grape juice basal medium pH 4.8. In exponential growth phase, cells were harvested, washed and resuspended in citrate buffer 0.05 M pH 5.0 during 2h at 20ºC for starvation. Cells were removed and the proteolytic activity was assayed in the supernatant by Cd-ninhydrin method. NMF was dissolved in citrate buffer pH 5.0 at concentrations of 100, 150, 200 and 500 % with respect to original concentration in wine. Same concentrations of NMF were dissolved in the *O. oeni* supernatant and incubated 60 min at 30°C. I-ACE activity percentage of each NMF solutions was measured by Cushman and Cheung method.
The untreated NMF solutions, at concentrations of 100, 150, 200 and 500 % showed I-ACE inhibitory percentage of 19.42, 45.39, 50.53 and 49.84 respectively. Protease activity detected in *O. oeni* supernatant, achieve a maximum of 2.08 mmol/l. NMF treated with *O. oeni* supernatant, showed an increase of free amino acid concentrations of about 30 %, indicating peptide hydrolysis. The I-ACE activity percentage of NMF treated with *O. oeni* exoprotease, achieving values of 35.56; 50.53, 65.83 and 52.34 for the 100; 150; 200 y 500 % of NMF concentrations respectively.
The I-ACE activity of the NMF of argentinean red wine was demonstrated for a first time. Maximum I-ACE inhibitory percentage was detected whit the NMF at double concentration with respect to wine. *O. oeni* X_2L exoprotease have an effective action on peptide hydrolysis of NMF and increase I-ACE activity in all samples assayed, possibly due to either a increase of the concentration of bioactive peptides or a release of peptides with higher activity.

FIS3

Nitric oxide production through heterotrophic nitrification pathway in two Azospirillum species differing in nitrite-reducing ability

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Nitric oxide (NO) is a gas present in animals, plants and microorganisms, involved in several physiological processes. In plants, one of the most important processes NO regulates is root morphogenesis. In bacteria, NO is produced by denitrification (i.e. stepwise reduction of NO3- to NO2-, NO, N2O, and N2). Besides, nitrite produced by nitrification (i.e. oxidation of NH4+ to NH2OH, NO2-, and NO3-) can be reduced to NO by a nitrite reductase. Both, nitrification and denitrification can be accomplished simultaneously by the same microorganism. It has been stated that *Azospirillum brasilense*, a plant-growth promoting rhizobacteria, can produce NO by aerobic or anaerobic denitrification, and also under conditions allowing the heterotrophic nitrification pathway. On the other hand, A. oryzae, a novel specie of *Azospirillum*, is able to reduce nitrate but not nitrite during denitrification. Taking into account the connection between the two NO-producing pathways at the intermediary level, our aim was to study NO production by *A. brasilense* Sp245 and *A. oryzae* through heterotrophic nitrification. Both strains were grown in aerated liquid media with NH4+ as N-source. At the end of log-phase growth, NH2OH and NO2- were quantified colorimetrically, and NO was determined with the NO-specific probe 4,5-diaminofluorescein diacetate (DAF-2 DA) using a fluorescence microplate reader. NO production induced by NH2OH or NO2- was also determined. In addition, an in silico search for ammonium monooxygenase (AMO) genes, which codify for enzymes that carry out the first step of nitrification, was performed in the *A. brasilense* Sp245 genome. Results showed that *A. brasilense* produced both NH2OH and NO2- at ÌM level, while NH2OH was not detectable and NO2- was accumulated in A. oryzae-culture supernatants. However, both microorganisms produced NO at the same level. NO production was induced by hydroxylamine and nitrite in A. brasilense but not in *A. oryzae*. Analysis in silico showed three putative AMO genes in *A. brasilense.* Overall, these results suggest an active heterotrophic nitrification pathway in *A. brasilense*. This pathway seems not to be functional in *A. oryzae*, thus it is suggested an alternative pathway for NO synthesis in presence of NH4+ and aerobic conditions. *This work was supported by ANPCyT and UNMdP. AADP and CMF are doctoral fellows of ANPCyT*

FIS4 - ORAL

Periplasmic nitrate reductase activity in *Azospirillum brasilense* affects siderophore production

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Iron (Fe) is an essential nutrient for almost all living organisms. In soils its natural abundance is high but in spit of this characteristic, aerobics and neutral pH environments greater reduces its bioavailability. Microorganisms had developed strategies to obtain Fe, being siderophores production one of the main used strategies. These are low molecular mass compounds, with high affinity by Fe ions. Nitric oxide (NO) is a small diffusible gas molecule present in animals, plants and even microorganisms. It participates as a regulator of numerous metabolic processes. It has been demonstrated that NO regulates *fur* activity, a general regulator of iron absorption in bacterium, linking in this way the signalling molecule NO with the iron acquisition from external environment. *Azospirillum* is a free living soil bacterium with the capability to colonize roots and promote general enhanced development of plants. Its capacity to fix atmospheric N2 requires a number of ferric-enzymes. On the other hand, it has been proved that *A. brasilense* growing in aerobic-NO3- -medium produces high quantity of NO, whereas its isogenic mutant Faj164 Nap^- (periplasmic nitrate reductase minus) produces only 5% of NO compared to the wild type. The objective was to evaluate siderophore production in *A. brasilense* Sp245 and Faj164 strains which differ in their capability to produce NO. Siderophore production was evaluated in Nfb medium without Fe in Petri dishes with $NO3^-$ or $NH4^+$ as nitrogen source and crome azurol S (CAS) as indicator. Results indicate less siderophore production in mutant Faj164 compared to wild type strain in $NO3^-$ medium. On the contrary, when Nfb-CAS had $NH4^+$ there were no differences in siderophore production between strains. In conclusion in A. brasilense Sp245 siderophore synthesis may be related to the NO levels produced by the bacterium. *This work was financed by ANPCyT and UNMdP. AADP is doctoral fellow from ANPCyT.*

aFIS5

PERIPLASMATIC PROTEINS OF GRAM-NEGATIVE BACTERIA INVOLVED IN THE BIOGENESIS OF METALLO-β-LACTAMASES

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All proteins fold to a characteristic native functional conformation, a situation that requires *in vivo* the participation of molecular chaperones which help promoting proper protein folding and prevent aggregation of misfolded states. Although a plethora of studies exist on the role of cytoplasmic chaperones, much less is known about periplasmic folding assistants. We analyzed here whether the biogenesis of metalo-β-lactamases (MβLs) requires assistance in the periplasmic compartment of Gram-negative bacteria. We first studied the biogenesis of the MβL GOB in *Escherichia coli* strains deficient in otherwise suggested periplasmátic chaperones such as DsbA, DsbC, DsbG, PpiA, PpiD, SurA, FkpA and DegP. Productive folding of this enzyme was evaluated by expressing the cognate gene (including its leader sequence) in the above mutants and analyzing its effects on ,β-lactam antibiotic resistence. Expression of the *gob* gene in any of these mutants did not result in a significant decrease of cefotaxime resistance when compared to the wild-type strain, suggesting that none of the above periplasmatic proteins participate individually in GOB folding, or the presence of redundant functions among them. In order to identify novel periplasmatic folding assistants, we generated MudJ random insertion mutants in GOB-producing *Salmonella enterica* var. Typhimurium 1344 and selecting for clones with reduced cefotaxime resistance. One insertion was localized in the phsB gen, which codes for a FeS protein and forms part of the *phsABC* operon that encodes the subunits of thiosulfate reductase responsible for H_2S production during anaerobic respiration. Our results suggest that this FeS protein may also function in the productive folding of metalloproteins such as MβLs in the periplasm of Gram-negative bacteria.

FIS6

Inactivation of type I polyhydroxyalkanoate synthase allowed the finding of other possible type II synthases

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Pseudomonas sp. 14-3 produces high levels of polyhydroxybutyrate (PHB). The cluster of genes involved in PHB biosynthesis (*phaRBAC*) are located on a genomic island being the PHB synthase (PhaC type I) the key enzyme. The wild type strain produces only PHB, the most common polyhydroxyalkanoate (PHA), from octanoic acid, but not from glucose. Inactivation of the *phaC* gene showed the ability to produce medium chain lengh PHA (PHAmcl) in this mutant, indicating the existence of another PHA synthase. Using PCR techniques, two putative PHA synthase genes (*phaC*mcl) were obtained from the wild type genomic DNA. One of them presented strong homology to type II PHA synthase genes of *Pseudomonas*, and the second one showed no homology with any of the synthases characterized until now. To analyze the functionality of these genes we followed two different strategies. In first place, the phaC genes were cloned into plasmid pBBR1MCS2 to complement a PHA-negative mutant of *Pseudomonas putida*. The second strategy consisted in mutate the two candidate genes using crossover PCR deletion techniques and screen for the lost of the ability to produce PHA. For the screening of acquisition or lost of the capability to synthesize PHA we used Nile red and Nile blue staining. Complementation of *Pseudomonas putida* KT2440 with *phaC* type I gene of *Pseudomonas* sp. 14-3 lead to the production of PHB instead of PHAmcl, showing a hierarchical control of type I synthase.

FIS7

Sucrose synthase expression and polysaccharides accumulation are enhanced under organic-carbon induced growth of filamentous cyanobacteria

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Occurrence of cyanobacterial blooms constitutes a major concern for human and livestock health and environment preservation. The model filamentous cyanonabcerium Anabaena variabilis reduces its doubling time from ca 30 h to 8 h when organic carbon is supplied to the culture medium, at the time that glycogen accumulation takes place. In this work we analyzed the effect of fructose on carbon metabolism in A. variabilis. We show that the nitrogen and carbon sources and light regulate the expression of the sucrose synthase (SuS, -D-glucosyl transferase, EC 2.4.1.13)αUDP-glucose: D-fructose 2-encoding gene (susA), in a similar way that they regulate the accumulation of polysaccharides. Furthermore, glycogen content in an Anabaena sp. mutant strain with an insertion inactivation of susA was lower than in the wild-type strain under diazotrophic conditions, while both glycogen and polysaccharides levels were higher in a mutant strain constitutively overexpressing susA. We also show that there are soluble and membrane-bound forms of SuS in Anabaena. Taken together, these results indicate that SuS is involved in the sucrose to polysaccharides conversion under fast-growing conditions induced by the availability of organic carbon, combined nitrogen and light.
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FIS8 - ORAL

Flour carbohydrate co-metabolism by *Lactobacillus plantarum* CRL 778 and *Saccharomyces cerevisiae.*

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The use of lactic acid bacteria (LAB) and yeast in wheat dough fermentation is a new option to obtain products with improvement dough properties, flavor, nutritional value and shelf life. These positive effects depend on the stability of LAB/yeast association. The interaction between them depends on both the competition for the carbon source (maltose, sucrose, glucose and fructose) and the sensitivity to the end products formed during fermentation. The aim of this study was to determine the sugar co-metabolism by *Lactobacillus* (L.) *plantarum* CRL 778 (from fermented dough origin and potential application in bakery for their antimicrobial properties) and *Saccharomyces* (S.) *cerevisiae* sp (commercial yeast). The microorganisms were grown as pure and mixed cultures at 30 °C for 24 h in broth containing pancreatic digest of casein (Casitone, Difco), K_2HPO_4, KH_2PO_4, Tween 80 and each (0.5% w/v) maltose, sucrose, or a mix (1% w/v) of sugars (0.2 % maltose, 0.7% sucrose, 0.05% glucose and 0.05% fructose) in similar proportions to dough. Cell viability, pH, residual sugars and end products (lactic acid, acetic acid and ethanol) were determined by HPLC and enzymatic kits. Results indicate that all sugars were used by *L. plantarum* and *S. cereviciae* as carbon source for growing. The lactobacilli preferently fermented monosaccharides, showing a faster growth rate in presence of these sugars. *S. cerevisiae,* showed a slower maltose uptake respect to sucrose, which was rapidly metabolized during the first 8 hours, releasing glucose and fructose. Similar results were obtained for the mixed cultures, while lactobacilli showed slow sugar uptake and no monosacharide release.
Ethanol and lactic acid production were lower (30-70% and 40-60%, respectively) when using mixed cultures; however no significant differences ($p>0.05$) on the growth were observed respect to single cultures.

FIS9

Yarrowia lipolytica Sterol Carrier Protein-2 localize in the yeast peroxisomes and has a potential role in the organellar lipid traffic.

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Yarrowia lipolytica is one of the most extensively studied "non-conventional" yeasts which is currently used as a model for the study of protein secretion, peroxisome biogenesis, dimorphism, degradation of hydrophobic substrates, and several new fields. The entire sequence of the six *Y. lipolytica* chromosomes has been determined. The sterol carrier protein-2 (SCP-2) is a nonspecific lipid transfer protein that has been implicated in the transport and metabolism of cholesterol, branched-chain fatty acids, acyl-CoA conjugates, and other lipids. Its gene was found in genomes from the three superkingdoms of life. We previously have shown that *Y. lipolytica* SCP-2 (YLSCP-2) is a 128 amino acid protein, which is inducible by fatty acids and binds a variety of lipids [Ferreyra *et al.*, 2006. *Arch. Biochem. Biophys.* 453(2): 197-206].
The aim of this work is to analyze the intracellular distribution of YLSCP-2 and perform genetic and biochemical studies to evaluate its function *in vivo.* To estimate the cellular concentration of YLSCP-2, an ELISA was developed and applied to the analysis of the cytoplasmic and peroxisome enriched fractions of the yeast cells mechanically broken. It was found that after induction, YLSCP-2 accounted for 0.35 ± 0.01% (mean ± SE; n=5) of the protein content of the organelle fraction; whereas in cells grown in glucose this content was 0.07 ± 0.01% (mean ± SE; n=4). On the other hand, YLSCP-2 content of the cytoplasmic fractions were 0.22 ± 0.02 and 0.14 ± 0.02% (mean ± SE; n=5) of total cytoplasmic protein for induced and non-induced cells, respectively. However, based on the proportion of marker catalase activity found in the cytoplasm, significant YLSCP-2 leaking from the peroxisomal fraction during fractionation cannot be ruled out. This results confirmed that YLSCP-2 is strongly induced by palmitic acid and preferentially located in the peroxisomal fraction of yeast lysate. The magnitude of this induction is such that it exceeds several folds the expansion of the peroxisomal compartment elicited by the inductor. Moreover, the inferred concentration of YLSCP-2 in the peroxisome, roughly estimated herein as 35 ÌM, and the affinity of YLSCP-2 for long-chain fatty acids, are high enough to make the protein to function as a lipid carrier inside the organelle. The biochemical data presented here is complemented by a genetic approach: *Y. lipolytica* null mutants for the gene codifing YLSCP-2 are being generated in the lab to evaluate the physiological consequences of its absence.

FIS10

Biosynthesis of glycogen by *Rhodococcus* members

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Members of genus *Rhodococcus* are frequently found in soil and other natural enviroments where several stresses conditions are commons. These microorganisms belonging to the non-sporulating actinomycetes, have showed to be resistant to various extreme conditions such as desiccation, osmotic stress and nutrient-limitation. Thus, they had to develop metabolic strategies to cope with such environments. One of these mechanisms may be the accumulation of storage compounds that can be utilized by cells as endogenous carbon sources and electron donors during periods of nutritional scarcity. The purpose of this study was to examine the *R. jostii* RHA1 genome for the presence of key genes involved in glycogen metabolism and to analyze the physiological capability of this strain for accumulating this storage compound. In addition, others members of *Rhodococcus* genus were also analyzed.
Six *glg* genes encoding key enzymes for glycogen metabolism were identified in RHA1; *glgA, glgX, glgC, glgP, glgE* and *glgB*. Key conserved amino acid residues in these proteins were identified in order to support gene identification.
On the other hand, RHA1 was able to accumulate a polysaccharide after cultivation of cells on both, nutrient broth and minimal salts medium with gluconate as sole carbon source. The qualitative analysis of this polysaccharide reveled to be a glucose polymer, like glycogen. In addition, we found that other species of the genus as *R. opacus, R. ruber, R. fascians* and *R. erythropolis* were also able to accumulate glycogen. In all cases, glycogen was a minor component in comparison with other storage compounds like triacylglycerols (TAG). To our knowledge, this is the first report on glycogen accumulation by *Rhodococcus* members.
Since the biosynthesis pathways of glycogen could compete for common precursors with other compounds like TAG, we analyzed the metabolic relationship between these two storage compounds in *R. opacus* PD630 using cerulenin, an inhibitor of the fatty acid de novo synthesis. The inhibition of TAG biosynthesis and accumulation by cerulenin, caused a sligth increase in glycogen content as revealed by semiquantitative TLC analyses. These results suggested that the biosynthesis routes of glycogen and TAG compete for common precursors, although they are used preferentially for TAG biosynthesis by cells of *R. opacus.* The ability to synthesize glycogen seems to be a common feature in *Rhodococcus* members. Glycogen, in addition to other storage compounds may contribute to the adaptive responses of rhodococci to environmental conditions.

FIS11

The Ferredoxin (flavodoxin)-NADP(H) oxidoreductase of *Rhodobacter capsulatus.* Function and Regulation

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The purple bacterium *Rhodobacter capsulatus* has a remarkable capacity of adapting its metabolism to a broad range of environmental conditions. When illuminated in anaerobiosis this microorganism synthesizes ATP through a cyclic electron transport around a single photosyntem, whereas the presence of oxygen promotes the establishment of a respiratory metabolism.
We demonstrated before that *R. capsulatus* contains a ferredoxin(flavodoxin)-NADP(H) oxidoreductase (FPR), capable to reduce the flavodoxin NifF, involved in nitrogen fixation metabolism. The contribution of FPR to the cell antioxidant response was demostrated in enterobacteria, but its physiological role in photosynthetic bacteria is still poorly understood.
To characterize FPR metabolic function, we analyzed the interaction between the enzyme with the FdVI, which was proposed to participate in assembly of iron-sulfur clusters. Recombinant *Rhodobacter* FdVI was purified by combination of anionic exchange chromatography and molecular filtration. The oxidase activity of FPR mediated by Fd was monitored by following NADPH consumption under steady-state conditions. Measured rates agree well with the putative function assigned to this enzyme in the biosynthesis of Fe/S clusters. Aconitase and 6-phosphogluconate dehydratase, Fe/S containig enzymes, are sensitive to oxygen exposure. The ability of FPR/FdVI system to repair Fe/S oxidized clusters, was tested on *Rhodobacter* cells free extracts, but it was found to be unfunctional in reactivation of oxygen damaged Fe/S cluster in an *in vitro* reconstituted system.
Sequence analysis of promoter region of *fpr* gene showed three RegA binding consensus sequences. RegA/RegB is a two component system and has been involved in regulating the balance between the generation and utilization of reducing power in this bacterium. RegB is a kinase that sense the redox homeostasis cellular and oxygen level, inducing the phosphorilation of RegA.
To study the *fpr* gene expression we constructed translational and transcrptional *lacZ* fusion and introduced them in *Rhodobacter capsulatus* cells by biparental conjugation. β–galactosidase activity and FPR activitiy were made on wild type and *regA*-disrupted strains. Ours results suggest that the RegA/RegB system function as an aerobic activator of R. *capsulatus fpr* gene expression.

FIS12

YhfJ: a Lipoyl transferase Involved in the Exogenous Protein Lipoylation Pathway in *Bacillus subtilis*

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Lipoic acid (LA) is a sulfur-containing cofactor found in most prokaryotic and eukaryotic organisms. In *Escherichia coli* and other organisms lipoic acid is essential for the function of several key enzymes involved in oxidative and single carbon metabolism including pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase (2OGDH), branched-chain 2-oxoacid dehydrogenase (BCKADH), acetoin dehydrogenase (ADH) and the glycine cleavage system (GCS). The current model for protein lipoylation in *E. coli* involves two pathways. The exogenous pathway, in which LA enters the cell by diffusion and is attached to the apoproteins by lipoyl protein ligase A (LplA), provides a scavenging pathway for utilization of exogenous LA. The endogenous pathway is the major route of LA synthesis. It consists in two steps: the first one is the transfer of octanoate from octanoyl-ACP to the 2-oxoacid lipoyl domains and to H protein catalyzed by octanoyl-[acyl carrier protein]-protein transferase (LipB), and the second, is the conversion of octanoylated domains into lipoylated derivatives by lipoyl synthase (LipA), which catalyzes the insertion of sulfur atoms into the six- and eight-carbon positions of the corresponding fatty acids. In bacteria, enzymes involved in lipoylation have gained increasing attention because of their implication in pathogenicity.
We have previously constructed a *Bacillus subtilis ¢ywfL* mutant, NM51, which was dependent of the addition of LA for growth in minimal media. Its growth was also restored by the addition of acetate and branched-chain fatty acid precursors, indicating that this strain was deficient in PDH and BCKADH activities. However, 2OGDH activity is not affected in this mutant. NM51 sporulates poorly in Schaeffer's sporulation medium, and this phenotype was partially reverted by the addition of LA. These results showed that YwfL is involved in the endogenous protein lipoylation pathway in *B. subtilis*, so *ywfL* was called *lipL*. The aim of this work was to identify the enzyme/s of *B. subtilis* involved in its exogenous lipoylation pathway. Analysis of the genome sequence revealed that this microorganism possess two ORFs which encode products with homology to LplAs (*yhfJ* and *yqhM*). We constructed an *yhfJ* null mutant, NM60, which did not present any growth defects in minimal media nor was deficient in sporulation, as expected for a lipoyl ligase. However, a *lipL yhfJ* double mutant, NM67, was unable to grow in minimal media even if LA was added. Addition of acetate and branched-chain fatty acid precursors partially restored its growth, but the growth rate was much slower than the observed for the wild type. Activities of dehydrogenase complexes of NM51 and NM67 strains grown with or without LA were measured. These results indicate that YhfJ has lipoyl transferase activity which is responsible of the lipoylation of the dehydrogenase complex.

FIS13

Biogenesis of Metallo-β-Lactamases in Gram-Negative Bacteria

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The expression of periplasmic,β-lactam degrading enzymes (β-lactamases) constitutes the most common mechanism of antibiotic resistance in Gram-negative bacteria. The recent emergence of, β-lactamases with the ability to degrade carbapemens, the last generation of b-lactam antibiotics, is now worrisome. Some of these novel enzymes possess one or two Zn(II) ions in the active site, and are known as metallo-β-lactamases (MβLs). MβLs have been described in nosocomial strains of many pathogens including *Bacteroides fragilis, Pseudomonas aeruginosa, Aeromonas hydrophila, Serratia marcescens, Elizabethkingia meningoseptica,* etc, and their dissemination due to genes carried on mobile DNA elements represents an acute problem worldwide.
MβLs are synthesized as precursors in the cytoplasm, and must be therefore translocated across the inner cytoplasmic membrane by either the general secretory (Sec) pathway or the twin-arginine translocation (Tat) pathway to reach the final functional destination. The cytoplasmic traffic of the precursors must be coordinated with the secretion apparatus, a process in which SecB and/or other molecular chaperones may participate. We have recently characterized the MβLs GOB-18 from a clinical isolate of *E. meningoseptica.* This enzyme is the deepest branching member of the family and shows substitutions in amino acid positions highly conserved in other MβLs. Our in vitro studies showed that GOB has a novel active site and is a broad spectrum enzyme maximally active with one equivalent of Zn(II) (Moran-Barrio *et al, JBC,* 282(25), 2007).
In the present work we studied the chaperone requirements for GOB biogenesis and secretion in *E. coli.* The extent of GOB secretion was analyzed by the ability to confer cefotaxime resistance to the cells as well as by immunoblot analysis of periplamic contents in both wild-type cells and isogenic mutants deficient in components of different secretion pathways (Sec and Tat) or cytoplasmic chaperone systems (Hsp70, Hsp60 and TF). Our results indicate that the Sec machinery mediates the secretion of GOB in an extended conformation. In addition, we observed a fundamental role of the DnaK system in this process: DnaK itself was essential and the co-chaperone DjlA specifically participated in the secretion process. In addition, TF also participated, probably directing the precursor from the ribosome to the Sec machinery. On the contrary, GroEL/ES was dispensable for the secretion process. In summary, several interactions between different cytoplasmic chaperones and the Sec machinery are necessary for the secretion of a pathogenicity factor such as an MβL in Gram-negative bacteria.

FIS14

Cytoplasmic *Escherichia coli* adenosine diphosphate sugar pyrophosphatase binds to cell membranes in response to extra-cellular signals as cell population density increases

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Adenosine diphosphate sugar pyrophosphatase (AspP) is a member of the “Nudix” (Nucleoside diphosphate linked to some other moiety X) hydrolase family of enzymes that catalyses the hydrolytic breakdown of ADP-glucose (ADPG) linked to glycogen biosynthesis (Moreno-Bruna *et al.,* 2001). In a previous work we showed that AspP activity is strongly enhanced by both glucose-1,6-bisphosphate and nucleotide-sugars, and by macromolecular crowding (Morán-Zorzano *et al.,* 2007). In this work we show that AspP binds to cell membranes as bacterial population density increases, ca. 30% of the total enzyme remaining membrane-associated as glycogen depletes during the stationary phase. This process is not dependent on the stationary transcription factor RpoS, the producer of the bacterial quorum-sensing autoinducer 2 (LuxS), presence of glycogen granules or glucose availability, but is stimulated by small soluble heat labile molecule(s) occurring in cell free spent supernatants of stationary cultures that are acid stabile and base labile. These data further point to AspP as a highly regulated enzyme, and provide a first set of evidences indicating that glycogen metabolism is subjected to regulation by intercellular communication in *E. coli.*

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Morán-Zorzano, MT, Viale, AM, Muñoz, FJ, Alonso-Casajús, N, Eydallin, G, Zugasti, B, Baroja-Fernández, E & Pozueta-Romero, J (2007) *Escherichia coli* AspP activity is enhanced by molecular crowding and by both glucose-1,6-bisphosphate and nucleotide-sugars. *FEBS Lett* 581: 1035-1040.

FIS15

Effect of the addition of amino acids in relation to the growth of strains *Lactobacillus plantarum* in natural orange juice

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In a previous study we demonstrated the amino acids requirements of the strains of *Lactobacillus plantarum* N4 and N8 isolated from oranges. Six common amino acids were necessary for growth of both strains. The N4 strain other two amino acids required in addition to the six amino acids necessary for growth.

The objective of this study was to evaluate the growth parameters of *Lactobacillus plantarum* N4 and N8 from oranges from Tucumán, Argentina, in orange juice and in the same medium added with the sulphur amino acids methionine (non-essential) and cysteine (essential) and the aromatic amino acid phenylalanine (stimulatory). The physical and chemical changes produced in the natural media due to microbial growth were evaluated.

Natural orange juice was clarified and autoclaved at 1 atm of overpressure. Amino acids were individually added at 0.2, 0.5 and 1g/l. Bacterial cultures were inoculated at a rate of 2%, and incubated at 30°C for 24 h. Bacterial growth was determined by counting cfu/ml. In samples of culture supernatants residual glucose was determined during growth in the different conditions.

Lactobacillus plantarum N4 and N8 developed in natural medium with a growth rate of 0,30 and 0.36 h^{-1} reaching a final cell concentration of 8,6 x10^8 and 3.2x10^8 cfu/ml, respectively. No change in growth parameters of the strain N4 was observed by the addition of cysteine, methionine or phenylalanine to the natural medium. In presence of 0.5 and 1g/l of cysteine the N8 strain growth rate diminished to 0.15 and 0.14 h^{-1}, respectively. By contrast, 0.2g/l of cysteine increased its growth rate, reaching the maximum cell concentration at 14 h of incubation. Methionine enhanced the growth parameters of the N8 strain in all assayed concentrations. In presence of 1 g/l, the maximum final biomass (4.52x10^8 cfu/ml) and growth rate (0.42 h^{-1}) were reached. Phenylalanine also stimulated the N8 strain growth rate, especially when concentrations of 0.5 and 1 g/l were present. In these conditions the final biomass also increased. Initial glucose consumption was about of 11.8% for the N4 strain and about of 14% for the N8 strain, except in the medium with phenylalanine where it corresponded to 25%. The initial pH diminution during growth in the different assayed media was correlated with the N4 and N8 strains fermentative metabolisms. Considering the growth responses in the natural medium and added with amino acids, it possible to infer that the nitrogen composition of orange juice could be enough to satisfy the *Lactobacillus plantarum* N4 amino acids requirements, independently of their categories as essential, non-essential or stimulatory amino acid. However, the Lactobacillus plantarum N8 growth was more effective in the natural medium with amino acids, principally methionine or phenylalanine than without them.

FIS16

Tyrosine effect on the development of *Lactobacillus plantarum* strains isolated from apples

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Decarboxylation of aminoacids, such as tyrosine, histidine and ornithine, results in formation of the corresponding biogenic amine, tyramine, histamine and putrescine; wich are the most frequently encountered in fermented foods (fish products, meat, and cheese) and beverages such as, wine and beer. Toxicological problems may result from the ingestion of foods containing relatively high levels of biogenic amines. Because of their physiological activities, histamine and tyrosine have been the most studied. Amine production has been associated with protective mechanisms of microorganisms against an acidic environment. Lactic acid bacteria are able to grow in acid fruit juices and are generally recognized as safe. Biogenic amines are of concern in relation to food hygiene; the occurrence of relatively high levels of certain amines has been reported as indicators of a deterioration process and/or defective elaboration practices.

The objective of this work was to study the effect of tyrosine on the development of *Lactobacillus plantarum* strains 42, 44, 49 and 66, isolated from apples, in acidity conditions similar to fruit juices. The culture media contained in g/l: peptone, 5; yeast, 3; glucose, 0.5 and tomato juice, 2% v/v. The media was added with tyrosine, 1 g/l and pyridoxal phosphate, 0.006 g/l and pH was adjusted to 4.5. Cultures were incubated at 30 oC during 15 days. Cell growth was determined spectrophotometrically at 560nm, and pH measurements were carried out.

No difference was observed in the growth between the control medium and the supplemented with tyrosine after 15 days of incubation in all assayed strains. Maximum growth rate (μmax) of the strain 42 of *Lactobacillus plantarum* was similar in both media. In the strains 44, 49 and 66 μmax increased 8, 23 and 26 % respectively in the media with tyrosine if compared with control media (0.025, 0.026 and 0.031 h^{-1}, respectively). Initial pH (4.5) diminished to 3.8-4.1 at the end of exponential growth phase in the different media, and remained constant except in the strain 44. In this case an increased pH value was observed when tyrosine was present in the medium (4.20) with respect to control medium (3.99). This fact could be related with the tyramine production by the strain 44 during the stationary growth phase. Thus, the ability to produce biogenic amines could be a strain-dependent property in *Lactobacillus plantarum* from apples.

The aminogenic potential is a negative characteristic of lactic acid bacteria that constitute autochthonous microflora of fruits, excluding them of technological applications such as cider production.

FIS17

Unusual phosphate-dependent expression of *Escherichia coli* respiratory genes in stationary phase

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In *Escherichia coli*, the three types of respiratory components are: (1) dehydrogenases, which carry out the oxidation of organic substrates and feed electrons into mobile quinone pool, (2) quinones, which deliver reducing equivalents to the terminal oxidases, and (3) oxidases, which reduce the terminal electron acceptors. Bacteria alter the composition of the respiratory chain as part of its ability to adapt to different growth conditions. The amount of each component is strictly regulated to optimize the respiratory chain, and maintain the redox balance, according to the substrates present and the physiological needs of the cell. In response to growth arrest, the aerobic electron transport chain components are down-regulated. Previously, we have found that the ndh gene, encoding respiratory NADH dehydrogenase-2, was unusually expressed in late stationary phase when phosphate concentration was >37 mM. In the former conditions, we have also demonstrated that cells presented higher oxygen consumption rates, were more viable, and had a lower NADH/NAD^+ ratio (similar to exponential) than cells grown in sufficient phosphate media (2 mM). Here, the expression of ndh and the other respiratory chain genes (*nuoAB, sdhC, cyoA, cydA,* and *ubiC*) was tested under different phosphate concentration up to 96h. In addition, NADH and succinate dehydrogenase, and NADH oxidase activities were assayed. The presence of high phosphate concentration in the culture media maintains the dehydrogenase activities and respiratory genes expression for at least 96 h, excepting NADH oxidase activity and *cyoA* gene expression. In order to investigate if the phosphate-dependet expression was mediated by a global transcriptional factor, a comparative study of respiratory promotors was done. Then, *ndh* expression was assayed using strains deficient in FNR, IHF, ArcA, and Fis. Any of those regulators was the responsible of phosphate effect in stationary phase. The present results point that the salt composition of the minimal culture media should be carefully considered in the experimental design, especially when stationary phase events are studied. Our results also suggest that phosphate concentrations in stationary phase may act as a signal for the maintenance of aerobic metabolism by a not yet described mechanism. Moreover, the long-term effect of phosphate concentration on respiratory chain enzymes is promising to postulate an unknown role of the repiratory enzymes in late stationary phase.

FIS18

PHYSIOLOGICAL VERSATILITY OF RHODOCOCCUS SP. 602 TO SYNTHESIZE TRIACYLGLYCEROLS IN DIFFERENT CONDITIONS

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A challenge in biotechnological approaches is the selection of the correct microorganism. *Rhodococcus* sp. 602 is a microorganism isolated from a soil sample collected in a polluted area from the petroleum industry. This is an oleaginous strain with the ability to degrade a broad variety of pollutants.
The aim of this study was to test the physiological versatility of strain 602 to transform several substrates in triacylglycerols (TAG) in different culture conditions.
First, we studied the ability of this strain to produce TAG from different substrates; strain 602 synthesized TAG from gluconate, benzoate, n-hexadecane, 3-cyclohexylpropionic acid, octadecylamine, ethanol + glycerol, n-hexadecane + n-hexadecanol and naphthalene; but not from phenanthrene, anthracene nor fluorene. Second, we quantified TAG produced from some of these substrates. From gluconate or benzoate TAG reached 71,22 or 64,91% of cellular dry weight, respectively. When this strain was cultivated on naphthalene under nitrogen limiting conditions, TAG reached 48,8 % of total lipids and was constituted by fatty acids of C8, C10 and C12 chain length; which are shorter than those occurring in gluconate or n-hexadecane cultures (C14 to C18). In this work we propose a new pathway for degradation of naphthalene in *Rhodococcus,* where a mono-oxigenase would be involved: naphthalene → 1-naphthol → 1,2,3,4-tetrahydro-1-hydroxynaphthalene → 4-hydroxy-1-tetralone → central metabolism. In addition, we amplified a diacylglycerol acyltransferase/ wax esther synthase (WS/DGAT) gene from this strain, a key enzyme for TAG biosynthesis; which showed high homology to *atf*1 from *R. opacus* PD630 and ro00039 from *R. jostii* RHA1, two known oleaginous microorganisms. Third, we studied the ability of this strain to produce TAG under diverse culture conditions: NaCl 3% (p/v), pH 10 and 4° C. In all these conditions strain 602 remained metabolically active, and showed the ability to accumulate TAG.
The versatility of *Rhodococcus* sp. 602 to synthesis TAG from different substrates and under different culture conditions make this microorganism a good candidate for biotechnological production of bacterial oils from wastes of petroleum industry and other organic residues.

FIS19

AUTOAGGREGATION AND BIOFILM FORMATION IN *Sinorhizobium meliloti* ARE AFFECTED BY NaCl.

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Sinorhizobium (Ensifer) meliloti is a Gram negative soil bacteria which symbiotically associates with alfalfa roots. In resting conditions, bacterial suspensions of the *S. meliloti* wild type strain Rm2011 were able to autoaggregate, as a result of the adhesion among the bacteria. These cell-cell interactions were estimated with a quantitative autoaggregation assay. We found that autoaggregation was similar in bacteria grown until mid-exponential or stationary phases. Autoaggregation in Rm2011 *exoY,* a mutant strain unable to produce the exopolysaccharide succinoglycan (EPSI), was similar to the wild type strain, suggesting that succinoglycan may not be involved in the establishment of cell-cell interactions. The medium composition strongly affected autoaggregation and biofilm formation. The addition of NaCl (300 mM) to the medium caused a significant inhibition of autoaggregation in both strains. When cultured in Y manitol minimal media, both strains were able to form robust biofilms on polystyrene microtiter plates. The presence of NaCl notably altered the partition of bacterial populations into the planktonic and biofilm phases: low salt concentrations favored the formation of the biofilm phase, whereas an almost exclusive planktonic growth was observed in high salt concentrations (300 mM), in the wild type and mutant strains. Taken together, these observations suggest that NaCl-mediated inhibition of cell-cell interactions, may partly explain the reduced biofilm formation ability under high saline concentrations. On the other hand, succinoglycan may not be relevant for in vitro cell-cell interactions and biofilm formation. Supported by *SECyT-UNRC, ANPCyT-PICT and CONICET.*

FIS20

Nitric oxide production by *Methylobacterium extorquens* AM1

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Methylobacterium extorquens AM1 is the best-studied methylotroph, i.e. an organism that derives energy and, in many cases, cell carbon from reduced molecules that have no C-C bond. This bacterium is often pink, due to the presence of carotenoids, and is referred to as pink-pigmented facultative methylotrophs (PPFMs). PPFMs are ubiquitous in the phyllosphere where they can use the methanol released by the plant. *M. extorquens* has the ability to promote the growth of various plant seedlings. In plants, nitric oxide (NO) is involved in several metabolic pathways, like root growth, senescence and stomatal closure. The objective of this work was to study the NO production by *M. extorquens* AM1, and its relation with β-carotene synthesis. *M. extorquens* AM1 wild type (pink) and its mutant in β-carotene synthesis (white) were grown in AMS liquid media with methanol or methylamine as C-source and methylamine, NH_4^+ or NO_3- as N-source, at 28 °C with orbital agitation (250 rpm). NO production was measured in the middle and end of log-phase growth by Electronic Paramagnetic Resonance (EPR) and fluorometrically with the NO-specific fluorescent probe 4,5-diaminofluorescein diacetate (DAF-2 DA) using a fluorescence microplate reader. Bacterial growth was characterized in the different media by spectrophotometry and viable plate counts. NO-production was detected in media with methanol and not in media with methylamine. In methanol-NH_4^+, NO was produced at the same level (average 0.9 nM $NO.g^{-1}$ bacteria) for both strains only in the middle of log-phase growth. In contrast, NO was produced by both strains in the middle and end of log-phase growth, even at lower values in the end than the middle, in methanol-NO_3- media. NO levels were 10-fold higher with NO_3- as N-source. Overall, these results show evidence that *M. extorquens* AM1 is able to produce NO through different pathways depending on the N-source and irrespective of the bacterial β-carotene synthesis. *This work was supported by ANPCyT and UNMdP. MET is an undergraduate student of UNMdP. CMF is a doctoral fellow of ANPCyT.*

FIS21

Gloeobacter violaceus Sucrose Synthase: New Insights on the Origin of Sucrose Metabolism

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Sucrose is one of the most common nonreducing disaccharides found in nature. To date its biosynthesis was reported only in oxygenic photosynthetic organisms. On the other hand, sucrose cleavage through the action of sucrose synthase (SuS, A/UDP-Glucose: D-fructose 2- α-D-glucosyl transferase, EC 2.4.1.13) yielding a sugar nucleotide and fructose, was described in plants and in filamentous nitrogen-fixing cyanobacterial strains like Anabaena (Nostoc) sp. PCC 7119 and 7120, Anabaena variabilis, Nostoc punctiforme, and Nostoc commune. In Anabaena strains Sus was suggested to control sucrose cell level through the cleavage of the disaccharide and to be involved in the control of carbon flux in the N2-fixing filament. Recently, when new genome sequences became available, a homolog to Anabaena sp. PCC 7120 SuS gene (susA) was uncovered for Gloeobacter violaceus PCC 7421, a unicellular early branching strain of the cyanobacterial lineage. The deduced protein sequence is 71% identical to that of SusA. The functional identification of the orf was carried out by heterologous expression in Escherichia coli. The recombinant His-tagged fusion protein exhibited SuS activity with higher substrate affinity towards ADP than to UDP and shared immunological properties with PCC 7119 SusA. Sequence alignments and modular analysis of G. violaceus SuS showed that its predicted protein structure was similar to those present in filamentous strains. These results suggest that both the phylogenetic origin and the role of SuS in the control of the carbon flux in N2-fixing filaments might be revised to account for these new findings. Supported by PICT Nº 38144, PIP 6105, UNMdP and FIBA.

FIS22 - ORAL

Detection of an autolysin produced by a *Pseudomonas* strain under anaerobic conditions

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Autolysins like antimicrobial agents are substances that have biotechnological and clinical interest. Autolytic enzymes cleave specific components of the bacterial cell wall, peptidoglycan (PG), and perform many important physiological functions during cell growth and development such as cell division, sporulation and competence. However, deregulated activity of these enzymes can also lead to autolysis under stressful circumstances such as those encountered during stationary phase. *Pseudomonas* sp. 14-3 is an Antarctic non pathogenic strain that is able to accumulate polyhydroxybutyrate. During anaerobic growth was observed an autolysis phenomenon at early exponential phase. The supernatant of a lysed culture was used for several assays. Zymogram was performed with purified *Pseudomonas* sp. 14-3 PG as substrate in renaturing polyacrylamide gels and autolytic activity was visualized as a clear zone in a background of PG stained with a Methylene Blue/KOH solution with an apparent molecular mass corresponding to 28 kDa. Lytic activity of the supernatant was tested in vivo in *Pseudomonas* sp. 14-3 and *Pseudomonas aeruginosa* PAO1. *P. aeruginosa* is an opportunistic pathogen that is the major cause of morbidity and mortality in patients with cystic fibrosis. Exponential and stationary cells of both strains were exposed to the supernatant of a lysed culture and an inhibition growth zone was observed in aerobic and anaerobic conditions. A turbidimetric assay was used to monitor the time course of PG solubilization in both strains. Decrease in turbidity of a suspension of PG after exposition to autolysin was observed. Biofilm formation and persistence in an anaerobic environment is now considered crucial in chronic infection with *P. aeruginosa*. Microplate assay staining with crystal violet was used to analyze the effect of crude lysate on biofilm formation. We found that *P. aeruginosa* and *Pseudomonas* sp. 14-3 biofilms were dispersed. Identification of the possible autolysin induced during anaerobic growth of *Pseudomonas* sp. 14-3 by MALDI-TOF and PCR techniques followed by sequenciation is now in progress. Further studies of autolytic enzymes in combination with antibiotics therapy could be interesting in the treatment of *Pseudomonas* infections.

FIS23

Adhesion of dairy propionibacteria to intestinal mucus in the absence-presence of dietary cytotoxic lectins

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Food and the metabolites generated during digestion and gastrointestinal transit play a major role in the consumer´s health. Many antinutritional and/or potentially toxic compounds are daily consumed by humans and animals. Among them, plant lectins are specific carbohydrate-binding proteins that are widespread in the diet, being present in many food items such as vegetables, fruits, cereals, beans and seeds. They are highly resistant to inactivation by cooking and by digestive processes and therefore it is likely that the colonic epithelium is exposed to many lectins that have retained their biological activity. In the intestinal lumen, lectins could act as antiproliferative agents or as tumour promoters by stimulating cell proliferation. They can cause deleterious morphological and physiological changes in the intestinal mucosa such as inhibition of digestive enzymes, shedding of brush border membranes and shortening of microvilli that conduce to reduction of the absorptive function. In previous studies we have determined that dairy propionibacteria have the ability to bind and remove some dietary lectins, preventing their cytotoxic effects on intestinal epithelial cells (IEC). However, adhesion to intestinal cells and mucus, a desirable property of probiotic bacteria, could be affected by their interaction with lectins. In fact, it has been observed that adhesion of propionibacteria to IEC was reduced but not abolished after binding lectins. In the present study, we determined the effect of jacalin (AIL), concanavalin A (ConA) and peanut lectin (PNA) on adhesion capability of dairy propionibacteria to intestinal mucus isolated from mucosa walls and luminal contents. Sterile multi-wells plates were coated with both fractions of mucus (0,5 mg/mL) by an overnight incubation at 4°C and then propionibacteria (10^8 UFC/mL) previously labelled with FITC were added and incubated under microaerophilic conditions for 60 minutes at 37 °C. Lectins (100 μg/mL) and their complimentary sugars (0,5%), were added to mucus before or after bacteria. Adhered propionibacteria were quantified with an spectrofluorometer by measuring the fluorescence released after bacterial lysis with lysozyme. A decrease of bacterial adhesion to the mucus isolated from intestinal walls was observed when ConA, AIL and PNA got in contact with mucus before microorganisms whereas adhesion was not affected in the opposite condition. On the contrary, adhesion of propionibacteria to the luminal mucus was not affected by the presence of lectins suggesting a different mucus composition probably due to processing by microflora that use it as a source of nutrients in the intestinal lumen. Preincubation of propionibacteria with lectins also decreased their adhesion to mucus to different extents depending on the lectin assayed. Results suggest that consumption of foods containing these propionibacteria would be a tool to avoid lectins-mucosa interaction and their undesirable effects.

FIS24

Cytoplasmic chaperones involved in bacterial survival from extreme thermal challenge

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A number of treatments based in elevation of temperature constitute commonly employed means for the control of food- and water-borne poisoning or spoilage bacteria. Some of these technologies, such as Minimal Processing, involve the use of mild heat treatments at temperatures close above the enterobacterial adaptation zone, i.e., 50 to 60º C. Bacteria, however, may survive in substantial numbers these thermal treatments, challenging predictions on their bactericidal effectiveness. Different cytoplasmic chaperone systems participate in the adaptation and growth of bacteria under sub-lethal temperatures, but their roles in the preservation of cell viability under extreme conditions are not completely understood. Therefore, a detailed study of the bacterial mechanisms promoting increased resistance to heat inactivation may provide useful data for the design of safer treatments to control food-borne microbial risk simultaneously minimizing product over-processing. We investigated the roles and cooperation of the *Escherichia coli* cytoplasmic chaperones in cell survival from extreme heat stress by genetic procedures. Mutants lacking the σ^{32} stress factor (Δ*rpoH*), which are unable to fulfill the normal heat stress response, exhibited an increased rate of bacterial inactivation when exposed to 50º C. This increased heat sensitivity was comparable to that obtained for mutants specifically deficient in DnaK (Δ*dnaH*), one of the main cytoplasmic σ^{32}-dependent chaperones, thus pointing to this system as the main responsible for extreme heat resistance. Remarkably, mutants lacking DnaJ (Δ*dnaJ*), the physiological DnaK co-chaperone, were more resistant than Δ*dnaK* mutants to the heat challenge. However, Δ*dnaJ*Δ*cbpA* double mutants showed similar sensitivity as ¢dnaK cells, indicating synergistic actions between DnaJ and CbpA in cell survival from extreme heat stress. The overall data suggests that an “expanded” and fully functional DnaK system is pivotal for the preservation of cell viability from extreme thermal challenge.

FIS25

GENETIC AND BIOCHEMICAL CHARACTERIZATION OF AROMA COMPOUNDS BIOSYNTHETIC PATHWAY IN NON-STARTER LACTIC ACID BACTERIA

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The utilization of starter cultures in the production of fermented foods allows obtaining reproducible quality. However, the use of non-sterilized raw materials let to the growth of a microflora that could result beneficial or detrimental for human consumption. A desirable characteristic of these microorganisms is its capability of catabolizing citrate, which leads to the production of aroma compounds. By contrast, the production of metabolism by-products such as biogenic amines that could affect human health should be avoided.
In this work, we analyzed the bacterium *Enterococcus faecalis* which is present in cheese as a non-starter microorganism. Our aim was to study the contribution of *E. faecalis* to the organoleptic properties of diary products. In the first place, we isolated and identified this bacterium from traditional regional fermented foods. We evaluated then, the presence of genes involved in citrate fermentation (transporter, citH; citrate lyase, citDEF; oxaloacetate decarboxylases, citM and oad; as well as the transcriptional activator, citO) and aroma neutral compounds synthesis (alsSD). We also detected the presence of these genes in laboratory strains originally isolated from clinical sources. Interestingly, we determined that genes involved in citrate catabolism and aroma production are present in strains from both origins. Finally, we studied the resistance to acidic pHs of culture medium of a wild type strain compared to an alsSD deficient strain, demonstrating that the aroma synthesis route constitutes a mechanism of resistance to acidic stress due to production of neutral compounds.

4. Microbiología Molecular /
Molecular Microbiology

MOL1

AN ATYPICAL *csrA/rsmA* HOMOLOG ENCODED IN A RHIZOBIAL CRYPTIC PLASMID IS FUNCTIONAL IN *Pseudomonas fluorescens.*

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In prokaryotes, small regulatory RNAs (sRNAs) control mRNA translation mainly by either of two mechanisms: antisense binding or antagonism of protein repressors. In the latter, also known as molecular mimicry, sRNAs offer several binding sites to the regulator protein thus competing with mRNA binding. The prototype proteins of this kind of translational regulation are the global regulators CsrA (carbon storage regulator) from E. coli and RsmA (regulator of secondary metabolism) from *P. fluorescens* and *P. aeruginosa*. Although CsrA/RsmA homologs are encoded in chromosomes of a wide variety of Gram-negative and Gram-positive genera, certain phylogenetic groups apparently lack this type of regulator proteins. This is the case for alpha-proteobacteria. However, it has been recently reported the presence of an *rsmA* homolog in the replication region of a *Sinorhizobium meliloti* cryptic plasmid (Watson & Heys, 2006, Plasmid 55:87-98). As the genome of the alpha-proteobacterium *S. meliloti* does not encode obvious *csrA/rsmA* homologues, this may represent an event of recent horizontal gene transfer. Thus, we were interested to study if this *rsmA* homolog (*rsmASm*) is expressed and functional as a translational regulator.
The predicted *rsmASm* sequence is 58% identical to that of *Xanthomonas axonopodis* pv. *citri*, but it has an unusual C-terminal extension that is predicted to fold into an extra alpha-helix. Sequence alignment, secondary structure prediction and homology-based modelling of R*smASm* tertiary structure suggest that the key residues for mRNA binding are conserved and correctly positioned in the RNA pocket. However, plasmid pBB84, containing a 4.5 kb fragment of the cryptic plasmid with the replication functions and rsmASm, failed to complement *P. fluorescens* rsmA mutants. As rsmASm is located just upstream and divergently from the repA gene, we thought it could be subject to a negative control mechanism of plasmid copy number. When the *rsmASm* locus was subcloned into the pME6000 vector, *P. fluorescens* mutants lacking rsmA and the antagonistic sRNAs were fully complemented. This was confirmed by detection of rsmA-dependent phenotypes (exoprotease activity, HCN production and antibiotic production) and by gain of control of target translational fusions (aprA'- and hcnA'-'lacZ). Thus, rsmASm is able to bind and prevent translation of *P. fluorescens* biocontrol mRNAs. At least for hcnA'-'lacZ and exoprotease targets, the rsmA antagonistic sRNAs RsmX/Y/Z were able to neutralize *rsmASm* repression, suggesting that *rsmASm* also recognizes *P. fluorescens* molecular mimic sRNAs. Functional complementation was correlated with Western blot detection of the RsmASm.

MOL2 - ORAL

Structural and functional characterization of DesK, the membrane fluidity sensor of *Bacillus subtilis*

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Bacillus subtilis, a gram-positive bacterium, frequently encounters stress conditions in its natural environment, the soil. In order to detect and respond to these stressful and variable conditions, it employs two-component signal transduction systems. The *B. subtilis* Des pathway is composed of the membrane Δ5-acyl lipid desaturase, Δ5-Des, and the two component system DesK/DesR. DesK is a histidine kinase located in the membrane and DesR is a cytoplasmatic response regulator that binds specifically to the Pdes promoter. Induction of the Des pathway is brought about by the ability of DesK to assume different signaling states in response to changes in membrane fluidity. This could be accomplished by regulating the ratio of kinase to phosphatase activities. An increase in the proportion of ordered membrane lipids favors a kinase-dominant state of DesK, which undergoes autophosphorylation followed by the transfer of the phosphate group to the cytoplasmic response regulator DesR. Phosphorylated DesR binds to Pdes and interacts specifically with the RNA polymerase to turn on des transcription. Activation of des results in the synthesis of Δ5-Des, which introduces double bonds in the acyl chains of membrane lipids. These newly synthesized unsaturated fatty acids decrease the phase transition temperature of the phospholipids, favoring the phosphatase activity of DesK on DesR-P and turning off transcription. We undertook structural studies in order to characterize the signaling states corresponding to the autokinase, phosphotransfer and phosphatase activities associated with the cytoplasmic region of DesK and to gain further insights into the mechanism by which this sensor protein can adjust its signaling state in response to changes in membrane lipid fluidity. Here, we describe the crystal structure of the complete soluble domain of DesK in two conformational states. Structure-inspired hypotheses for the distinct catalytic mechanisms and for signal transduction through the membrane to the cytoplasmic domain will be discussed.

MOL3

Design and activity of novel antimycobacterial compounds

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With a death toll of more than two million deaths per year worldwide, *Mycobacterium tuberculosis*, the causative agent of human tuberculosis, is the deadliest bacterial pathogen. In spite of the efforts to eradicate this illness, the number of cases is rising. Two of the factors that are responsible for this increase are the few drugs available for an efficient treatment and the appearance and dissemination of multi-drug resistant strains. There is an on-going effort to develop new drugs by either identifying new targets or improving the efficiency of drugs currently in use. Two examples of the latter are ethambutol, an anti-tubercular drug of clinical use, and econazole, an anti-fungal compound with recently described anti-tubercular activity. In both cases, the targets have not been conclusively demonstrated although both are being used as scaffolds for the design of novel compounds with improved and more specific activity. Here we report the synthesis and anti-tubercular activity of two series of compounds, one of them -1, 2, 3 triazoles- made by "click chemistry", and the second one by N-modification of alkyl diamines.

Thirty-eight triazole compounds were tested against different strains of opportunistic (*M. avium*) and pathogenic (*M. tuberculosis*) mycobacteria. Two of the synthesized molecules (A4Z3 and A7Z1) showed activities at lower μg/mL concentration for *M. avium* with one of them (A7Z1) displaying high activity on *M. tuberculosis*; being more active that known anti-fungal azoles (econazole and clotrimazole) used as control drugs.

Thirty-four diamines were also assayed for their anti-mycobacterial activity on *M. avium* and *M. tuberculosis*, with very promising results since six of these molecules exhibited strong activity (Minimum Inhibitory Concentration, MIC, ranging from 0.8 μg/mL to 6.25 μg/mL) against both species. Moreover, two other molecules exhibited good activity specifically against *M. avium* (MIC≤6.25 μg/mL).

While 1, 2, 3 triazoles are also active against a panel of multi-drug resistant (MDR) *M. tuberculosis* strains, diamines are in the process of being evaluated. None of the molecules of both groups showed any citotoxicity when assayed on Vero cells, suggesting that a therapeutic use is possible.

In summary, the molecules reported here are worth considering as scaffold for further improvement in their activity as well as in animal trials.

MOL4

Characterization of a modified lipoprotein of sporulation in *Bacillus subtilis*

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In response to nutrient starvation and high cell density, a developmental program called sporulation is initiated in *Bacillus subtilis.* This process has become a paradigm of prokaryotic cell differentiation. An early event in this process is the formation of an asymmetric septum that divides the cell into two compartments, a smaller one, called prespore and a larger mother cell. The mother cell will assist in the prespore developmental process and will eventually lyse, releasing the dormant environmentally resistant spore. The differential behavior of each cell is generated by the activation of specific RNA-polymerase sigma factors in each compartment. The spores retain a specific sensory mechanism enabling them to germinate. The germination process is characterised by a series of degradative events leading to the loss of typical spore properties and outgrowth into a new vegetative cell. To identify hypothetical lipoproteins involved in sporulation, we labelled *B. subtilis* cultures with tritiated palmitic acid and found a putative lipoprotein expressed under the control of the mother cell specific sigma factor σ^E. We demonstrated that the protein detected is GerM, previously reported to be necessary for both spore formation and germination. A version of GerM mutated in its putative lipidation site (the Cys13 changed to Ala) called GerM1 was not able to incorporate tritiated palmitic acid. A mutant strain expressing *germ1* evidenced impaired sporulation efficiency. Measurements of sporulation parameters showed low levels of glucose dehydrogenase activity and dipicolinic acid content in sporulating *gerM1* cells. A sporulation blockage was confirmed analyzing the expression profile of genes controlled by different sigma factors sequentially activated during the developmental process. Addition of germinants to the mutant spores resulted in the triggering of an incomplete germination with a normal loss of heat resistance (an early event) but significantly deficient in loss of spore refractility (a later event). These phenotypes are similar to those of a *gerM* null strain, suggesting that the lipobox site of this lipoprotein is critical for its proper function.

MOL5

Hypermutability and adaptive phenotypes in *Pseudomonas aeruginosa* isolates from individuals with cystic fibrosis

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Pseudomonas aeruginosa, is an opportunistic pathogen causing many types of difficult-to-cure infections in immunological compromised patients. *P. aeruginosa* is the most relevant pathogen producing pulmonary chronic infections in patients with Cystic Fibrosis (CF). During CF chronic infection *P. aeruginosa* diversifies into phenotypes with particular traits that are not displayed in isolates obtained from other infections. Among them, there are mucoids, avirulent variants, hypermutators and isolates with altered antibiotic susceptibility. Importantly, this phenotypic diversification favors *P. aeruginosa* long-term persistence making it impossible to eradicate by any known therapy. Since the acquisition of these phenotypes mostly involves loss-of-function mutations in targets genes, it has been suggested that this phenotypic diversification is catalyzed by hypermutator strains, which are mainly deficient in the DNA mismatch repair system (MMR). Indeed, the link between antimicrobial resistance and hypermutators was strongly suggested. Moreover, we have previously established a linkage between hypermutability and mucoid conversion as well as emergence of avirulent variants via quorum sensing inactivation, working with a *P. aeruginosa* MMR-deficient strain *in vitro*.
In order to gain further insights in the role of the hypermutability in *P. aeruginosa* phenotypic diversification in vivo, we analyzed the association between hypermutator phenotype and mucoid (*mucA* mutants), avirulent (*lasR* mutants) and multi drug resistant (*mexZ* mutants) variants in a collection of *P. aeruginosa* isolates obtained from CF patients. We examined 40 isolates from 27 patients chronically infected with *P. aeruginosa* who are assisted in local Hospitals. According to SpeI macrorestriction fragment profiles we observed that most patients were infected by different *P. aeruginosa* clones as keeping with previous reports. We observed that 20 isolates (50%) were hypermutators and 14 patients (55.5%) had hypermutators. Afterward, loss-of-function mutations in the coding region of *mucA*, *lasR* and *mexZ* were scored by PCR amplification and direct nucleotide sequence analysis. Only one isolate (2,5%) showed unaltered sequences in the three genes, displaying at the same time a non mutator phenotype. Also, 75% of the isolates showed mutations in *mexZ* of which 40% were also hypermutators. In addition, we observed that 65% of isolates showed mutations in *mucA*, of which 46% showed a hypermutator phenotype. Interestingly, a lower percentage of isolates (35%) harboured mutations in *lasR*, however, 80% of them were found to be hypermutators. We are currently investigating the molecular bases of the hypermutability in the 20 hypermutator isolates by complementation assays and sequencing of the main MMR components.

MOL6

Characterization of two *Bacillus subtilis* strains spontaneously resistant to cerulenin.

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Fatty acid biosynthesis is catalyzed in bacteria by a series of soluble proteins which together constitute the type II fatty acid synthase (FAS II). This essential pathway is the target of several antibiotics. The first step in the fatty acid elongation cycle is the condensation of malonyl-ACP with acyl-ACP to generate 3-keto-acyl-ACP two carbons longer. This step is catalyzed by the 3-keto-acyl-ACP synthases. In *B. subtilis* there is only one condensing enzyme, termed *FabF*, able to elongate medium and long-chain acyl-ACPs. This enzyme is found as a heterodimer, and is specifically and irreversibly inhibited by the mycotoxin cerulenin. Expression of fabF, together with most FAS II genes, is regulated by the global transcriptional repressor FapR. Activity of FapR is, in turn, modulated by the intracellular levels of malonyl-CoA.
We have previously described a *B. subtilis* strain containing the *fabF1* allele, which codes for the cerulenin-insensitive protein FabF[I108F]. This strain had a cerulenin MIC fourfold higher than the wild type strain. Although FabF[I108F] is functional both *in vivo* and *in vitro,* its ability to elongate long chain acyl-ACPs is impaired and the strain overexpresses the FAS II to compensate this deficiency. Expression of *fabF1* in a *fabF* background rendered the cells resistant to cerulenin, and suggests that the mixed dimmers are functional.
Here we report the characterization of another two spontaneous mutants of *B. subtilis* that exhibit a fourfold increase in the cerulenin MIC. Sequence analysis of the *fabF* gene of each strain revealed a single base change that resulted in the substitutions Leu111Phe and Ile108Met, respectively. We renamed these alleles *fabF2* and *fabF3,* respectively. The aminoacids 108 and 111 are constituents of the hydrophobic pocket that accommodates the acyl-ACP. Similar to what was proposed for the *fabF1* allele, we suggest that changes for bulkier aminoacids are linked to a smaller active site cavity that prevents accommodation of the antibiotic. Opposite to what was observed in *fabF1* cells, genes coding for the FAS II were not overexpressed in *fabF2* and *fabF3* strains, as evidenced by reporter gene analysis. A second striking difference between *fabF1* and the other two mutant alleles is that overexpression of *fabF* together with either *fabF2* or *fabF3* is lethal. Reporter gene analysis indicated that in these strains, FAS II genes are downregulated, a condition associated with a diminution of malonil-CoA contents. We hypothesize that the mixed dimmers between FabF and either FabF[L111F] or FabF[I108M] have a greatly increased malonyl-ACP decarboxylase activity.
These results provide major insights into the key elements that contribute to the activity of this important class of enzymes and the mechanisms associated with the resistance to cerulenin.

MOL7 - ORAL

The unique features of a Twin-arginine translocation component are essential for survival in haloarchaea.

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The twin arginine translocation pathway (Tat) transports proteins into and across cell membranes of prokaryotes and chloroplasts in a folded conformation. Among all organisms, haloarchaea are the only known organisms that utilize this pathway to translocate the majority of their secretory proteins, which makes it essential for survival.

The haloarchaeon *Haloferax volcanii* has a Tat machinery composed of two TatA (TatAo and TatAt) and two TatC (TatCo and TatCt) homologs. Haloarchaeal TatC proteins show unique modifications. TatCt presents 14 transmembrane segments (TMS) instead of the typical 6 TMS of all other known TatC proteins, probably due to gene duplication. In order to understand the significance of these modifications we analyzed the effect of different truncations in the TatCt protein of *H. volcanii*. We observed that over expression of the N-terminal 6 TMS was sufficient to support normal growth, but this truncation could not complement for a tatCt knock out when expressed at chromosomal levels. Interestingly, the C-terminal region (6 or 8 TMS) was not capable to sustain cell growth, even when over expressed. Sequence alignments showed that the C-terminal portion of TatCt lacks three residues (L16, D211 and Q215 in *E. coli*) that are conserved among all prokaryotes and that seem to be important for Tat protein translocation. In addition, a region comprising the third periplasmic loop and part of the 5th TMS shows 100% conservation in the N-terminal region and is not present in the C-terminal half of TatCt. These sequence variation might be responsible for TatCt C-terminal inability to complement for a *tatCt* deletion. Our observations suggest that the N-terminal region performs an essential function of TatCt that the C-terminal region lacks. Future research will focus in determining the molecular basis of these differences. This analysis could reveal traits that make this transport pathway effective in secreting large number of substrates, which may represent a useful Biotechnology tool.

MOL8

Tetracycline resistance mediated by *tet*(M) gene in lactobacilli isolated from kefir grains

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The importance of probiotics in food industry is growing nowadays, since probiotics have shown to be benefical to health. Kefir is a probiotic fermented milk beverage achived by the use of kefir grains, which are composed of yeast and bacteria, including species of lactobacilli.

Lactobacillus species have been traditionally used to produce a wide variety of fermented foods and have a long history of safe use, reason for which are generally recognized as safe (GRAS status). Therefore the characterization of these bacteria, particularly in regard to antimicrobial resistance, is often neglected. Since the strong expansion of the probiotic market and the increasing drug-resistance reported in probiotic bacteria, they represent a potential source for the spread of antibiotic resistance genes along the food chain to the consumer raising major concerns with regard to food safety. The EFSA (European Food Safety Authority, 2005) suggest the QPS-status (Qualified Presumption of Safety) which provide a qualified generic approval system that would harmonise the safety assessment of microorganisms throughout the food chain. For being considered for QPS-status, the only qualification that might be attached by common lactobacilli used in dairy applications is the evidence of the absence of acquired antibiotic resistance.

In the present work, strains belonging to the genera *Lactobacillus* isolated from two different kefir grains were analysed for the presence of resistance to tetracycline, which has been used in animal husbandry as growth promoter. The minimal inhibitory concentration (MIC) of tetracycline has been valued by the broth microdilution method. We found that all the studied isolates from one of the kefir grains were resistant to the antibiotic (MIC 64 to > 256 μg/ml), whereas the isolates from the other grain were all susceptible (MIC < 1 μg/ml). Then, we aimed to unravel the genetic basis on those strains that displayed atypical phenotypic resistance to tetracycline. We analysed the presence of genes encoding tetracycline resistance by PCR assays, and by cloning and sequencing, we confirmed that all the resistant strains carried the *tet*(M) gene encoding a ribosomal protection protein. It remains unanswered the potential of these tetracycline resistant isolates to transfer the *tet*(M) gene to other bacteria. These findings reassert the fact that antibiotic resistance genes are present in food-associated bacteria.

MOL9

Elizabthekingia meningoseptica's resistance to carbapenems, a case of redundancy?

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Elizabethkingia meningoseptica is a gram-negative rod widely distributed in nature. It is known to cause meningitis in premature and newborn infants. In adults, cases of pneumonia, endocarditis, postoperative bacteremia, and meningitis have been reported, usually associated with a severe underlying illness. The organism is usually multiresistant to antibiotics typically prescribed for treating gram-negative bacterial infections, including extended-spectrum β-lactam agents and aminoglycosides, and thus constitutes a clinical concern.
E. meningoseptica is resistant to all β-lactams antibiotics, apparently as a consequence of the expression of three cromosomically encoded β-lactamases: a class D serine-β-lactamase CME, and two metallo-β-lactamases (mβ1s), BlaB of subclass B1 and GOB of subclass B3. All mβl-producing bacteria so far investigated produce a single mβ1 accompanying a serine-β-lactamase. Given that the substrate profile exhibited by BlaB and GOB is similar, we decided to investigate the involvement of each of these mβ1s in carbapenem resistance in *E. meningoseptica.*
Phenotypic assays suggested that resistance to carbapenems is exclusively associated with mβ1 production, CME being responsible for the hydrolysis of penicillins and cephalosporins. RNA expression of *bla*CME, *bla*BlaB and *bla*GOB genes was tested by qRT-PCR, revealing similar levels of transcripts for the three genes. Exposure of *E. meningoseptica* to imipenem and cefotaxime resulted in no induction of any of the *bla* genes. In stationary phase, the levels of BlaB RNA manifested an abrupt increase with respect to CME and GOB. Quantitative immunoprecipitation of GOB from an *E. meningoseptica* crude extract showed no diminution in imipenemase activity. These results suggest that GOB is not implicated in in vivo resistance to imipenem. Therefore, BlaB would be the mβl responsible of clinical resistance to carbapenems, probably implicated in a stationary-phase defense mechanism. As for other subclass B3 enzymes, the role of GOB in the cell remains to be elucidated.

MOL10

PROTEINS INVOLVED IN NADP+/H METABOLISM AFFECT THE INDUCTION STATE OF THE *soxRS* REGULON IN *Escherichia coli*

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Bacteria have evolved sophisticated molecular mechanisms to monitor oxidant levels and to activate antioxidant defence genes in response to specific signals. Two transcription regulators serve in *Escherichia coli* as redox sensing proteins under oxidative stress: SoxR and OxyR (both produced constitutively and activated upon exposure to superoxide- and hydrogen peroxide-generating agents, respectively). These transcription factors become active when they are oxidized: the [2Fe-2S] clusters in SoxR and two cysteine residues in OxyR. During aerobic growth the S-Fe centres of SoxR dimer are maintained in the inactive reduced form by a reducing activity dependent on NADPH and dedicated protein/s, although the entire mechanism remains unclear.
When oxidized SoxR, activates the transcription of the *soxS* gene. The SoxS protein induces transcription of the *soxRS* regulon, whose products act collectively to avoid and repair oxidative damage. Superoxide and NADPH/NADP+ appear to be specific signals. We studied the response of the *soxRS* regulon under overexpression of proteins involved in reactions with NADPH in the presence or in the absense of superoxide. The overexpression of the own malic enzyme and plant ferredoxin in *E. coli* changed the activation state of SoxR. These proteins participate in reactions that involve $NADPH/NADP^{+}$ either as substrate or partner.
High levels of pea ferredoxin in *E. coli* led to a rise of SoxS in the absense of stress, whereas the increase of malic enzyme resulted in down-regulation of SoxS after oxidative stress. These changes were observed by measuring β-galactosidase activity from a transcriptional *soxS* fusion and by western blot. Overexpression of proteins that changes the NADPH pool affects SoxS expression, either in the ground state or in the presence of compounds that generate superoxide, indicating that the levels of NADPH act as a sole signal to trigger the *soxRS* response. These observations are consistent also with oxidation of the SoxR [2Fe-2S] clusters probably due to interferences with the NADPH pathways of responsible protein(s) that maintain SoxR in the reduced form.

MOL11 - ORAL

Specific amino acid residues in GolS metal-binding loop determines the metal selectivity

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Metal-dependent transcriptional regulatory systems are the major cellular mechanism that bacteria employ to control metal homeostasis and/or resistance to harmful non-essential transition elements. *Salmonella* has two transcriptional regulators of the MerR family that respond to monovalent metal ions: CueR, which is involved in copper homeostasis, and GolS, which is responsible for gold detoxification. In contrast to CueR, that has similar affinity for both cations, GolS is 52 times more sensitive to Au than to Cu. A detailed analysis of the C-terminal metal-binding loop of these proteins (between two conserved Cys: C_{112} and C_{120}) shows some differences that could account for cation-specificity. Indeed, while CueR harbor a CPGDDSADC sequence, a CAGDALPDC sequence is present in GolS, highlighting the switch between P_{113} and P_{118}, and the presence of a charged D residue in position 116 in CueR, that in GolS is an A.
To investigate the role of this loop in metal discrimination we first generated chimeric GolS and CueR sensors in which their native metal-binding loop were replaced by the one present in CueR or GolS, respectively. We observed that the GolS chimeric protein (with the metal-binding loop of CueR) was able to activate the expression of the GolS-controlled gene *golB,* by the addition of Au or Cu ions in a similar manner than the induction of a typical CueR-regulated gene. Conversely, expression of the CueR-controlled gene *copA* was induced by the CueR chimeric protein (with the metal-binding loop of GolS) only by the addition of Au ions. In addition, we constructed by site-directed mutagenesis GolS mutant proteins in which the amino acids within the metal binding loop of the sensor that distinguish the GolS grove was replaced with those present in CueR-like regulators. The response of each mutant regulator to Au, Cu or Ag ions was analyzed by monitoring golB expression. While induction of *golB* was only achieved by Au in the wild type strain and in most GolS mutants, it was induced to a similar extent either by the addition of Au, Cu or Ag in a $A_{113}P$ mutant strain.
Our results indicate that in GolS Au-selectivity is achieved by the amino acid residues encompassing the C_{112}-C_{120} loop, and highlight the relevance of position 113 for the discrimination between the monovalent metal-ions from group IB of the periodic table.

MOL12

The involvement of *Pseudomonas aeruginosa* CrbB and NtrC response regulators in the regulation of choline utilization and phosphorycholine phosphatase expression

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P. aeruginosa is an opportunistic pathogen that primarily affects cystic fibrosis and immunocompromised patients, is ubiquitous in nature and has the capacity to survive in different nutrient-limited environments. When utilizing choline, as carbon or nitrogen or as carbon/nitrogen source, the bacteria synthesize among other proteins, a phosphorycholine phosphatase (*PchP*) which is repressed by the presence of succinate/NH_4 in the culture medium. Recently, the gene coding PchP (pchP) has been identified and its transcriptional regulation has been studied. The *pchP* promoter (P1) has also been identified and all the studies indicated that it is a σ^{54}-dependent promoter. In *P. aeruginosa,* the two-component regulatory systems, CbrAB and NtrBC, play an important role in the regulation of bacterial utilization of substrates that provide both carbon and nitrogen, in a σ^{54}–dependent manner.
The aim of this work was to study the physiological role of CbrB and NtrC in the choline utilization and in the choline-mediated induction of *pchP.* To investigate this, wild type PAO1 (WT), Δ*cbrB* and Δ*ntrC* strains were used. To study the *pchP* regulation, a transcriptional fusion (P1::*lacZ)* was constructed and introduced in the chromosome of each strain.
CbrB was shown to be essential for the choline utilization, since the Δ*cbrB* strain was unable to use it as carbon/nitrogen source. However, the Δ*cbrB* strain utilized choline as a sole nitrogen source only if a succinate was present. This result suggested that NtrC was possibly involved in the regulation of choline catabolism. Therefore, the ability of the Δ*ntrC* strain to grow on choline was examined. The growth was comparable to WT in both, HPi-BSM plus choline or plus choline/NH_4. No growth was observed in HPi-BSM plus choline/succinate. The results indicated an important role of NtrC and CbrB in the utilization of choline in response to nitrogen and carbon starvation, respectively. To explore the effect of CbrB and NtrC on *pchP* transcription, βGal activity was determined in the following strains: WT, Δ*cbrB* and Δ*ntrC* with the P1::*lacZ* fusion in the chromosome. These strains were grown in HPi-BSM with choline, choline/NH_4 or choline/succinate. The Δ*ntrC* P1::*lacZ* strain grown in media with choline or choline/NH_4 showed approximately 30-38% of the activity observed in the WT. With choline as nitrogen source, Δ*cbrB* P1::lacZ produced a βGal activity similar to the WT. Thus, it could be concluded that NtrC is a *pchP* promoter activator. In addition, our results allowed us to propose a model of a molecular mechanism that involves the global role for both CbrB and NtrC in the choline metabolism and, the role of NtrC in *pchP* regulation.

MOL13 - ORAL

PARALLELS AND DIFFERENCES IN COPPER RESISTANCE AMONG ENTEROBACTERIAL SPECIES

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Copper is an essential trace element employed in all domains of life, but its intracellular levels must be carefully handled to prevent the formation of reactive oxygen species through a fenton-like reaction. In Gram-negative bacteria copper resistance is primarily handle by the cue regulon. In *Escherichia coli* the *cue* regulon is composed by the copper sensor/regulator CueR that, upon detecting the presence of copper ions in the bacterial cytoplasm induces the expression of two target genes, *copA* and *cueO. copA* codes for an integral inner-membrane copper-transporting P-type ATPase, which ensures removal of excess Cu(I) from the cytoplasm. *cueO* codes for a periplasmic multi-copper oxidase responsible for the control of copper levels through an oxygen dependent cuprous oxidase activity in aerobic conditions. Under anaerobic conditions *E. coli* also relies on a second system to increase copper resistance, composed by the CusCFBA efflux pump, which is transcriptionally controlled by the two component system CusR/CusS. *Salmonella* harbours all the cue components but lacks the cus system. Despite of this, *Salmonella* is much more resistance to copper in anaerobic conditions than *E. coli*.
In this work we identified a novel, *Salmonella*-specific CueR-regulated gene, *cueP,* coding for a periplasmic protein that increase resistance to copper both in aerobic and anaerobic conditions. We also observed that CueP overexpression partially restored resistance to copper to a *cueO* deleted strain. We hypothesized that in *Salmonella* CueP could functionally substitute the *E. coli* cus system for copper resistance in anaerobic conditions. To test this, we replaced the entire *E. coli* chromosomal *cus* locus for the wild-type copy of the Salmonella *cueP,* including its own promoter. The CueR-dependent expression of *cueP* in this strain partially suppressed the lack of the cus locus for copper resistance in anaerobic conditions. Moreover, overexpression of *CueP* completely restored resistance to copper in a *cueR cus* double mutant strain in aerobic conditions, supporting its role as a copper-resistance factor in the bacterial periplasm.
Our results highlight important differences in copper handling between related bacteria, and suggest that, contrary to other enterobacterial species, *Salmonella* have evolved a single pathway to overcome copper excess both in aerobic and anaerobic conditions.

MOL14 - ORAL

DISSEMINATION OF THE Tn*402* TRANSPOSON FAMILY AMONG BACTERIAL GENOMES

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The Tn*402* transposon consists of four genes, *tniA, B, Q* and *R*. It has been first described in the IncP1 plasmid R751 from *Enterobacter aerogenes,* which carries a class 1 integron; however, only derivatives of the Tn*402* transposon were found among clinical isolates. Tn*402* belongs to the Tn*5053/402* family of transposases that recongnize a relatively specific target, the *res* site. Previously, it has been reported that Tn*402* was an ancient vehicle for the spread of resistance genes. In addition, the Tn*5053/402* transposon family is also responsible of the dissemination of the mercury resistance due to the association of the transposition module *tni* with the *mer* operon. Most Tn*5053/402* family members are located in plasmids that belong to the IncP-1beta incompatibility group helping in the horizontal transference of the transposon.
The aim of this work is to understand the role of the Tn*402* in the bacterial kingdom as well as to evaluate its presence in different niches.
We searched for the TniR sequence protein from the Tn*402* tranposon in complete (n = 736) and partial (n = 1181) bacterial genomes available in the GenBank. Only 30 genomes encoded TniR with a sequence identity higher to 80%; but only 13 genomes also encoded for the TniA, TniB and TniQ proteins indicating that the *tni* module was complete and its dissemination is limited to a 1.3% of the bacterial genome. In parallel, we searched for the presence of the *tniR* and *tniA* genes in 98 environmental isolates recovered from low antropic environmental niches by the PCR technique using specific primers. Nineteen out of 98 (19.4%) harboured an identical copy of the *tniR* gene but only 3 also had the *tniA* gene indicating that 3.1% of the isolates had the transposon *tni* module. Six of 19 (31.6%) bacterial genomes had the *mer* operon associated to the transposon; similarly there was a 26% prevalence of the operon in the environmental samples indicating a global spread of this genetic element. Phylogenetic analysis based on the TniR sequence showed that the Tn*5053/402* had two main lineages with no consistent pattern based on the procedence of the microorganism or the association to the antibiotic o mercury resistance. Our analysis also showed that while the TniR protein is widely encoded in different bacteria from very dissimilar niches, the *tni* module of the Tn*5053/402* family is not. Since the Tn*402* transposon has been always found disrupted at the *tni* module is it possible to assume that its survival in a bacterial genome is compromised.

MOL15

BORN TO BE POLYMORPHIC: THE OUTER MEMBRANE PROTEIN CARO FROM *Acinetobacter baumannii* EXISTS AS A FAMILY OF MULTIPLE ALLELES.

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Bacterial cells have evolved a range of mechanisms which allow them to adapt the functionality of their outer membrane proteins (OMP) in response to conditions prevalent in the environment. Sequence diversity in OMP-coding genes have been shown to result from the expression of alternative surface variants encoded in the genome; and/or the short-term evolution of mutable regions of surface components.
In this work, we focused on the study of the evolution of the gene coding for one of the major OMP of the emergent nosocomial pathogen *Acinetobacter baumannii,* which we previously designated CarO. CarO was shown to be a member of a new family of heat-modifiable outer membrane proteins restricted to the family *Moraxellaceae* among the °-Proteobacteria (Mussi et al., 2005). It was first identified as a protein associated with carbapenem resistance since its disappearance from the outer membrane due to the inactivation of its coding gene by IS resulted in the acquisition of carbapenem resistance(Mussi et al., 2005).
We analyzed here the genetic variation of *carO* within a wide collection of *Acinetobacter* clinical strains from three different important urban centers of distinct provinces of Argentine, such as Buenos Aires, Rosario and Entre Ríos, recovered from 1990 to 2006. Direct nucleotide sequence determination identified 4 clearly distinguishable *carO* gene groups (I-IV) encoding unique amino acid sequence variants among the whole *A. baumannii* population. Sequence comparisons between the *CarO* variants revealed that genetic variation in carO is not randomly distributed but strikingly concentrated in three regions designated variable region (VR) 1, 2 and 3, comprising 36% of the whole *carO* sequence. Also, phylogenetic analysis showed that there is complete *carO* - allele interchange between A. baumannii strains. Recent data concerning the physiological role of the *A. baumannii* variant II indicate that *CarO* is involved in the permeation of the basic amino acids L-ornithine and L-arginine (Mussi et al., 2007), compounds structurally related to carbapenems. Furthermore, the finding that strains harbouring variants I and III are unable to grow on L-ornithine as sole carbon source, and concomitantly present low MICs levels for carbapenems, are in agreement with a differential substrate specificity. We also found that the related species *Acinetobacter* DNA genomospecies 3 and the non - pathogenic bacterium *A. baylyi* possess a *carO* version with 72.7-88.4 and 63-68 % of identity, respectively, compared to the variants present in *A. baumannii.* Functional analysis revealed that an *A. baylyi carO* mutant exhibits reduced growth rate only in the basic amino acid L-arginine. Moreover, fitness experiments revealed the importance of the presence of *CarO* in minimal media, reinforcing the notion that *CarO* protein is related to the internalization.

MOL16

MOLECULAR CHARACTERIZATION OF *STREPTOCOCCUS UBERIS* STRAINS ISOLATED FROM BOVINE MASTITIS IN ARGENTINEAN CENTRAL DAIRY REGION

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Bovine mastitis, an inflammation of the mammary gland, is the single most important factor contributing to economic losses to the dairy industry. Several streptococcal species are capable of causing infections that result in mastitis, including *Streptococcus uberis , S. agalactiae* and *S. dysgalactiae.* Among these species, *Streptococcus uberis* is particularly worrisome due to the fact that this so-called 'environmental streptococcus' is ubiquitous in the dairy environment and is predominantly associated with sub-clinical mastitis cases, resulting in reduced, and poor quality milk yields. The factors contributing to the pathogenesis of *S. uberis* disease are not well understood, although several potential virulence determinants have been described. Some of the potential virulence determinants include hyaluronic acid capsule, plasminogen activating protein, PauA, neutrophil toxin, lactoferrin binding proteins, CAMP-factor and a phosphorylating glyceraldehyde-3-phosphate dehydrogenase. The development of vaccines against mastitis-causing pathogens has been slow, partly due to the prerequisite that infection must be controlled without induction of a significant inflammatory response, since this in itself contributes to the disease condition. The aim of this work was to study the occurrence of seven virulence determinants (pau, pauB, hasA, hasB, hasC, skc y cfu) among 61 isolates of *Streptococcus uberis* strains isolated from bovine mastitis in Argentinean central dairy region, by PCR. Forty strains (66%) yielded an amplicon size of 1200 bp for pauA gene, whereas no strain showed pauB gene. The skc gene could be observed for 37 (60.7%) *S. uberis*. Twenty one (34.4%) strains showed cfu gene. Forty one (67.2%) and thirty five (57.4%) strains showed hasA and hasB gene, respectively. Amplification of the hasC gene resulted in 53 (86.9%) strains with amplicons sizes of 300 bp. hasABC+ genotype was the most prevalent, present in 32 (52.5%) strains. pauA + hasA +, hasB + y hasC + skc+ cfu+ genotype was the prevalent, present in eight strains (13%). On the other hand one strain was negative for the seven virulence factors. The genotypic results of the present study might help to understand the distribution of potencial virulent determinants of *S. uberis* isolated from dairy central region of Argentina and might be the base for preventive strategies. Result of the present investigation would allow future work to better understand pathogenic mechanisms of *S. uberis* infection.

MOL17

Role of exopolysaccharides on biofilm formation in *Sinorhizobium meliloti.*

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Bacterial surface polysaccharides are crucial for the establishment of a successful symbiosis between rhizobia and legumes. They were shown to be also critical for biofilm formation in several bacterial species, which is defined as a bacterial community surrounded by a polysaccharide matrix usually attached to a surface. *S. meliloti* is able to produce two exopolysaccharides, succinoglycan and EPS II. Both polymers are produced as high- and low-molecular-weight (HMW and LMW, respectively) fractions; however, only the LMW forms of either succinoglycan or EPS II are active in nodule invasion. Succinoglycan is normally produced by *S. meliloti* Rm1021; however, EPS II synthesis only occurs under phosphate limitation, in the presence of an intact copy of *expR* or when *mucR* is mutated. *mucR* is a transcriptional regulator of exp genes (responsible for the production of EPS II) and an activator of the biosynthesis of succinoglicano. Therefore, *mucR* regulates the production of both exopolysaccharides in *S. meliloti.*
S. meliloti is able to sense nutritional and environmental conditions in the culture medium and respond abandoning the planctonic state and forming biofilms. Bacterial adhesion is modulated according to environmental changes through the regulation of exopolysaccharide biosynthesis. However, *mucR* expression (evaluated by using a lacZ transcriptional fusion) was not changed by addition of sucrose (0.3 M), NaCl (0.015 M), phosphate (25 mM) and calcium (7 mM) to the RDM medium, conditions in which biofilm formation is increased (Rinaudi et al., 2006). mucR expression is reduced 44% in biofilms compared with planctonic cells in exponential phase growing in *Rhizobium* Defined Medium (RDM). Additionally, we have shown that in a minimal medium low in phosphate (0.1 mM) the Sin/ExpR quorum sensing system controls biofilm formation through EPS II production. Moreover, synthesis of succinoglycan is not essential for biofilm formation but it would slightly affect its structure during the first days post-inoculation (Rinaudi et al., 2008 III ASM Conference in Cell-Cell Communication in Bacteria). The *mucR* mutant, which is able to produce succinoglycan and only HMW EPS II, showed the same low levels of biofilm formation compared with the Rm1021 strain. By CLSM we observed that the *mucR* mutant formed small channels in the culture chamber, but failed to develop microcolonies, a phenotype analogous to the one observed in the non-EPS II-producing parental strain Rm1021. Taken together, our results suggest that i) under various environmental conditions, MucR would not play an important role in biofilm formation on an abiotic surface by *S. meliloti* and ii) while the LMW-EPS II, would be necessary for attachment to abiotic surfaces, succinoglycan biosynthesis leads to unstable biofilms and it may play a role on biofilm dispersal but not in biofilm formation.

MOL18 - ORAL

Cytolocalization of the *Salmonella enterica* PhoP response regulator *in vitro* and *in vivo.*

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The PhoP/PhoQ two-component system controls the expression of essential virulence traits in the pathogenic bacteria *Salmonella enterica serovar Typhimurium.* Environmental deprivation of Mg^{2+} activates the PhoP/PhoQ signal transduction cascade, which results in an increased expression of genes necessary for survival inside the host. The intravacuolar cellular compartment where *Salmonella* resides after its entry in mammalian host macrophages (named SCV for *Salmonella* containing vacuole) is a physiological environment which triggers the PhoP/PhoQ two-component system. PhoQ is the sensor protein that interacts with extracelular Mg^{2+} and induces a specific PhoP phosphatase activity, controlling the phosphorylation state of the transcriptional regulator PhoP. However, it was demonstrated that PhoP overexpression can substitute for PhoQ- and phosphorylation-dependent activation. Preferential or dynamic localization of signal transduction proteins has been demonstrated not to be exclusive of compartmentalized eukaryotic cells. Many bacterial processes involve asymmetric localization of protein activity i.e. proteins involved in chemotaxis, development or signal transduction.
In order to investigate the localization of PhoP and the effect of the input signal and the phosphorylation state on its spatial distribution, we set up the FlAsH labeling technique, which adds a hexa-aminoacid motif to the target protein.
We examine PhoP cytolocalization in response to extracellular Mg^{2+} limitation *in vitro* and to the SCV environment in macrophage cells. We show that in these PhoP/PhoQ-inducing environments PhoP displays preferential recruitment to one cell pole, while being homogeneously distributed in the bacterial cytoplasm in repressing conditions. This localization disappears in the absence of PhoQ or when a non-phosphorylatable PhoPD52A mutant is expressed. However, when PhoP transcriptional activation is achieved in a Mg^{2+}- and PhoQ-independent manner, PhoP regains asymmetric polar localization.
These results indicate that PhoP cellular location is dynamic and conditioned by its environmentally-defined transcriptional status, showing a new insight in the PhoP/PhoQ system mechanism.

MOL19 - ORAL

LOST IN TRANSLATION: NrfA, A PUTATIVE RNA CHAPERONE FROM *SINORHIZOBIUM MELILOTI,* AND ITS AUTOREGULATION STORY.

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In the genome of the nitrogen-fixing bacterium *Sinorhizobium meliloti* 1021, there is an ORF of 80 aminoacids, annotated as NrfA, with ~47% (37/80) identity to Hfq, a key player of riboregulation in *E. coli.* As we look forward to determine a role for this rhizobial protein, we seek to understand the *nrfA* expression pattern. A chromosomal *nrfA-lacZ* transcriptional fusion revealed that *nrfA* expression is induced ~3-fold in late-exponential phase in rich medium, whereas a chromosomal *nrfA'-'lacZ* translational fusion was expressed at a relatively constant level along the growth curve in the same medium. This suggests a kind of post-transcriptional regulation of *nrfA* expression. At this point we asked if the NrfA protein itself was involved in this regulatory phenomenon. To this end, we studied the behaviour of the translational fusion in an *nrfA-* background and we observed: first, that b-galactosidase activity from nrfA'-'lacZ followed a similar pattern to that of the transcriptional fusion with a ~3-fold induction in late-exponential phase; second, that irrespective of the growth phase the absolute b-galactosidase activity increased ~5-fold over the corresponding fusion in the wild type strain. Finally, when we complemented this strain with a low-copy plasmid bearing a wild type nrfA copy under the control of its own promoter, we could restore unvariable nrfA'-'lacZ expression. These observations point to an autoregulatory mechanism. The secondary structure prediction of the *nrfA 5´*-UTR shows the presence of both sequence and structural motifs similar to that of *hfq 5´*-UTR in *E. coli*, which is known to be autoregulated at the translational level. Altogether, our results support the hypothesis that *S. meliloti nrfA* expression is translationally controlled, directly or indirectly, by its own protein product.

MOL20 - ORAL

Non-hydrolyzable antisense oligonucleotides direct *aac(6')-Ib* mRNA cleavage by RNAse P.

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Resistance to aminoglycosides (Ag) is mostly due to a wide variety of modifying enzymes. Spread of *aac(6')-Ib* among pathogenic bacteria is a growing concern as it generates resistance to the clinically important Ag amikacin (Ak). A possible strategy to overcome this problem is to silence *aac(6')-Ib.* RNA molecules complementary to single stranded regions of *aac(6')-Ib* mRNA, carrying the consensus sequence for RNAse P ACCA in it's 3'-end were designed. These External Guided Sequences (EGS) were assessed in vitro and in vivo for their ability to direct RNAse P digestion of the messenger. Two of them, EGSA2 and EGSC3, were able to reduce Ak resistance. Degradation of EGS is a problem to further develop this technology. To face this we designed antisense compounds with the EGSC3 sequence using non-hydrolysable nucleic acid analogs. Phosphorotioates, 2'-O-Methyl RNA and Locked Nucleic Acids (LNA) were assayed. Among them, only oligonucleotides composed of LNA derivatives and deoxinucleotides (DNT), LNA/DNT EGS, were able to direct RNAse P-mediated precise cleavage of *aac(6')-Ib* mRNA. Time course experiments showed that mRNA cleavage mediated by LNA/DNT EGS occurred at a similar rate to RNA EGS. Deoxyoligonucleotides with LNA substitutions in different positions of the EGS were designed and assayed for binding to the messenger and mRNA cleavage in the presence of RNAse P. Results showed that configuration of substitutions were very important for both inducing RNAse P mediated cleavage of the messenger and for binding of these EGS to *acc(6')-Ib* mRNA. Stability of LNA/DNT EGS and RNA EGS to degradation in pure bacterial cultures was assessed. Importantly, LNA/DNA EGS were more resistant than RNA EGS to degradation if exposed to pure Escherichia coli cultures. This is the first report EGS of nucleic acid analogs being able to direct mRNA degradation by RNase P. Our results suggest that LNA/DNA EGS might be an effective tool for *aac(6')-Ib* silencing by an RNAse P mechanism.

MOL21

The Superoxide Dismutase of *Rhodobacter capsulatus* is Regulated by the RegB/RegA Two-Component System.

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Rhodobacter capsulatus is a gram-negative phototrophic bacterium that can thrive under a broad range of enviromental conditions. When the oxygen tension is low, this bacterium synthesizes ATP through a light driven, anoxygenic electron transport around a single photosystem, whereas in the presence of air it shifts to a respiratory metabolism after expression of oxidases and dehydrogenases. Following the establishment of respiration, oxygen-centered derivates such as superoxide, hydrogen peroxide and hydroxyl radical are expected to be produced by reduced intermediates of the electron transport chain. These toxic species are refered to as Reactive Oxygen Species (ROS) and can damage proteins, nucleic acids and cell membranes. The cells have evolved a number of enzymatic and nonenzymatic antioxidant defense mechanisms, which reduce the harmful effects of ROS and maintain the cellular homeostasis between pro-oxidants and antioxidants. The first line of enzymatic antioxidant defense in most aerobic organisms is made up of one or more superoxide dismutases (SODs), a metallo enzyme which catalyze the disproportionation of superoxide to yield oxygen and hydrogen peroxide. *R. capsulatus* contains a single, oxygen-responsive superoxide dismutase (RcSOD) with cambialistic properties. RegB and RegA constitute a regulatory system that provides an overlying layer of redox control on a varirty of energy-generating and energy-utilizing processes such as photosynthesis, carbon fixation, nitrogen fixation, hydrogen utilization, aerobic and anaerobic respiration, denitrification, electron transport and aerotaxis.
The presence of putative RegA binding sites located upstream the *sod* gene of *R. capsulatus* was deduced after *in silico* analysis. To visualize the influence of RegA *on sod* gene expression, a 588 bp DNA fragment from the upstream region of the *sod* was cloned. Translational *lacZ* fusions were constructed and introduced in *Rhodobacter capsulatus* by biparental conjugation. Measurement of β-galactosidase, SOD activity and immunostaining were performed to estimate the RcSOD expression levels both in the wild-type strain SB1003 and the *regA*-disrupted strain MS01 cultured under different aeration conditions. Our results suggest that the RegB/RegA two-component system act as an anaerobic repressor of R. *capsulatus sod* gene expression.

MOL22 - ORAL

Some insights of the mechanism of action of salivaricin CRL 1328 against pathogens

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Salivaricin CRL1328 is a bacteriocin produced by *Lactobacillus salivarius* subsp. salivarius CRL1328, a human vagina isolate, that inhibits the uropathogens *Neisseria ghonorroeae, Enterococcus faecalis, E. faecium, Staphylococcus saprophyticus* and *Streptococcus agalactiae.* The final objective of our research group is the design of a probiotic product containing beneficial microorganisms, and also salivaricin, for the prevention and/or treatment of urogenital infections. In previous studies we characterized the genetic locus responsible for the bacteriocin production. Salivaricin CRL1328 is a two peptide bacteriocin whose activity depends on the complementary action of two peptides (SALα and SALβ). These peptides were chemically synthesized and their biological activity was confirmed.
In this work, the mechanism of the bactericidal action was studied, by using the biologically active synthetic salivaricin CRL1328 and the urogenital pathogen *E. faecalis* as sensitive strain. In addition, the optimal peptide ratio for bactericidal effect was analyzed. The transmembrane electrical potential ($\Delta\psi$) was recorded by measuring the fluorescence of 3,5-dipropylthiadicarbocyanine Iodide [DiSC3(5)] using the ionophore valinomycin as control. On the other hand, the transmembrane pH gradient (ΔpH) was determined by monitoring the fluorescence of the pH-sensitive fluorescent probe carboxy-fluorescein diacetate succinimidyl ester (cFDASE) with nigericin as control. A SALα/SALβ peptide ratio of 1 to 1 was required for optimal antimicrobial effect, while the individual peptides showed no activity. Salivaricin dissipated $\Delta\psi$ of *E. faecalis* glucose energized cells in 50 mM K-HEPES (pH 7.0) and reduced the cFDASE fluorescence. Taken together, these data demonstrate that salivaricin CRL1328 dissipated the membrane potential and pH gradient in energized cells, leading to the collapse of the proton motive force. This action would be most likely because of the pore formation in the cytoplasmic membrane of target cells which allows the efflux of ions. Further studies are being performed to determine if this mechanism of action is similar when interacting with other uropathogens.

MOL23 - ORAL

GGDEF/EAL domains and motility in *P.putida*: a novel protein involved in motility regulation

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Chemotaxis allows motile bacteria to respond to chemical gradients and concentrate themselves near the source of nutrients. Microorganisms belonging to *Pseudomonas* species are known to be able to grow in a wide variety of substances, including many that are considered environmentally dangerous. Unlike previously characterized chemotactic behaviors in *Pseudomonas* strains, chemotaxis of *P.putida* CF600 to methyl phenol was shown to be dependent on its metabolism and did not seem to require a specific chemoreceptor

Since in *E.coli* metabolism-dependent taxis responses are mediated by Aer, a protein closely related to chemoreceptors that contains a FAD-binding PAS domain, we analyzed the *P.putida* genome in the search of aer-like genes. Three aer-like genes were found, and next to one of them, within the same transcriptional unit, a protein containing PAS-GGDEF-EAL domains was found and called our attention.

In the last years GGDEF and EAL domains have been associated to diguanylate cyclase and phosphodiesterase activities, respectively. These enzymatic activities would participate in control of intracellular levels of a recently described second messenger for prokaryotes, c-di-GMP. It is known that levels of c-di-GMP can modify cells behavior and motility, therefore PP2258 was thought to be involved in energy taxis via regulation of intracellular levels of di-c-GMP. To test this hypothesis, in this work biochemical properties of PP2258 were studied using as a first approach over expression of the protein both in *E.coli* and *P.putida* and *in vivo* assays. Null mutants in PP2258 show defects in motility.

When over expressed in E.coli or P.putida cells, c-di-GMP levels were notably increased compared to control cells, although accumulation of c-di-GMP was much lower in *E.coli* than in *P.putida* extracts. A point mutation in GGDEF domain, which is supposed to be involved in c-di-GMP synthesis, causes a decrease in this accumulation, although does not suppress it. On the other hand, a mutation in EAL domain, which is supposed to be involved in its hydrolysis, led to an even higher c-di-GMP accumulation. Together, these results suggest that PP2258 could have both, diguanylate cyclase and phosphodiesterase activities.

5. Microbiología Ambiental / Environmental Microbiology

AMB1

ANTIMICROBIAL SENSIBILITY OF *Clostridium perfringens* STRAINS ISOLATED FROM FOODS IN SAN LUIS, ARGENTINA

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Clostridium perfringens is a gram positive bacilli, anaerobic and spores former that is common causes of enteritis and enterotoxemias in both animals and humans. The food poisoning results from ingest of contaminated foods with strains of *C. perfringens* that have the enterotoxin (CPE). This disease is not frequently diagnoses in our country because it is self-limited; but it can be risky in people with immunodeficiency, old men and children. There are evidences of the use of the antibiotics to feed animals destined to the human consumption. This can develop in some cases antibiotic resistance. The resistance to tetracycline has been evidenced frequently by other authors. The resistance to metronidazole is not frequent and it is the antibiotic that is normally used for clinical treatment in affections by anaerobe. The objective of this work was to determine the resistance to tetracycline and metronidazole of strains of *C. perfringens* enterotoxigenics and not enterotoxigenics food's isolated in San Luis. Twelve strains of *C. perfringens* were used, these were beforehand characterized and isolated from sample of meat, spice and dehydrated soups, six of them enterotoxigenics and other six not enterotoxigenics. These strains were previously grown in thioglycolate broth with indicator at 37 ºC for 24 h in anaerobiosis. The minimal inhibitory concentration (MIC) was determined in broth to test two antibiotics; using a inocula of 5 x 10^5 UFC/ml and antibiotics concentrations from 0.5 to 128 μl/ml, this was incubated in anaerobiosis at 37 ºC during 24 h. After incubation, was carried out visual reading, the MIC value is concentration of the tube where turbid is not observed at simple view. Each tube compared with a control of growth without antibiotic to evaluate the intensity of development. Only a strain presented resistance moderated to tetracycline with MIC of 8 μg/ml corresponding to a strain not enterotoxigenic. Finding two strains with moderate resistance (≥1 μg/ml), an strain enterotoxigenic and another not, and nine strain with high resistance (≥2 μg/ml), five enterotoxigenics and the remainder not enterotoxigenic. On the basis of these results we concluded that there is not considerable difference in the distribution of resistance between enterotoxigenic and not enterotoxigenic strains. The resistance to metronidazole is significant when compared with previous studies. The strains that presented higher resistance to metronidazole also presented moderated resistance to tetracycline, in agreement with other author's studies. It is important to note that resistance occurs one hour after treatment with antibiotics.

AMB2

Natrialba magadii (an halophile archaebacteria) as a model to understand microbial UV-C tolerance and survival in extraterrestrial environments.

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The *Halobacteriaceae* family (order *Halobacteriales*) comprises a group of extremophile archaebacteria who inhabits in environments with high salt concentrations (3.4-5.1 M NaCl range for optimally growth). Some halobacterial species are acidophilic or alkaliphilic, growing in a pH below 5 or in pH optima between 9 -11 respectively.
It is known that halophile archaebacteria are inhabitants of halites and ancient evaporites in Earth. Since evaporites have been detected in Martian meteorites, these organisms are proposed as plausible inhabitants of Mars-like planets or other extrasolar planets.
Ultraviolet (UV) radiation is an important environmental factor, because it can act as a genotoxic agent inducing DNA damage, or damaging a wide variety of proteins and lipids. In fact, high exposure to UV-C (wavelengths ≤ 290 nm) is lethal to most of the terrestrial biological systems.
Because halophiles are exposed to intense solar UV radiation in their natural environment they are generally regarded as relatively UV tolerant.
In an extraterrestrial environment the large amount UV radiation coming from certain type of stars (during events named "flares"), could set a limit for the development of extraterrestrial life. It is uncertain how these UV events can affect the evolution of life in these environments beyond the Earth, since life is particularly vulnerable to ultraviolet radiation.
In this work we analyze the effect of UV-C on the haloalkalophile microorganism *Natrialba magadii*, who was isolated from Magadi lake in Kenya, Africa (ATCC 43099).
To this end cultures of *N. magadii* were grown to mid-exponential phase (around OD 600= 1) at 37.0 °C, in rich media (pH 10.0) containing (in g/l): yeast extract, 20.0; NaCl, 200; Na2C03, 18.5; and exposed to a Phillips 15W Hg lamp (254 nm emission peak) with constant mixing. Aliquots of the irradiated culture were withdrawn after different irradiation times, and the effect of the UV treatment was assessed by diluting the sample and following the changes of the growth kinetics in media of identical composition. Growth was monitored by increasing in optical density at 600 nm.
Preliminary results show that even after significant UV damage, as judged by the absence of detectable growth for more than 30 hours, the surviving cells were able to resume growth with nearly normal kinetics.

AMB3

Niche oriented selection of phenol-degrading populations in bioreactors

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The distribution and abundance of species have important implications for the functioning and stability of many ecosystems. Here we test the variability of the functional diversity of an activated sludge acclimated to degrade of phenol, by following the dynamics of the gene coding for the multicomponent phenol hydroxylase (LmPH) as a function of the concentration of phenol in the feed. LmPH catalyzes the conversion of phenol to catechol, the rate-limiting step in the phenol degradation pathway in the environment.
Two lab-scale activated sludge systems specialized in phenol degradation were subjected to a gradual increase in phenol load. Two libraries of LmPH gene were constructed at different times of reactor operation. Aminoacidic-derived sequences of a total of 124 clones were separated into seven groups with a phylogenetic distance of 0.12, which likely represent ecologically relevant variants of the enzyme. Seven specific sets of LmPH primers were used to follow the dynamics of LmPH gene diversity, using real time PCR assays at 6 different times corresponding to different loads of phenol, from 100 to 1500 mg/L. The same sets of primers were used for the quantification of LmPH variants in two control reactors, which were maintained with a constant phenol concentration of 100 mg/L. We found a replicable coexistence of redundant species, with their relative abundance determined by the concentration of phenol in the feed. Comparison of the abundance patterns of phenol-degrading species suggests a considerable degree of determinism, where phenol-degrading populations are recruited from the existing pool, each with their particular ecological traits that determines their relative abundance in the community. We conclude that in the absence of immigration, niche differentiation has a strong influence in the assembly of phenol-degrading communites.
To test this prediction we pursued the isolation of representatives of bacteria containing each of the different detected LmPH gene. Thirteen isolates were obtained using a wide range of culture conditions, finding at least one representative of six of the seven populations. These isolates are currently being characterized for their kinetics properties of phenol degradation and for their kinetics of growth and inhibition by phenol.

AMB4

ANTIBIOTIC RESISTANT *Escherichia coli* IN WATER AND FISH SAMPLES FROM SAN JUAN RIVER, SAN JUAN, ARGENTINA.

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Antibiotics generally used in medicine and vet, had been detected in sewage with deficient treatment. Spilling of these wastewaters in aquatic environments generates a serious damage for ecosistem, leading to proliferation and dissemination of patogenic bacteria resistant to antibiotics. The aim of this study is to determine the presence of antibiotical resistance of *Escherichia coli* (E. coli) strains isolated from water and fishes` tissues (Genus: Astyanax) in the San Juan river`s lower basin. Samples were collected in the outfall of Ignacio de la Roza Dam (Pinar), and in the latitude of Albardon and San Martín`s bridges. Pinar was chosen as pristine zone, because it is an uninhabited place, and Albardon and San Martin because they receipt diffuse contamination from marginal ranches without sewer. Three water samples and three fishes were taken in each place every two months, during 2007. Fishes were sacrificed by medular cutting; the intestine and a piece of muscle were extracted before 24 hours from collection. The tissues were homogenized in sterilized sodium chloride saline solution. Water samples and homogeneized solution were diluted and inoculated by spread plate method using EMB agar (Britania). *E.coli* strains were confirmed through INVC and Gram`s tinge. Each strain was tested by the disk diffusion method using Muller Hinton agar (Britania) to evaluate their suceptibility to eigth different antibiotics: nalidixic acid, ampicillin, amikacin, chloramphenicol, nitrofurantoin, colistin, gentamicin and cefalothin. Islotes were considered resistant or sensitive according to standards suggested by NCCLS (2000). The results obtained show different levels of resistance to all antibiotic analysed for *E.coli* isloted from water and tissues samples. In this way, ampicillin and amikacin resistant strains were found in water and fishes from Pinar, previously considered as a pristine zone. In samples collected from Albardon and San Martín, appears resistance to all antibiotics with significant seasonally variation. These results allow to confirm the presence of resistant antibiotic strains of *E.coli* in water and fishes collected from San Juan river`s lower basin. These facts represent a serious sanitary and ecology risk and deserve attention, due to the introduction of resistence plasmids into natural environments.

AMB5 - ORAL

DIVERSITY OF AMMONIA OXIDATION BACTERIA (AOB) IN AN INDUSTRIAL FULL-SCALE ACTIVATED SLUDGE AT THE EDGE OF NITRIFICATION FAILURE

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Ammonia removal via nitrification is an important service provided by many wastewater treatment plants. It is a two-step process, usually rate-limited by the oxidation of ammonia to nitrite performed by ammonia-oxidizing bacteria (AOB). Frequent instability of biological nitrification is brought about by small changes in environmental conditions, such as temperature, pH and the presence of toxic compounds. The way in which the environment will affect the process will ultimately depend on the diversity and ecophysiology of nitrifiers existing in each particular ecosystem. Therefore, there is a strong need to understand the ecological basis for instability that would allow predicting and eventually avoiding operational failures.
We have investigated the process of ammonia oxidation in a full-scale activated sludge from an oil refinery, which receives a high load of free ammonia, hydrocarbons and phenol and suffers from repeated periods of nitrification failure. A significant correlation between NH_4^+-N and phenol concentration in the treated wastewater was observed. NO_3^--N and phenol concentration exhibited the same correlation, albeit negative. We performed a thorough molecular diversity analysis of ammonia oxidation bacteria present during a period of full nitrification. DNA was isolated from a sludge sample from the aeration basin of the WWTP and used as template for PCR, with primers targeting highly conserved regions in the gene coding for the enzyme ammonia monooxygenase (*amoA*). PCR-amplified fragments of 453 bp were used to construct a clone library. Nucleotide sequences and the derived amino acid sequences were determined for a total of 110 clones. *amoA* genes were divided into only two OTUs separated by a genetic distance of 0.06. Dominant OTU (77% of the clones) was related to *Nitrosomonas europaea*, whereas the second OTU was affiliated with *Nitrosomonas nitrosa* linage.
Novel PCR primer sets were designed to target signature DNA sequences in the *amoA* gene of the two detected taxons and quantified using real time PCR. The proportion of cells belonging to taxon 1 was estimated as 0.7% of total cell quantified with primers targeting total bacterial 16S rRNA. The percentage of AOB was 0.9% based on 16S rDNA assay with CTO primers. A value of 2.4% was obtained for the quantification of bacteria belonging *Nitrosomonas* genus by fluorescence in situ hybridization (FISH) using the *Nitrosomonas*-specific probe Nso190.
According to these results, AOB cell numbers are approximately three times lower than the predicted values determined by a theoretical process-based estimate of AOB biomass. If other nitrification processes are discarded, we propose that AOB adapted to perform under harsh conditions have very low yield, providing one possible explanation for why nitrification is unstable in the presence of toxic compounds such as phenol.

AMB6

ANTIMICROBIAL ACTIVITY OF EXTRACTS OBTAINED FROM PLANTS AGAINST AIR MICROORGANISMS IN ARCHIVES

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A large number of particles of different origin, shape and size are suspended in the air of outdoor and indoor environments, they constitute the atmospheric aerosol. Under optimal environmental conditions, air microbiota may coexist with objects and collections of archives with causing significant damages. Yet, in the extent there are changes affecting environmental conditions; microorganisms may exert negative effects, both from the point of view of biodeterioration. Natural products obtained from plants, constitute an alternative and useful source in controlling biodeterioration not being harmful to the environment. The aim of this paper was to study the antimicrobial activity of hydroalcoholic extracts of *Cichorium intybus* L., *Arctium lappa* L., *Centaurea cyanus* L., *Plantago major* L., *Medicago sativa* L., *Allium sativum* L., *Pinus caribaea Mor., Eucalyptus citriodora* Hook and the essential oil of *Piper auritum* Kunth against *Bacillus polimixa*, *Enterobacter agglomerans* and *Streptomyce*s sp. isolated from indoor atmosphere of National Archive of Cuba. Samples were collected from the indoor environment by using a biocollector (Grille or Rack II, 30 L/ min) and Petri dishes with nutrient agar for their isolation. The equipment was placed at 1.5 meters above the floor level, following a diagonal method which included 5 points by triplicate in each deposit. Petri dishes were incubated at 28°C during 4 days so as to carry out the recount of colonies. The antimicrobial activity of the extracts was determined by hole-plate diffusion methods and minimum inhibitory concentration (MIC). Each test was performed in triplicate. Assayed extracts showed good biocide activity.

AMB7

MICROORGANISMS PRESENT IN ARCHIVES. THEIR PARTICIPATION IN THE PROCESSES OF BIODETERIORATION AND HEALTH IMPACT

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Microorganisms present in environments that hold materials belonging to the cultural heritage may be causing the biodeterioration of valuable collections and affect the health of people working in these environments. The objective of this work was to study the microbial concentration and microbiological control of air and of different materials (maps and pictures) in deposits of the Historical Archive of the Museum of La Plata, Argentina and the and define the potential risk that microorganisms represent to the documentary heritage biodeterioration and health. For microbiological control of airborne contamination Petri dishes were used. They were exposed following the Omeliansky methodology and for the documents swab sampling method was used. Taxonomical identification of microorganisms isolated was performed. The fungal genera that predominated in the air for both deposits were *Aspergillus*, *Penicillium* and *Cladosporium*, coinciding with the genera found on the documents. Most fungi isolated degraded cellulose and produced pigments and acids. In the air gram-positive bacteria predominated in both deposits and identified genera were *Streptomyces*, *Bacillus*, *Streptococcus* and *Staphylococcus*. The genera of bacteria isolated from documents were *Clostridium*, *Bacillus* and *Streptomyces*, all of them with known proteolytic and/or cellulolytic activity. The bacteria and fungi isolated in these studies are capable of producing diseases. There were high microbial concentrations in the National Archive of the Republic of Cuba deposits and, in particular, levels of bacteria were significantly higher than those found in the Historical Archive of the Museum of La Plata.

AMB8

SPATIAL VARIATION IN BACTERIOPLANKTON COMPOSITION ALONG THE RIO DE LA PLATA ESTUARY

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Bacterioplankton are major biogeochemical agents in aquatic ecosystems that play a key role in organic matter remineralization and nutrient cycling. The aim of this work was to characterize the bacterioplankton composition distributed along two transects of approximately 160 km (north and south) and two depths (surface and bottom) from Río de la Plata Estuary. Physicochemical properties of water samples were investigated, distribution of bacterial abundance was evaluated by epifluorescence microscopy after both 4,6-diamindino-2-phenylindole (DAPI) and acridine orange (AO) staining, and DNA total analysis was performed by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified of 16S rRNA gene fragments. Two statistical methods were used to analyse the DGGE banding pattern: Shannon-Weaver index of general diversity (H) and Principal component analysis (PCA). Total bacterial counts ranged from 7.5×10^5 to 4.4×10^6 cells ml^{-1}. The values bacterial densities were higher at the south transect than at the north one, although the highest values were observed at both surface and bottom salinity fronts. The bacterial assemblage of the different samples showed a characteristic and reproducible DGGE fingerprint. Between 5 and 13 bands were found in each sample. The numbers of bands as well as the values of H diversity index were generally higher in bottom samples compared to surface samples. PCA analysis of DGGE profiles revealed a marked homogeneity in bacterial composition of north area, whereas, bacterial assemblages in south area were more heterogeneous. This pattern was particularly associated with physical-chemical parameters such as temperature and salinity. Results indicated differences in bacterioplankton community structure in the two analyzed areas. These variations were consistent with the different hydrography of the area, since the south zone is affected by diluted waters which come from the discharge of the estuary.

AMB9

Bacterial diversity associated to arsenic contamination in Salar de Ascotán, Chile.

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Andean Puna is a special place with unique geological, geomorphologic and climate characteristics. These characteristics promoted the formation of extensive salt flats in the Andes in conjunction with geothermal processes and evaporation of water rich in arsenic and other compounds (borates, nitrates, etc.). Microbial diversity inhabiting these environments is poorly studied, however, these ecosystem provide a unique collection of habitats for studying microbial diversity in relation to salinity and arsenic deposits.
Bacteria have been identified that can oxidize and/or reduce arsenic compounds for energy conservation in many environments. Activities of mobilizing and precipitation of arsenic were demonstrated in contaminated sediments of River Loa and Salar de Atacama, in northern Chile. We analyzed the planktonic bacterial assemblages inhabiting Salar de Ascotán, a salt flat of the II Region of Chile, by PCR- denaturing gradient gel electrophoresis (DGGE) and sequencing of 16S rRNA gene fragments. Laguna Turquesa samples was specially analyzed because their high concentration of total arsenic (28 mg ml^{-1}). Culturable, arsenic-precipitating cells were detected by most-probable-number (MPN) incubations. The presence of yellow precipitate was considered as a positive result. DGGE approach was used to characterized bacterial population in sediment samples, as well as those obtained by enrichment cultures at different temperatures (4 and 30 ºC), and concentrations of As(III) and As(V), incubated in aerobic and anaerobic conditions. From a total of nearly one hundred of bands obtained in DGGE profiles, only 40% could be reamplified and sequenced, obtaining sequences with identities between 94 and 99% with those deposited in database. From these, nearly to 70% belong to uncultured bacteria (DGGE bands or clones). *Bacillus*, *Alkaliphilus* and *Paraliobacillus* were found as bacterial isolates and cited by other authors as halotolerant or halophile, metabolically related to metallic compounds reductions and isolated from extreme environments.
Respect to the incubation temperature, with exception of only one sequence (unc. marine bacterium clone A3, identity 98 %) found at 4 and 30 ºC, the rest of the sequences grouped in one or other temperature condition. Conglomerate analysis in order to compare DGGE profiles at different enrichment conditions, allow finding a major grouping of similar profiles by temperature, clearly observing 4 groups or nods that differed at more than 50% of maximum distance.

AMB10

Design and Optimization of Real Time PCR Assays for the Quantification of Aromatic Ring-Hydroxylating Dioxygenase Genes in Marine Sediments

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In aquatic ecosystems, sediments can serve both as reservoirs and as potential sources of contaminants to the water column. In many sites along the Patagonian coast, we detected between one and eleven different polycyclic aromatic hydrocarbons (PAHs), some of them in concentrations exceeding those recommended by sediment quality guidelines to protect aquatic life. Using culture independent methods, we detected in these sediments eight different types of aromatic ring-hydroxylating dioxygenase (ARHD) genes, which code for enzymes involved in the first step of PAH biodegradation pathways. Of these, five were classified as novel gene types, as they form deeply rooted branches (less than 70% identity at the amino acid level) with previously described ARHD sequences. In accordance, we have designed eight Real Time PCR (qPCR) assays, including primers and TaqMan probes, for the quantification of these genes in environmental samples. The design took into account annealing and thermodynamic properties of the primers-probe system aiming to increase assays performance. Given the high cost of the TaqMan probes, the assays were first tested in conventional assays with the DNA-binding fluorophore EvaGreen. Annealing and plate-read temperatures and magnesium ion concentrations were optimized for each assay. Sensitivity and linearity were tested with standard curves built with plasmids carrying the appropriate gene fragments previously obtained by cloning and confirmed by sequencing. Specificity was tested using environmental DNA as template by running melting curves against the corresponding standards and by agarose gel electrophoresis.
Preliminary assays were performed using DNA samples directly extracted from coastal sediments with different levels of PAH contamination. The number of ARHD gene copies per microliter of extracted DNA was estimated for each assay at two DNA concentrations. Similarly, 16S rRNA gene copy number was estimated using universal eubacterial primers. These values were used to estimate the relative abundance of the different ARHDs in the microbial community, with respect to 16S rRNA gene abundance. In certain chronically polluted sites, values for an individual gene type could reach up to 1800±200 copies per microliter of extracted DNA, representing the 0.07% of bacterial 16S rRNA genes detected in the same sample. Our results indicate that the assays are sensitive enough to detect genes present at very low frequencies in the community, and will be fundamental to analyze the relative abundance of these genes in different coastal environments. These qPCR assays will also allow to analyze the dynamics of bacterial populations carrying these functionally relevant genes in microcosm experiments, in order to identify the ecological role and environmental needs of these populations present in the sediments. In addition, these ARHD qPCR assays will be used to identify clusters of catabolic genes in a metagenomic fosmid library.

AMB11

Diversity of Aromatic Hydrocarbon-Degrading Genes in Sub-Antarctic Coastal Marine Sediments

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Functional characterization of aromatic hydrocarbon-degrading bacterial populations can be achieved by studying genes encoding for aromatic ring-hydroxylating dioxygenases (ARHDs), enzymes responsible for the first step of their biodegradation pathways. We amplified ARHD gene fragments from total DNA extracted from intertidal sediments of Patagonia, using a degenerate primer set designed to specifically target these catabolic genes in Gram-positive bacteria. Two gene libraries were constructed by cloning the amplification products obtained from two sediment samples of Ushuaia Bay (Tierra del Fuego), retrieved at the same site but two years apart. Clones carrying amplification products produced due to mispriming events were detected and excluded from further analysis using the TBLASTX program of BLAST. Bioinformatic tools were used to describe and compare both clone libraries. The DOTUR software identified 15 different groups of genes, which were called gene types I to XV. In order to infer the possible function of these gene fragments, their sequences were compared to four different databases: NCBI, COG (Clusters of Orthologous Groups: a phylogenetic classification of proteins), KEGG (Kyoto Encyclopedia of Genes and Genomes: a metabolic pathway database) and GO (Gene Ontology: an ontology-based gene products database).
Deduced amino acid sequences of group XIII showed 85% identity (94% similarity) at the amino acid level with the dioxygenase IpbAa from *Pseudomonas putida* RE204 (AAC03436), a cumene-degrading bacteria. The remaining sequences showed less than 78% amino acid identity to ARHDs published in the NCBI database. An alignment of the deduced amino acid sequences from clones with ARHD sequences obtained from the NCBI database, showed conserved key amino acid residues associated to the active site of these enzymes. On the other hand, searches performed in COG, KEGG and GO databases resulted in all sequences matching ring-hydroxylating dioxygenases, and E-values were lower than 10^{-4}, 10^{-17} and 10^{-16}, respectively, indicating a very low probability of matching these genes by chance. Gene libraries were compared by calculating diversity and similarity indexes. Both libraries were equally diverse (Shannon's diversity index between 1.86 and 1.89), showed low dominance indexes (Simpson's dominance index between 0.14 and 0.21) and were moderately similar (Chao's Jaccard abundance-based similarity index: 0.59).
Most ARHD gene fragments analyzed in this study were highly divergent from those previously described, showing that there is still much uncertainty about aromatic hydrocarbon catabolic pathways in marine environments. Further research aiming to quantify the abundance of these functional genes will be performed, in order to assess the ecological role of these populations in the degradation of these harmful pollutants in marine sediments.

AMB12

POLLUTION LEVELS OF STREAMS DRAINING TO THE RÍO DE LA PLATA BETWEEN BUENOS AIRES AND LA PLATA CITIES.

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Main streams and canals flowing to the el Río de la Plata from Riachuelo (Buenos Aires) to arroyo El Gato (La Plata) were sampled to survey pollutants content and potential impact on coastal population and receiving waters. Physical and chemical parameters (temperature, pH, DO, BOD, ammonia, nitrate, phosphate) and biological pollution indicators (Heterotrophic Plate Count, CFU, PCAgar; Total Coliforms, MPN, BGLB broth; Fecal Coliforms, MPN, EC broth; *Escherichia coli*, MPN, EC-MUG broth and fecal Enterococci, MPN, Chromocult) were measured. In most of the sites high levels of pollution could be detected (DO ≤1mg/l; BOD ≥25 mg/l; ammonia ≥5 mg/l; phosphate ≥0.5 mg/l; plate counts ≥5 x 106 UFC/ml; TC ≥1 x 10^4 NMP/100ml y FC ≥2 x 10^3 NMP/100ml), similar to urban wastewaters without treatment, according to the local population density and industrial development. Only the streams Pereyra, Villa Elisa and Rodríguez which drain rural zones, showed natural running waters values (DO ?4 mg/l; pH ?8; BOD ≤10 mg/l; plate counts ≤10^6 UFC/ml; TC ≤5 x 10^2 NMP/100 ml and FC ≤10^2 NMP/100 ml), even with some human activity influence.

AMB13

MICROBIAL WATER SAFETY IN THE SOUTH COASTAL ZONE OF THE RÍO DE LA PLATA.

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Water samples of the Río de la Plata were taken at 500, 1500 and 3000 m from the coast, in transects perpendicular to the shore line, from Palermo to Punta Lara. Fecal pollution indicator bacteria were counted by a standard technique (*Escherichia coli* (EC), MPN, APHA, 9221 B1 F) and by miniaturized standard techniques (*Escherichia coli*, MPN, ISO 9308-3 and fecal Enterococcus (FE), MPN, ISO 7899-1). All the analyzed samples showed microbial indicator numbers between 3.6 x 101 and 2.4 x 106 EC/100 ml (MPN, APHA), 7.9 x 10^1 and 2.2 x 10^6 EC/100 ml (MPN, ISO) and ≤ 7.5 x 101 and 9.4 x 105 FE/100 ml (MPN, ISO). Results by different techniques showed good agreement. Correlation coefficient between EC-APHA and EC-ISO counts were 0.91 and between EC-ISO and FE-ISO counts were 0.85. According to EC counts, only two of the samples were acceptable for safe recreational uses and half of them were not able to be used as a source for drinking water production.

AMB14 - ORAL

Characterization of bacteria isolated from pristine High Altitude Andean Wetlands resistant to extreme environmental conditions

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High Altitude Andean Wetlands (HAAW) is a system of shallow oligotrophic lakes originated in the tertiary age distributed across The Puna, the high plateau of the South American Andes from Bolivia to Argentina. These lakes are scattered throughout the region at altitudes from 3,600 to 4,600 meters. Microbial communities within these aquatic ecosystems are tolerant to wide fluctuations of environmental factors in addition to steady-state extreme conditions such as high UV radiation (maxim UV-B W/m^2 in situ 10,78), high heavy metal content (mainly arsenic 0,8 to 11,8 mg/L), high salinity (0,4 to 117 ppm), oligotrophic and low phosphorus (i.e. 0,02 mg/L).HAAW ecosystems are totally unexplored with no access roads, which provide an unique opportunity to study the ecosystem interactions and responses to environmental stress. Salar de la Laguna Verde is a system of four oligotrophic shallow lakes (L.) (L. Aparejos, L. Azul, L. Negra and L. Verde) distributed along 100 km^2. L. Catal belongs to Salar del Hombre Muerto in Catamarca and L. Vilama is located in Jujuy. The exploration at the microbial diversity of HAAW could give new insights to the mechanism of adaptation and tolerance to extreme environments.
Aim: The aim of this work was to isolate bacteria from different wetlands and to study the diverse response to UV-B and antibiotics resistance of isolated microorganisms. Experimental Design: Water and sediment samples were exposed to UV-B radiation at similar doses than natural environmental conditions. Most resistant bacteria were isolated by their intrinsic resistances to UV-B, salinity and antibiotics resistances. All of them have been identified by 16S rDNA sequence.
To study UV-B resistance, the microorganisms were grown in adequate medium at 30 ºC in flask with constant agitation. The cells were harvested at exponential phase. Pellets were washed in 0.9 % NaCl and fractions of each cell suspension were transferred to sterile quartz tubes. Tubes were exposed under UV-B lamps and radiation doses were quantified by a radiometer. Survival percentages were determinated by colony forming units at different expositions times.
The antibiotic resistance was determitated by disk diffusion methods on Müeler-Hilton agar according to the current recommendations of the National Committee for Clinical laboratory Standards (NCCLS) for susceptibility.
Results: Until this moment we have isolated 155 strains distributed in the following taxonomic groups: Gamma, Beta, Alpha-proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Archaea. These results showed a prevalence of Firmicutes (43%) and Gamma-proteobacteria (32%). We established the presence and correlation of resistances to UV-B radiation and antibiotics in Andean wetlands and that wetland water and sediments in these environments are a source of antibiotic resistant bacteria.

AMB15

Diversity of rhizobia that nodulates soybean which were isolated from the soils under different management systems

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Bacteria undergo genetic modifications while living in their natural habitat, the soil, as a result of horizontal DNA transfer and or natural mutations or rearrangements. This might generate diversity in soils allowing this the isolation of natural mutants that may outperform wild type strains, at least under certain circumstances. This might be enhanced by soil management since it induces changes in the soil environment. The purpose of this work is to evaluate and compare the diversity of rizobia isolated from soils cultivated under conventional and minimum tillage. Rhizobia were isolated from soils with different management history. The Most Probable Number was estimated as described by Woomer (1994). Plants were inoculated with soil dilutions and were grown along a 45 day period in the greenhouse under controlled conditions. Then, the plants were harvested and after surface sterilizing the nodules bacteria were isolated from their interior. Only part of the analysis has been performed. Our preliminary findings indicate that those soils under conventional tillage, with soybean as the preceding crop, had a lower number of rhizobia, than soils under minimum tillage with maize as the preceding crop. Under minimum tillage, there were a higher number of bigger colonies and an equivalent number of two different types of colonies most probably representing *B. japonicum* and *B. elkanii.* Under conventional tillage there were a similar number of big and small colonies but the number of watery colonies was higher than mucoid ones. The diversity found among organisms isolated from the first and last dilution series was different under both management systems. We characterized by means of a PCR-multiplex reaction that specifically identifies representatives of any of the three genera *B. elkanii*, *B. japonicum* and *B. lianingense.* 80 isolates and among them 75 were *Bradyrhizobium.*

AMB16

"Developing strategies to assess the distribution and bioremediation potential of chemotactic vs non chemotactic strains"

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The ability of bacteria to follow gradients of chemicals and concentrate themselves near nutrients or away from toxic substances is called chemotaxis and represents a powerful strategy for survival. On the other hand, it has been shown that some contaminant-degrading bacteria display chemotaxis towards the specific contaminants in laboratory studies. The chemotactic ability might represent a crucial factor in the efficiency of bioremediation in natural environments.
The general aim of our work is to establish how the chemotactic behavior affects the rate of hydrocarbon degradation in environment-like setups, and to that end we intend to develop methods to assess the distribution of different strains in those setups.
Our model system is *Pseudomonas putida* KT2440 (che+) and a generally non-chemotactic derivative (che-), both transformed with a plasmid that confers the ability to grow using short-chain alkanes as their only carbon source. Preliminary experiments were performed using other carbon sources for growth and as chemoattractants since they were handled more easily than the highly volatile octane.
In order to analyze the distribution pattern of che+ and che- strains in co-inoculation experiments, we used two variants of the plasmid named pFULL and pDEL, the latter of which carried a specific deletion. Two pairs of primers were used to amplify fragments from the plasmids, one pair whose target is missing in pDEL and the other one targeted to both templates.
Petri dishes containing different solid or semi-solid supports on which the carbon sources were evenly distributed were inoculated at the center with mixtures of the strains che+/pDEL and che-/pFULL. Samples were taken at different times and at several distances from the point of inoculation and amplification reactions were performed with these samples using both pairs of primers. The ratio che+/total cells was calculated for each sample in order to see the population composition for each time and space point. In soft agar plates, with citrate as the sole carbon source, the population became enriched in che+ cells as they followed the self-generated gradient of nutrients. We discuss the relative distribution of che+ and che- strains in setups intending to mimics soil environment and that are more comparable to real environmental situations than the traditional soft agar plates.
As a first approach to compare the degradative efficiency of both strains, parallel experiments were performed in which they were inoculated separately, and the carbon source exhaustion along the whole available space was determined.
These methods represent a useful tool that will be used in future microcosms assays to analyze the distribution of inoculated cells in order to evaluate the relevance of chemotaxis in hydrocarbon degradation.

AMB17

BACTERIOPLANKTON: SAMPLE PROCESING AND 16S RIBOSOMAL GENE AMPLIFICATION

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Bacterioplankton diversity provides information about the environment evolution since it is the first step on the food chain. Almost 99% of the environmental bacteria are not culturable. Molecular methods give us the tool to obtain their genomic and identification. There are a wide range of celular lysis protocols and molecular tools available. The aim of this study was to define reliable methods for DNA purification and successful PCRs, suitable for water samples from Embalse del Río III, Córdoba.
Three different methods were assayed: A. Centrifugation of 30 – 1000 ml of water. DNA extraction according to Pitcher et al (1989); B. Filtration of 250 – 1000 ml water through GF/C and 0.22 um policarbonate filters. Filters processed after Fuhrman et al (1988); C. Centrifugation of 2 – 10 ml of water and DNA purification according to Boström et al (2004). From DNA obtained by each method, the 16S DNAr gene was amplified by PCR technique with specific primers. Three different DNA polymerase were used: Taq (Invitrogen), Pfx50 (Invitrogen) and Expand Long (Roche). E. coli DNA was used as positive control. With method A, 11% (5/42) positive PCRs were obtained with Taq and 23% (3/13) with Pfx50. With method B, amplification was 0% (0/14) with Taq and 45% (19/42) with Pfx50. Method C gave positive amplification in 26% (13/50) of the samples with Pfx50 and 71% (10/14) with Expand Long.
According with these results we employed the Boström et al. protocol in combination with the use of Expand Long DNA polymerase for the PCR technique. The later sequenciation gives us information about bacterioplancton community in the sampling moment.

AMB18

ACTIVITY OF SOIL ENZYMES IN GRAZED ECOSYSTEMS OF NORTHEASTERN CHUBUT PROVINCE, ARGENTINA.

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Soil enzymes have an important role in nutrient cycling and organic matter decomposition. We analyzed dehydrogenase, β-glucosidase, protease, alkaline and acid phosphatase activities in the soil, and their relationships with soil organic carbon (C), total nitrogen (N) and pH at three sites across a grazing pressure gradient in northeastern Patagonia. At each site, five surface soil samples were taken from both plant-covered patches (PCP) and inter-canopy areas (IC) randomly selected. A sharp change in dehydrogenase, ,-glucosidase, and alkaline phosphatase activities existed between PCP and IC, with significantly higher values in the former. Strong positive correlations were observed among such variables and between these and soil organic C. At all sites, alkaline phosphatase activity was 2 to 3-times larger than acid phosphatase activity. Moreover, for the latter there were no significant differences among sites. With increasing grazing pressure, C and N decreased significantly in PCP, and protease activity in IC. The C/N ratio increased significantly with grazing pressure in bare interspaces, but remained unchanged in canopy soils. The pH values increased with grazing, in both PCP and IC. This agrees with findings of other studies explaining this increase by animal excrement and urine input, followed by a rapid urea-N hydrolysis. The high dehydrogenase, β-glucosidase, and alkaline phosphatase activities in PCP may be explained by preferential resource accumulation, soil moisture, and milder climatic conditions beneath the plant canopy promoting microbial processes. The low acid phosphatase activity could be attributed to the high soil pH, which may favor microorganisms producing alkaline-active enzymes. The decrease in protease activities observed in soils with increasing grazing, which agrees with results obtained in other semi-arid areas, suggests that N mineralization and N availability may be affected by grazing. This is also supported by a diminution in N. In addition, intense grazing induces changes in vegetation resulting in plant litter with more recalcitrant organic material that could explain the increase in C/N ratio. The variations in the patterns of enzyme activities with grazing, such as those observed in this study, are valuables to assess the effects of different management regimens in semi-arid environments.

AMB19

BIOFILM DEVELOPMENT IN MATERIALS USED IN DRINKING WATER DISTRIBUTION NETWORKS.

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In numerous industrial systems including the drinking water distribution networks, bacterial biofilms constitute a serious problem. Bacterial cell attachment to metallic or polimeric surfaces may result in microbial corrosion resulting in the damage of pipeline. These biofouling activities can be described in terms of the physicochemical properties that are associated with bacterial metabolism and biofilm development.
In this work, we analized the biofilm formation capacity (BFC) of bacteria present on drinking water of La Plata city. We used different materials such as gray fundition, galvanized steel, commercial copper and polyethylene. We submerged each material in non flow drinking water for one month. Bacteria attached to the surface were removed by scraping using 1 ml of sterile water. Aliquots of serial dilutions were spread onto nutrient agar plates and incubated at 30ºC for 4 days. The number of cfu/ml was determined and colonies with different phenotype were selected for further analysis. The bacterial number was higher in gray fundition (3.10 x 10^5 cfu/ml)compared with the other materials (1.31 x 10^4 cfu/ml on galvanized steel and 3.4 x 10^3 cfu/ml and 4 x 10^3 cfu/ml on commercial copper and polyethylene respectively). Different bacterial isolates were selected from each material on the basis of colony morphology, colour and Gram stain. We observed white, yellow smooth, yellow rough, cream and pink colonies on gray fundition, white and pink colonies on galvanized steel, white and yellow smooth colonies on commercial copper and only pink colonies on polyethylene. To analyze the BFC we used a microtiter plate assay. We determined the relation between the cells growing in the attached state, evaluated by staining with crystal violet, to cells growing in the planktonic state ($BFC=Abs._{attached\ cells\text{-}crystal\ violet}/OD._{planktonic\ cells}$).
White and cream colony forming bacteria were Gram positive cocci while pink and yellow colony forming bacteria were Gram negative bacilli. The high biofilm performance with BFC values ≥3 was observed for bacteria isolated from gray fundition (yellow rough colonies) and pink colony forming bacteria from gray fundition, galvanized steel and polyethylene. White colonies forming bacteria showed intermediate performance with BFC values ~1, and the other phenotypes showed low biofilm performance (BFC values ≤1). Then, gray fundition was the preferred material for colonization showing the high bacterial number and bacterial isolates with highest biofilm formation capability. These kind of analysis are important for a better knowledge of the initial stages of the biocorrosion of drinking water network.

AMB20

ISOLATION OF DEGRADING-BACTERIA TOLERANT TO 2,4-DICHLOROPHENOL AND 2,4,6-TRICHOROPHENOL FROM SAN ROQUE RESERVOIR (CÓRDOBA, ARGENTINA)

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Aquatic environments are exposed to mixtures of pollutants affecting ecosystem structure and function. Some pollutants such as chlorophenols are highly toxic to biological communities due to their persistence and bioaccumulation capacity. Moreover, chlorophenols have been declared environmental hazards worldwide. There is still limited insight into the long-term effects of diffuse contamination by chlorophenols in water environments. Bacteria play a key role in ecosystem stability as they are responsible for organic carbon and nutrient recycling. As regards pollution impact assessment, bacterial communities seem to be a promising target group of organisms due to their increased tolerance and adaptation to contaminant exposure. Biodegradation is one of the strategies used for reducing the toxicity of their immediate surroundings and bringing about pollutant transformation. The aims of this study were to select bacteria tolerant to 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP) and to evaluate their degradation ability. Bacteria were isolated from San Roque reservoir sediments. San Roque is a highly eutrophicated reservoir in Córdoba Province (Argentina) receiving urban and agricultural untreated effluents. Sediment samples (1% w/v) were exposed to 50 mg/L of 2,4-DCP and 2,4,6-TCP with agitation (150 rpm) at 20 ºC for up to 5 days. From bacterial counts, we isolated strains and evaluated their tolerance to each of the compounds assessed. Tolerance was defined as the maximum growth observed on the plate at a certain concentration and assessed by a plate serial dilution technique for each compound (National Committee, 1999). Degrading ability was determined in synthetic minimal medium with different concentrations of 2,4-DCP and 2,4,6-TCP. These bacteria-degrading chlorophenols were characterized and identified by conventional biochemical methods and the API system. The isolated strains tolerated up to 200 mg/l of 2,4-DCP and 700 mg/L of 2,4,6-TCP. All the strains were able to degrade 50 mg/L. Tolerant bacteria were gram-negative and non-fermenter. Lower tolerance and biodegradation rates were observed for 2,4-DCP rather than for 2,4,6-TCP, threrefore, 2,4-DCP showed a higher inhibition effect over the bacterial community. These results suggest that tolerance to high pollutant concentrations and the ability to biodegrade the compounds studied might contribute to restore ecosystems and natural conditions.

AMB21

Preliminary studies of phosphate solubilizing peanut rhizobacteria in microcosm assays.

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Peanut (Arachis hypogaea L.) is a widespread leguminous plant of great agricultural and economic significance. Argentina is one of the main peanut producer countries in the world and 94% of its production is concentrated in Córdoba province. Due to intensity of agricultural practices, peanut soils of Cordoba have decreased their availability of phosphorus to plants. The aim of this work was to analyze the effect of phosphate rhizobacteria in peanut growth in microcosm experiments. For this study, a peanut cultivated soil from cropping area of Cordoba with low content of phosphorus was employed. Three native isolates obtained from peanut soils of Cordoba were selected considering their phosphate solubilization ability and other PGPR properties: J49, J157 and S57. The formers produced the highest phosphate solubilizing halo in Frioni`s medium and all of them showed siderophore production. Isolate J157 showed antibiosis against *Sclerotinia minor* and *S. sclerotiorum*, two phytopatogen fungus of high incidence in peanut crop of Córdoba. On the other hand, isolate S57 demonstrated indolacetic acid phytohormone production.
Peanut seedlings were inoculated with a bacterial suspension (10^9 ufc/ml) and sown in sterilized plastic cups filled with sterilized soil with low phosphorus content. Five replicates were performed for each treatment. The treatements were as follows: 1.Non-inoculated plants (negative control) 2. PO4KH2 fertilized treatment: non inoculated seedlings watered regularly with PO4KH2 (0,002M), 3. $(NH_4)_2PO4$ fertilized treatment: pots containing non inoculated seedlings and $(NH_4)_2PO4$ (70 kg/ha), 4.Plants inoculated with *Pseudomonas fluorescens* (“Rizofos”), 5. Peanut seedlings inoculated with isolate J49. 6. Peanut seedlings inoculated with isolate S57, 7. Peanut seedlings inoculated with isolate J157. Plants were grown under controlled environmental conditions, watered regularly with sterilized tap water and, twice a month, with Hoagland´s phosphorus free medium. The plants were harvested at 60 days post-inoculation and plant and root dry weight, aerial plant length, plant and soil phosphorus content were determined.
Results showed that plant dry weight was statistically higher in peanut plants inoculated with isolates J49 and S57 while aerial plant length was higher in pseudomonads and ammonium phosphate plant treatments. No differences were observed between the treatments and negative control in root dry weight and, on the other hand, phosphorus content was highest in potassium phosphate fertilized treatment.
Although field experiments are necessary, results show that peanut soil in the province of Cordoba harbor rhizobacteria with major PGPR properties which represent a potential source of new strains that could be used as biological inoculants in agriculture.

AMB22

GROWTH PROMOTING OF MAIZE BY PHOSPHATE SOLUBILIZING BACTERIA

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The possibility of using soil microbes that promote nutrition and development of plants provides new alternatives for increasing the yield of major agricultural crops and avoid the indiscriminate use of chemical fertilizers. The soils involved in these practices, have high contents of insoluble mineral phosphates, but they are generally deficient in soluble phosphates (Pi), essential for plants and microorganisms development. Plant growth–promoting rhizobacteria (PGPR) are soil free-living bacteria that increase or facilitate, directly or indirectly, the plant growth. They have a range of properties, including P-solubilization, nitrogen fixation, and production of phytohormones, antibiotics and siderophores. The implementation of native PGPR strains as inoculants in region crops, is particularly interesting since they are adapted to soil type and able to compete efficiently with the native microflora. In our laboratory a collection of rhizobacteria from Puna soil (NOA) was isolated. We use phosphate-solubilizing activity, growth in phosphorus limitant concentration and siderophores production as selection criteria. From 45 isolates, 4 microorganisms were selected according to the mentioned criteria. They were identified as *Serratia marcescens* EV1, *Pantoea agglomerans* EV2, *Pantoea agglomerans* EV3 and *Pantoea agglomerans* EV4, by molecular techniques (access number FM202483 to FM202486). The growth-promoter effect was determined by inoculating bacteria on maize plants grown under controlled conditions in glasshouse. Nutrient solution with $Ca_3(PO_4)_2$ as a sole phosphorus source was added periodically. Root and shoot system dry weight was evaluated after 30 days incubation as plant growth measure. The isolated strains increased significantly ($p< 0.05$) dry weight regarding to control. *Pantoea agglomerans* EV4 was selected as those of major capability strain to promote root and shoot development.
In short, results from these experiments suggest that inoculation of PGPR with different beneficial properties, like P-solubilization, should be the future trends of bio-fertilizer application for sustainable crop production.

6. Interacciones Procariota-Eucariota / Procaryote-eucaryote Interaction

INT1

Study of antigens selectively expressed in the infective phenotype of *Bordetella pertussis*

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The low efficacy in preventing colonization of the pertussis vaccines in use have led to disease re-emergence. Re-emergence of pertussis may be explained by several factors: an increase of asymptomatic adult carriers, expansion of strains with certain antigenic variations among others. The iron limitation is a condition that an infecting microorganism face inside the host. By mean of comparative proteomic and bioinformatics we identified antigens that although present in the infective phenotype are absent in current vaccines. Among them we selected two, supposedly exposed on the surface of the bacteria, to study their potential as vaccine components, namely, a putative iron binding protein, and a putative exported protein with remarkably high expression under physiological conditions. The proteins proved antigenic in mice and the induced antibodies recognized the respective native protein on surface of *Bordetella pertussis*, with confirmed overexpression in bacteria adapted to the iron limitation. When tested in vitro both kind of antibodies displayed agglutinin and opsonic activity, the main biological activities against *B. pertussis* colonization. We then checked whether the genes are conserved among circulating strains by screening a panel of clinical isolates by PCR. Importantly, we found them present in all the strains tested. Taken together our results point at these antigens as promising new vaccine candidates.

INT2

Effects of volatile compounds emitted by plant growth promoting rhizobacteria (PGPR) on the biomass of Basil plants (*Ocimum basilicum* L)

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The study of biotic interactions mediated by chemicals is one area of knowledge that most new developments has been presented in recent years. These interactions are variable between species, as well as the effects caused; that are associated with induced or constituent metabolites in response to different stimulus. These compounds, mainly secondary metabolites produce morphological and behavioral changes in the involved organism, whether plant, animal or microorganisms.
There are microorganisms with beneficial effects on the ground that may have considerable potential as biocontrol agents and/or biofertilizers; this specific group of bacteria that promote growth in plants is known as "plant growth promoting rhizobacteria" (PGPR). The application of rhizobacterias has resulted in an increase in the emergency, biomass, developing root systems and increases in crop production of commercial interest. So far, there are not recorded studies on the direct effects of volatile compounds emitted by these bacteria in aromatic and medicinal plants. In this regard, studies were conducted in double partitioned Petri dishes, where in one area were inoculated with the rhizobacteria *Bacillus subtilis* GB03 and in the other were placed sterile basil seeds (*Ocimum basilicum*); in these way, there were no physical contact between bacteria and plant. After 15 days total fresh weight, leaf surface, shoot and root dry weight, stem and root length was registered. Overall, it was noted that plants in contact with the volatile compounds emitted by the PGPR strain doubled the leaf surface regarding control plants. The total fresh weight of both the stem and root showed the same trend, reflecting the effect on the dry weight.
These results suggest that the volatile compounds emitted by rhizobacterias increase production of biomass. The use of these microorganisms as biofertilizers particularly in this kind of crop would bring great benefits, since they have positive effects on plants and do not produce any detrimental ecological impact on the environment or human health.

INT3

Colonyzation of *Lycopersicon esculentum* by *Burkholderia tropica*

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As agricultural production is intensified over the past few decades, producers became more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. However, increasing use of chemical inputs causes several negative effects, i.e., development of pathogen resistance to the applied agents and their nontarget environmental impacts. Furthermore, the growing costs of pesticides, particularly in less-affluent regions of the world, and consumer demand for pesticide-free food has led to a search for substitutes for these products. The use of biofertilizer and biocontrol organisms is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture. These beneficial bacteria are usually referred as PGPB (plant growth-promoting bacteria). These organisms are able to colonize roots and, some of them, are also able to colonize plant tissues (endophytes). Despite their different ecological niches, have been described different mechanism to promote plant growth and control of phytopathogens. The degree of tissue colonization shows the capacity of these bacteria to adapt to that specific environment and to establish a non pathogenic association. Several techniques have been employed for the isolation of endophytic bacteria. The most common procedure are based on surface disinfection of plant tissues and further bacteria enumeration in specific media. These techniques, although able to estimate the colonization degree of plants do not provide information on colonization sites or type of tissue that have been infected. To obtain such an information methods that allow a direct *in situ* identification and localization of endophytes have been developed. In the present study microscopic *in situ* localization of *Burkholderia tropica* (labeled with a gus reporter gene) has been performed in tomato plants (*Lycopersicon esculentum*, cv. *platense italiano*) inoculated with this organism. Our results can be summarized as follows: emerging roots and root hairs were heavily colonized (>10^5 CFU.gram fresh tissue^{-1}); stem tissues were also colonized (~10^4-10^5 CFU.gram fresh tissue^{-1}).

INT4

Protein-protein interactions indentified for effector proteins of the phytopatogen *Xanthomonas axonopodis* pv. *citri*

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Xanthomonas axonopodis pv. *citri* (Xac) cause citrus canker, a serious disease of the citrus genus that results in important losses in citriculture regions. Xac is a bacteria Gram (-) and uses secretion systems for translocation of pathogenicity and avirulence proteins to the plant cells. There are many genes characterized like elicitors of the host plants response and/or of hypersensitive response (HR) in non host plants. In the sequenced genome of Xac several genes have been identified as putative effectors proteins that exert their function in the plant cell, among these are *XAC3090*, *avrXacE1* (*XAC0286*) and *avrXacE2* (*XAC3224*). *XAC3090* presents six domains of leucine rich repeats (LRR), while avrXacE1 and avrXacE2 show similarity with avirulence proteins from other plant pathogens. We isolated *XAC3090*, *avrXacE1* and *avrXacE2* of Xac and constructed mutants in these genes, using a suicide vector transferred by biparental mating. For disease symptoms assays, bacterial suspensions were infiltrated into leaves of host plant orange (*Citrus sinensis*). The results showed that there are not differences in the lesions produced by the infection with Xac-XAC3090 in host plants; while Xac-avrXacE1 and Xac-avrXacE2 showed different phenotypes than Xac wild type. Xac-avrXacE1 produced a darkness lesion than Xac wild type; and inoculation with Xac-avrXacE2 generated a necrotic lesion in citrus leaves. The bacterial growth in citrus leaves observed for mutants were similar to the Xac wild type. Two-hybrid assays in yeasts were carried out to characterize the interaction of XAC3090, AvrXacE1 and AvrXacE2 with possible target sequences in a library of total genomic DNA from Xac. Shortly, *XAC3090*, *avrXacE1* and *avrXacE2* were cloned in the vector pOBD; and a library of total genomic DNA (Xac chromosome plus plasmids) containing fragments of 500 to 3,000 bp was cloned into the vector pOAD. Two-hybrid assays were performed by using simultaneous screening of two reporter genes under the control of different inducible promoters (*GAL1-HIS3* and *GAL2-ADE2*). This simultaneous screening reduced the number of false positives. 192 positive clones were evaluated by sequencing. 38 positive preys for AvrXacE1 were found, among these preys we found the molecular chaperone DnaK (XAC1522) and the avirulence protein Hbss3.0, 40 preys for XAC3090 that showed interaction with a two component system sensor histidine kinase (XAC3643), and 22 preys for AvrXacE2 that revealed interaction with the enzyme cellulase (XAC2522). Among the hypothetical protein there are several preys that present domains involved in protein-protein interaction. These results suggest that these effector proteins could have different roles in pathogenicity.

INT5

Effect of inoculation with two *Azospirillum brasilense* comercial brands in *Festuca arundinacea* Schreb.

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Azospirillum brasilense contributes to increase yields of cereal and forage grasses by improving root development in properly colonized roots. There is lack of information in the temperate Argentinian region that allows to recognize the effectiveness of the inoculation with *Azospirillum brasilense* in forage grass *Festuca arundinacea* Schreb. The aim of this study was to evaluate the effect of two comercial brands and three doses of inoculant with and without nitrogen fertilization on the Azospirillum rhizosphere colonization and dry matter accumulation (DM kg/ha) of Festuca arundinacea. Plots of 5.25 m2, with a density of 600 viables seeds /m2, were sown in Santa Catalina (Bs. As.) on a typical Argiudol soil. The dose-based inoculant was that recommended for wheat (1 1/100 kg seed). The values informed for dry matter accumulation (DM kg/ha) relate to the sampling conducted over the period December 2006-April 2007. During the same period, the rhizosphere colonization was quantifyed by MPN in NFB medium. Treatments combined two comercial brands x three doses of innoculant (1 / 2 dose-basis, dose basis and double dose), with and without nitrogen fertilization(250 kg N/ha). The experimental design was a randomized block with four replicates. The data were analyzed using ANOVA, and comparison of means test ($p > 0.05$). Simple correlation between response variables (rhizosphere colonization and dry matter accumulation) was performed. No statistical significance was detected between control and any of the treatments involving *Azospirillum.* Higher values of DM accumulation were only obtained with nitrogen fertilization. In addition, there was no significant correlation between *Festuca arundinacea* dry matter accumulation and rhizosphere colonization. Experimental evaluations will be continued in order to assess the bacteria behaviour in different seasons.

INT6

XacFhaB, a non-fimbrial adhesin of *Xanthomonas axonopodis* pv. citri is involved in citrus canker disease development.

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Bacterial attachment to host tissues by various adhesins is a first step in the pathogenesis of many animal pathogens, but a role for attachment in plant pathogenesis is less clear. In *Bordetella pertussis*, an animal pathogen, the non-fimbrial adhesin FhaB is secreted in a Sec-dependent manner by the two-partner secretion system, which involves an outer membrane-associated accessory protein (FhaC partner protein). *Xanthomonas axonopodis pv. citri* (Xac), is the casual agent of citrus canker, which affects most commercial citrus cultivars resulting in significant losses worldwide. This phytopathogen encodes FhaB (XacFhaB) and FhaC (XacFhaC) homologs, in this work we analyzed the role of these proteins in the pathogenicity process. We observed an increase of XacFhaB mRNA levels in a medium that mimics the intercellular space of leaves and also in infected leaves, suggesting a role for XacFhaB in pathogenicity. To evaluate the participation of XacFhaB and XacFhaC in this process, we constructed deletion mutants of these genes (ΔXacFhaB and ΔXacFhaC). These mutants and Xac wild type (WT) were infiltrated in citrus leaves and we observed that at low bacterial concentrations despite infiltrated areas were similar, canker lesions produced by ΔXacFhaB mutant were smaller and appeared in a more dispersed fashion than typical lesion observed with WT bacteria. The bacterial number of ΔXacFhaB mutant recovered from these infected leaves was significantly fewer than that of Xac WT. The lesions and the bacterial growth in citrus leaves observed for ΔXacFhaC mutant were similar to the Xac WT. The virulence of ΔXacFhaB and ΔXacFhaC was also determined spraying it on leaves surface, a method that resembles the natural infection. In this case, we observed that ΔXacFhaB produced less number of cankers than Xac WT and the epiphytic fitness of ΔXacFhaB was also affected. The number of cankers and epiphytic fitness of ΔXacFhaC mutant was also impaired but in a less extension than for ΔXacFhaB mutant. Collectively these findings demonstrate that XacFhaB has an important role in X. axonopodis pv. citri pathogenicity and that XacFhaB protein could be partially transported by XacFhaC protein, nevertheless XacFhaC is not the principal secretion partner of XacFhaB. Having establish that XacFhaB is necessary for X. axonopodis pv. citri virulence in citrus leaves, we next analyzed at which of the different stages of the pathogenic process XacFhaB is involved and observed that this protein is necessary for bacterial adherence to leaf surface and for cell to cell aggregation involved in biofilm formation. In addition, we observed by swarm plate analysis that ΔXacFhaB mutant has a higher cell mobility which may explain the canker dispersed phenotype produced by this strain. This work demonstrated that non-fimbrial adhesins have an important role in plant pathogen adherence and pathogenicity.

INT7

Occupation of peanut root nodules by opportunistic Gammaproteobacteria

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Peanut (*Arachis hypogaea* L.) is a widespread leguminous plant of great agricultural and economic significance that can be used as food crop or as source of several food products. Argentina is one of the main peanut producer countries in the world and 94% of its production is concentrated in Córdoba province. Bacteria that nodulate peanut in natural environments from all over the world have been classified as *Bradyrhizobium* (*Arachis*) sp., but species names have not been defined yet. To assess the diversity of bacteria associated to *A. hypogaea* L. nodules in the peanut producing area of the province of Córdoba, we have characterized a collection of isolates obtained from surface-sterilized root nodules. Besides to the slow-growing *Bradyrhizobium* spp., fast-growing bacteria were also recovered from the nodules. The 16S rDNA sequences of seven fast-growing strains were obtained and the phylogenetic analysis revealed that these isolates belong to the Phylum Proteobacteria, Subclass Gammaproteobacteria, and include *Pseudomonas* spp., *Enterobacter* spp. and *Klebsiella* spp. These strains were unable to induce nodule formation in *Arachis hypogaea* L. plants, but enhanced plant yield upon inoculation. Nodulation *nodC* gene could neither be amplified by PCR nor detected by Southern blotting in any of the isolates. Analysis of nitrogen fixation genes showed that *nifH* gene was detected in the *Klebsiella*-like isolates. Furthermore, these strains were able to grow in a nitrogen-free culture medium. These results suggest that the increase in the shoot dry weight of plants inoculated with these strains could be related with their ability to fix nitrogen inside the plant tissues.
Simultaneous and delayed co-inoculation of the Gammaproteobacteria strains and *Bradyrhizobium* sp. SEMIA 6144 conducted in peanut plants revealed that the fast-growing strains were able to colonize nodules even after their formation. The results obtained in this work are supporting the idea that the Gammaproteobacteria recovered from peanut nodules are best described as opportunistic bacteria and not genuine peanut symbionts, being necessary the cooperation of host-endophyte-rhizobia for nodule occupation.

This work was supported by CONICET, ANPCyT, SECyT.

INT8

Cholesterol rich domains are involved in filamentous hemagglutinin mediated-attachment of *Bordetella pertussis* to epithelial cells.

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B. pertussis is the etiologic agent of whooping cough, a reemerging infectious disease. Adhesion to respiratory epithelial cells is a central phenomenon in the pathogenesis of *B. pertussis*. Previous work of our group have showed that cholesterol rich domains in the plasma membrane known as lipid rafts are involved in *B. pertussis* interaction with host cells. However B. pertussis adhesins implicated in this interaction has remained elusive. We here investigated whether filamentous hemagglutinin (FHA), the major adhesin of this pathogen, is involved in this cholesterol dependent interaction. Human respiratory epithelial cells (A549) treated with and without cyclodextrin, a cholesterol sequestring drug, were incubated with purified *B. pertussis* FHA. Cell associated FHA was assessed both by fluorescence microscopy and flow cytometry. The results showed that the depletion of cholesterol led to a significant decrease in the amount of cell associated FHA. Using a cold detergent extraction method we furthermore demonstrated that FHA is isolated in association with detergent resistant membrane fractions -DRMs- (so called lipid raft) of A549 cell unless DRMs were isolated in the presence of saponin, a lipid raft disrupting drug, in which case FHA lossess its capacity to associate with these domains. Our results seems to indicate that *B. pertussis* interaction with cholesterol rich domains might centrally depends on FHA although we cannot exclude the possibility that other adhesins were also involved.

INT9

Role of bacteria cell surface structures in Xanthomonas axonopodis pv. citri biofilm formation

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A biofilm is a community of bacteria embedded in an extracellular matrix that can be attached to a surface. This structure is believed to provide protection to bacteria from environmental aggressiveness. Previous works in other microorganisms have shown that external bacterial proteins are implicated in the first steps of biofilm formation. Xanthomonas axonopodis pv. citri (Xac) is the causative agent of citrus canker. Previous work from our laboratory demonstrated that this bacterium is able to produce biofilm both in vitro and in vivo. To evaluate the participation of different external protein structures in the adhesion step we generated three different mutants: Xac fliC (flagelin gene), Xac flgE (hook gene) mutants, both involved in flagella structure, and Xac fhaB encoding a non fimbrial adhesin. In quantitative violet crystal experiments these mutants showed differences in adhesion compared to Xac wild type. We also used confocal laser scanning microscopy to analyze biofilm formation for these mutants. Differences were observed in the biofilm architecture between wild type and mutant strain. Swimming and swarming motility experiments also showed a marked alteration. In addition, we found differential behavior and symptoms development in host plant infection experiments.

INT10 - ORAL

Transcriptional regulation of RND drug efflux pumps in *Brucella suis*

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The resistance nodulation cell division-type (RND) efflux pumps are responsible for the multi-drug resistance phenotype observed in many clinically relevant species. Besides, RND pumps were implicated in physiological processes with a role in the virulence mechanisms of several pathogenic bacteria. Our previous results have shown the functionality of two RND pumps named BepDE and BepFG, involved in the resistance to structurally unrelated compounds in the intracellular pathogen *Brucella suis.* In other bacterial species the RND efflux pumps has been demonstrated to be transcriptionally regulated by proteins from the TetR family. The context of *bepDE* showed the presence of a gene encoding a putative repressor from the TetR family named BepR, while the regions around *bepFG* did not suggest the presence of any putative regulator. The aim of the present work was to determine the *bepDE* and *bepFG* promoter activities both in vitro and in the intracellular environment and analyse the function of BepR in the transcriptional regulation of bepDE. We constructed transcriptional fusions of PbepDE and P_{bepFG} predicted promoters to the GFP-promoter-less plasmid pKGFP. The fluorescence activity was evaluated in rich (TS, triptic soy broth) and modified minimal medium E (MME) using a multi-plate reader (Berthold Technologies). Although a significant level of $P_{bepDE\text{-}GFP}$ expression was observed in both media, the presence of stechiometric dose of bepR strongly repressed $P_{bepDE\text{-}GFP}$ expression, suggesting that BepR is a transcriptional repressor of *bepDE.* Interestingly, the presence of sodium deoxicholate (DOC), which is a substrate of both pumps released the repression mediated by BepR. We also evaluated the expression of BepR promoter (P_{bepR}) using the same reporter system. A significant increase in reporter expression from P_{bepR} when DOC was added to the culture media was also observed. Although bepFG expression from $P_{bepFG\text{-}GFP}$ showed undetectable level of expression in TS or MME media, P_{bepFG} activity showed 5-fold induction in a BepDE-defective mutant. HeLa cells infected with *B. suis* harbouring the $P_{bepDE\text{-}GFP}$ (and the same number of bepR copies), or the $P_{bepFG\text{-}GFP}$ construction showed reporter activity at early stages of infection (5 h) that sustained for 48 h. Similarly, P_{bepR} was clearly expressed in HeLa cells. Taken together, these results suggest that BepR acts as a local repressor of *bepDE* and *bepR* itself which can be released by the presence of DOC. Moreover a regulatory interplay between *bepDE* and *bepFG* was observed suggesting that a global regulatory system fine tunes the expression of efflux systems in *Brucella suis.*

INT11

CHARACTERIZATION OF MUTANTS IN Xanthomonas axonopodis pv. citri LIPOPOLYSACCHARIDE BIOSYNTHESIS

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Plants rely on innate immunity to defend against most potential pathogens. A broad range of pathogens is recognized by plants through so-called pathogen-associated molecular patterns (PAMPs), which are highly conserved fragments of pathogenic molecules usually playing pivotal roles in microorganisms. Lipopolysaccharide (LPS) is an essential outer membrane component of gram-negative bacteria. In plant pathogenic bacteria, LPS is an important virulence factor. LPS is also being increasingly recognized as a major PAMP for plants. The genes *wzt* and *rfb303*, present in *Xanthomonas axonopodis* pv. citri (Xac) genome, code for an ATP-binding component of the O antigen ABC transporter and a core glycosiltransferase, respectively. Xac mutant strains defective in *wzt* and *rfb303* genes were constructed.
The aim of this study is to determine the role of *Xanthomonas axonopodis* pv. citri LPS in citrus canker. The motilities of Xac wild-type, Xac-*wzt* and Xac-*rfb303* have been analyzed. Samples of over night cultures were stabbed in swimmer agar (0,3 % w/v) and swarmer agar (0,7 % w/v) plates. Xac-*wzt* showed loss of motility in these conditions compared with Xac wild-type and Xac-*rfb303*. In this context a western blot assay with antibodies anti-flagellin of *Serratia marcescens* was made. Xac-*wzt* expresses lower levels of flagellin than Xac wild-type and Xac-*rfb303.* No differences were observed in the adhesion capacity of the three bacteria strains to orange leaves and to a plastic surface. Protease secretion was determined on milk agar plates and no differences were observed between Xac wild-type and the mutants. The degree of host plants (*Citrus sinensis*) cell membrane injury induced by biotic stress was estimated through measurements of ion leakage from the cells into an aqueous medium. Xac-*wzt* exhibited the lowest values of injury index compared with Xac wild-type and Xac-*rfb303*. This was consistent with the phenotype of infected orange leaves. On the other side, infection of non-host plants of cotton with wild-type and mutant bacteria produced the typical hypersensitive reponse (HR). Trypan blue was used to selectively stain dead cells. Cotton leaves were stained with DAB to detect H_2O_2 accumulation throughout the inoculation site.
The Xac-*wzt* mutant was less virulent in orange plants and produced lower values of injury index than Xac wild-type and Xac-*rfb303* suggesting that Xac LPS is involved in pathogenicity during citrus canker disease. On the other hand, Xac wild-type, Xac-*wzt* and Xac-*rfb303* produced the HR in non-host plants of cotton.

INT12

Effect of biofertilization with *Azospirillum brasilense* on *Lolium multiflorum L* (italian ryegrass) pasture establishment

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Lolium multiflorum L (italian ryegrass) is the major cool anual forage widely distributed in temperate ecosystems of Argentina. To express its high forage production needs environments with good fertility. It was stated that *Azospirillum brasilense* acts as facilitator of the absorption of nitrogen in soils. The objective of this study was to determine if the biofertilization with *Azospirillum brasilense* can reduce by half the optimum amount of nitrogen fertilizer applied without producing significant differences on italian ryegrass establishment regarding the optimal nitrogen fertilization. This study was conducted at the FCA-UNLZ field on an Argiudol soil, and was sown on March 2008. Two cultivars representing the annual biotype, one diploid and other tetraploid, were used. The treatments were (1) complete fertilization (200 kg N / ha) (FC), (2) half fertilization (100 kg N / ha) (FM), (3) biofertilization with *Azospirillum* (100l/kg seed) (Az), (4) complete fertilization (FC-Az) + *Azospirillum*, (5) half fertilization + *Azospirillum* (FM - Az). Plots unfertilised and non inoculated were used as control. The response variables considered were: seedlings / m2, tillers/m2and establishment efficiency. The measurements were made from 28/3/08 to 19/5/08. The experimental design was a randomized block with four replicates. The data were analyzed using ANOVA and comparison of means tests ($p > 0.05$). At the end of the trial, the higher values for seedlings/m2 were acchieved by FC and Az-FM, for both cultivars. No differences were detected between treatments on tillers/m2
at the end of the trial. The highest establishment efficiency was obtained with FC-Az for the tetraploid cultivar. Regarding the conditions applied in this preliminary trial it may be concluded that the biofertilization with *Azospirillum* shows itself as an attractive technology in enhancing ryegrass establishment with lower nitrogen fertilization input.

INT13

Identification of novel adhesion molecules in *Brucella suis*

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Shotgun phage display cloning is a useful tool for studying interactions between bacteria and host proteins. Theoretically, these libraries consist of phages that together display all protein domains encoded by the bacterial genome. From such a library, polypeptides with affinity for another molecule can be isolated by affinity selection (panning). This procedure can lead to the selection of clones with a true binding ability. We are interested in discovering the factors that promote adhesion and invasion of the host tissues in the intracellular pathogen *Brucella suis.* The ability of *Brucella* to invade and survive within cells is the cornerstone of its virulence, yet the mechanisms underlying both processes are not clear.

It has been shown that Brucellae are able to adhere to cultured cells and that extracellular matrix components are involved in the interaction of *Brucella* with erythrocytes, macrophages, and epithelial cells. To identify bacterial adhesins, invasins, receptins and their minimal binding domains, a *B. suis* phage display library was constructed by cloning shotgun digested genomic DNA into the pG8SAET phagemid vector. The phages were panned several times against immobilized ligands. Fibronectin (an extracellular matrix protein), fetuin (a sialic-rich protein) and hyaluronic acid (a carbohydrate polymer that is a major component of the extracellular matrix in connective, epithelial, and neural tissues) were used as ligands in different panning experiments. After enrichment, the sequences of the inserted foreign DNA were obtained for ninety two positive clones, and five candidates were selected. The criteria chosen for selection of these candidates was: possesion of “overlapping inserts” (clones containing different inserts derived from the same gene), isolation resulted from different elution conditions (competence with free ligand, pH) and been originated after three rounds of panning.

The presence of the fusion proteins was corroborated by screening for expression of the E-tag, and specificity of the binding was determined for each individual clone. The possible role as adhesins of the candidates was further analized.

INT14

Nodulation kinetics of an *Ensifer* (*Sinorhizobium*) *freedi Tn5* - mutant (M25) altered in its survival ability

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Survival is an important character of organisms in terms of their ability to cope with the environment they encounter in the soil. However it is also an important biotechnological aspect considering inoculants and their shelf life, which is mainly related with the viable number of organisms, a key aspect of inoculation success. Bacterial survival depends among other things upon nutrient availability, the presence of competitive organisms, etc. The purpose of this work was to evaluate and compare the nodulation kinetics and nitrogen fixation ability of a *Tn5* mutant of HH103-1 (M25) compared to the wild type. Nodulation kinetics was assessed by growing soybean seedlings in growth pouches inoculated with increasing concentration of bacteria $1*10^4$, $1*10^5$, $1*10^6$, $1*10^7$, $1*10^8$, $1*10^9$. Nitrogen fixation was assessed in plants cultivated in modified Leonard jars along a 40 day period under controlled conditions. To estimate Nitrogen fixation, nodule number and dry weight, plant dry weight and nitrogenase activity was measured. We concluded that even though the inactivated gene in M25 is associated with the bacterial survival ability under conditions of starvation, the mutant efficiency as well as the nitrogen fixation ability, was similar to that of the wild type, even though the inactivated gene has been found to be expressed in the bacteroids of nitrogen fixing nodules.

INT15

Characteristics of a new Argentinean strain of Xanthomonas axonopodis pv. citri isolated from Citrus limon

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Citrus canker disease is caused by five groups of Xanthomonas strains that are distinguished primarily by host range, three are Xanthomonas axonopodis pv. citri (Xac A, A*, Aw) and two form a phylogenetically distinct clade and originated in South America, Xanthomonas axonopodis pv. aurantifolii (Xaa B y C). All strains of Xanthomonas cause hyperplastic cankers on citrus that are diagnostic of the disease, but host range varies among strain groups. It has been demonstrated, that pthA gene and its homologs encode the primary causal effectors of the citrus canker disease phenotype. Until now, has not been found citrus cultivars resistant to Xac nor have been isolated natural strains of Xac not pathogenic. Previously, we have characterized a variety of Xanthomonas isolated from citrus plants from Northwest Argentine with evidence of the disease. From these studies, we have molecularly identified an atypical Xac strain, which surprisingly induce a hypersensitive response (HR) on lemon leaves. The purpose of this study was to make a deeper characterization of this isolated through specific genomic fingerprints generated by rep- PCR method. These results confirm that this strain corresponds to X. axonopodis pv citri. rep-PCR with ERIC primers showed that this isolated can be identified from the other Xac based on the absence of a 1.6-kb fragment. This fragment was cloned and sequenced. Simultaneously, pathogenicity tests were conducted in other varieties of citrus, resulting grapefruit, tangerine and Mexican lime tolerant to this bacteria. Molecular techniques were used to determine the likely number of pthA homologs carried on this no pathogenic bacterium. The significance of this work is not only because a new Xac isolates causing HR on lemon plants were characterized, but also because allow progress in studying the genes that regulate the defense response to Xac infection.

7. Biotecnología / Biotechnology

TEC1

TOWARDS THE DEVELOPMENT OF FERRICYANIDE-MEDIATED ELECTROCHEMICAL RAPID-BOD BIOSENSORS

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Organic pollution in wastewaters is commonly determined through a standard method called 5-days biochemical oxygen demand assay (BOD_5) that correlates biodegradable organic matter in samples with dissolved oxygen consumed by microorganisms, after 5 days incubation. But O_2 low solubility in water quickly becomes the rate-limiting reagent in catabolism of organic matter, making difficult an active intervention for environmental monitoring and/or process control.
The use of a ferricyanide-mediated rapid BOD approach to overcome the oxygen limitation problems has been reported. O_2 was replaced by ferricyanide ion (with higher solubility) as alternative electron acceptor in the biochemical reaction, allowing the use of increased bacterial concentration and greatly decreased incubation times required to microbially oxidize significant amounts of organic substrate.
In this work we present a strain, called BO365, metabolism study, using ferricyanide respirometry and electrochemical techniques.
It has been found that the strain reduces ferricyanide, utilizing it as an electron acceptor, to ferrocyanide; when a + 500 mV potential is applied, the ferrocyanide is reoxidized to ferricyanide producing a current that would be proportional to the amount of carbon sources consumed by the strain.
Significant currents have been detected in short incubation times, beginning from one hour incubation, supplying to the samples carbon sources as glucose, sucrose, lactose, levulose, succinic acid, L- glutamic and a standard solution, GGA, containing 150 mg glucose/l + 150 mg glutamic/l; this standard solution is employed in the BOD_5 test as a good proceedings and inocule quality control.
In order to standardize the incubations times, we looked for an adequate compound. Usually for this purpose azide sodium is employed, but this product is toxic and expensive. We have found that iodine povidone (PERVINOX®), from common use and not toxic, inhibits the ferricyanide reduction up to one hour after its addition; this has not been observed employing azide sodium at a final concentration of 0.2%.
One of the advantages of the use of microbial sensors, opposite to other biosensors as the enzymatic ones, with major commercial success, is the low specificity. This advantage is important in the determination of BOD and toxicity.
In this work we also tested the currents decline when incubating the strain with a toxic compound, potassium dichromate, a strong oxidizing and carcinogen agent.
Our ultimate goal is the design of a microbial sensor that enables a rapid BOD and/or toxicity determination in wastewaters, treatment waters and water natural sources samples.

TEC2

Whey as a potential low-cost growth medium for *Lactococcus lactis* fermentations

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Bacteriocins are proteinaceus antibacterial compounds that can be produced by lactic acid bacteria commonly present in foods. They are degraded by proteolytic enzymes of the gastrointestinal tract and seem to be non-toxic and non-antigenic to animals. Thus, they can be used to enhance the safety and shelf life of many foods.
One of the most industrially relevant bacteriocins is nisin, which is produced by some strains of *Lactococcus lactis*. Nisin is active against some Gram-positive bacteria, including pathogenic and food spoilage microorganisms such as *Staphylococcus aureus* and *Listeria monocytogenes*. Nisin is a safe and effective food preservative, and it has been used for decades in many countries. Use of nisin was approved in 1988 by the US Food and Drug Administration (FDA) for pasteurized cheese spreads. Subsequently, FDA approved nisin use in other foods. Uses of nisin to control spoilage LAB have been identified in beer, wine, alcohol production and low-pH foods such as salad dressing.
The production of bacteriocins is normally performed in complex growth media: de Man Rogosa and Sharpe (MRS), All Purpose with Tween (APT), Elliker, Brain Heart Infusion (BHI), Tryptone Glucose Extract (THE), Trypticase Soy Broth (TSB) and Trypticase Soy Broth Yeast Extract (TSBYE). Although these media promote growth and relatively high bacteriocin levels, their cost make them insuitable for a large-scale production.
The sweet whey is the waste of the cheese production and contains high lactose and salts concentrations and with a low protein content. Approximately, 9 Kg of whey for every kilogram of cheese is obtained and the cost associated with disposing this large volume of whey is substancial. Furthermore, the high chemical oxygen demand of whey make its disposal a pollution problem. Therefore, whey as fermentation feedstock has been of industrial interest for many years.
In the present work, we studied new formulations of growth media whey-based for *Lactococcus lactis* subsp. lactis ATCC 11454 fermentation. Growth rate, nisin production and lactose consumption using different formulations were tested. Batch cultures pH-controlled were grown in raw and deproteinezed sweet whey, with and without supplementation of an extract of soy protein. Aditionally, we tested the effect of enzymatic digestion of the whey.
Soy protein supplementation of whey in a ratio of 1:2 increases the growth and production of the nisin producing strain. About 90% of lactose in whey was utilized in this fermentation. No difference observed when sweet or deproteinezed whey was used. Also, no effect was detected when whey with and without enzymatic digestion was used.
This study shows the potential of whey as an inexpensive basal medium to produce nisin by Lactococcus lactis, turn on a pollutant in a resource.

TEC3

Characterization of Two Glycosyl Hydrolases from *Shewanella* sp. G5

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β-Glucosidases (EC 3.2.1.21) are a heterogeneous group of enzymes with a broad substrate specificity range over different aryl- and alkyl-β-D-glucosides. A *Shewanella* sp. G5 strain was isolate from the intestinal content of *Munida subrrugosa* in the Beagle Channel, Tierra del Fuego (Argentina); this marine bacteria was able to grow at 4 and 20 ºC using cellobiose as carbon source. The enzyme was quantified and expressed in UI (μmol min^{-1}) from assays that determined the temperature effect at 5, 10, 15, 25, 30, 37 and 45 ºC and pH effect at 3, 4, 5, 6, 7, 8 and 9; using p-Nitrophenyl-β-D-glucopyranoside (pNPG, Sigma). Zymograms assays were performed using a fluorescent substrate 4-methylumbelliferyl-β-D-glucopiranoside (4-MUβG, Sigma). A 100% and 80% of relative activity value were obtained at 37 ºC, pH 8; and 25 ºC, pH 6, respectively. Zymograms assays using 4-MUβG showed the presence of two enzymes, with molecular weight about of 70 and 114 kDa respectively. These two β-glucosidases were characterized molecularly in previous studies by the sequencing of two encoding genes.

TEC4

Effect of vine crop level and yeast inoculation on growth kinetic and biota extension of lactic acid bacteria associated with winemaking. A study in Patagonian red wines.

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During their growth in wine, lactic acid bacteria (LAB) metabolize malic acid to lactic acid. This so-called malolactic fermentation (MLF) is an important step in the production of red wines since it improves wine stability and quality. In winemaking MLF is directly linked to LAB growth and specific activity of the cells and many winemaking variables that affect MLF evolution also affect wine quality. A good understanding of the effect of these factors on the MLF behaviour and quality of wine will emerge only after the kinetics of LAB are quantitatively measured and correlated with chemical analysis of fermenting juice and wine.

In this work, the influences of the vine crop level and the yeast inoculation on LAB growth kinetics and biota extension during winemaking were evaluated in 2007 vintage. Pinot noir grape musts from vines with different crop levels, high (8.000 kg/ha), middle (6,000 kg/ha) or low (5.000 kg/ha), were inoculated with a commercial *Saccharomyces cerevisiae* starter (F15 of Laffort) and fermented at pilot scale (100L). Additionally, a grape juice from high crop level vines was also spontaneously fermented at the same scale. AF progress was followed by °Bmé measuring and MLF by paper chromatography. Musts and wines samples were taken periodically during and after AF, spread in duplicate onto YEPG agar (total yeast), L-Lysine agar (non *Saccharomyces* yeast) and MRS and MRS plus tomato agar (total LAB) and incubated three days under aerobic (yeast) or ten days under anaerobic (LAB) conditions at 25ºC and the viable cell (CFU/mL) counts. Anaerobic gram positive and catalase negative bacilli and cocci were assigned to the LAB. The physicochemical characterization of musts and wines were performed according to OIV methods and their sensorial evaluation by a panel of trained wine testers.

The results showed that in all fermentations the viable population of LAB increased during AF following a typical microbial growth pattern of batch culture until the end of FA, after this a significant and sustained decrease of viable LAB populations was observed in all wines; additionally, the LAB biota extension was always significantly greater in conduced high crop level Pinot noir must fermentation than in the other conduced and spontaneous fermentations reaching maximum populations from average values of 10^5, 10^2 and 10^4cfu/mL, respectively. Bacilli and cocci proportions were also different between wines. In all cases, the fermenting must pH increased 0,4 units throughout FA so, it may be that AF and MLF carried out simultaneously.Mostly ,the wines showed normal enological parameters. Although the final pH was practically the same in all young wines some sensorial properties which could to be related to the BAL biota such as wine flavour were significantly different. In addition, under assay conditions the analized variables influenced on some particular aspects of the LAB biota and qual.

TEC5

Design and optimization of recombinant *Escherichia coli* strains for the synthesis of polyhydroxyalkanoates

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The general objective of this project is the development of processess for the industrial production of polyhydroxybutyrate (PHB) from agroindustrial residues or byproducts using recombinant *E. coli.* This study is centered on the construction of *E. coli* strains for the stable and efficient production of PHB from glycerol by inserting pha genes in the bacterial chromosome and manipulating cellular metabolism to optimize carbon and energy usage in polymer synthesis.

We had previously constructed a recombinant E. coli strain (K24) carrying genes *phaB*, *phaA* and *phaC* from *Azotobacter* sp. FA8 downstream from a T5 phage promoter, under *lac* operator control (pJP24). In previous studies performed with isogenic strains we observed that strains carrying the *ldh* mutation, affecting lactate synthesis, accumulated more PHB. In order to study if this effect was the same in other genetic contexts it was transferred to strain K24 by generalized transduction, giving strain K24L. This strain was found to accumulate more polymer than its parental strain in 3 litre fermentor cultures.

Based on these results *ldh* was chosen as a target for the insertion of *pha* structural genes in the *E. coli* chromosome. Several strategies are currently under way to achieve this. This will allow the insertion of the genes in the chromosome while generating an *ldh* mutation in the same step, that is expected to increase carbon and reducing power flow towards polymer synthesis. The strategies involve three steps. The first contemplates the construction of a plasmid containing the pha genes and an antibiotic resistance cassette flanked by FRT sequences. These sequences are recognized by a yeast recombinase and allow the elimination of the antibiotic resistance gene after the construction is completed. This plasmid is used as a template for the amplification of the pha genes together with the antibiotic resistance gene, using primers containing regions of homology with *ldh.* The amplification fragment is then inserted into the chromosome by means of the red genes from phage lambda. In this last stage, the resistance cassette is eliminated using the yeast recombinase. The final product is a recombinant *E. coli* strain containing the pha genes inserted in the chromosomal ldh gene, and no antibiotic resistance genes.

We expect that this strain will be adequate for the efficient and stable synthesis of PHB from glycerol. This will create new alternatives for the obtention of PHB, a bioplastic, and at the same time will broaden the possibility to obtain a greater added value for the main byproduct of biodiesel synthesis. On the other hand, the use of this strain minimizes the potential environmental and sanitary risks implied by the industrial use of bacterial strains containing transferrable antibiotic resistance genes.

TEC6

Diversity and geographical distribution of indigenous *Saccharomyces cerevisiae* strains from the Comahue winegrowing region (Argentinean North Patagonia).

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One of the most significant technological advances in winemaking has been the control of the microbiological process by grape must inoculation with selected *Saccharomyces cerevisiae* strains. Today, the use of indigenous wine yeasts selected from each winegrowing area is a widespread enological practice. These local yeasts are presumed to be more competitive than commercial yeasts because they are better adapted to the ecological and technological features of their own area. Comahue region is one of the most Southern winegrowing regions in the world. In this region, located in Argentinean Patagonia, spontaneous and conducted grape juice fermentations are carried out to produce mostly dry red wines. The aim of this work was to characterize the indigenous wine *Saccharomyces cerevisiae* diversity within Comahue region in order to develop regional starters. Six cellars located in four different winegrowing areas, Colorado (RC) and Negro (HC) River Upper Valleys, in Rio Negro Province, and El Chañar (Ch) and Añelo (Ñ) in Neuquen province, were selected and 19 spontaneous and conduced red wine fermentations carried out in them during 2002 to 2006 vintages were sampled at different stages. Yeasts were isolated onto YEPD agar plates and 523 isolates assigned to *S. cerevisiae* species using ITS1-5.8S-ITS2 PCR-RFLP method were characterized at the strain level using combined mtDNA-RFLP and killer behaviour against K2 type toxin patterns. Twenty three commercial starters often used in the cellars were also characterized at strain level.

Ninety six different combined patterns, i.e. 96 strains, were discriminated among the isolates analyzed, eight from which corresponded to commercial strains (233 isolates, 45% of the total biomass) evidencing some commercial *S. cerevisiae* strains are capable to remain in the environmental winery over a large period of time. On the other hand, the results reveal a great diversity of enological indigenous strains (88 different patterns among 290 isolates, R=3,29) and a higher variability within the *S. cerevisiae* population associated with CR area (R=2.08) than the ones associated with HC (R=3,53), Ch (R=3,84) and Ñ (R=7,00). Additionally, all spontaneous fermentations were carried out and completed by a few dominating strains (two to three) associated with a variable number of secondary strains. Some of these dominant indigenous strains remained in the environmental cellar over the period of time studied and they were also identified in conduced fermentations. Finally, the evaluation of the relatedness among strains using Principal Coordinates Analysis from combined molecular and killer data allowed the clustering of the indigenous *S. cerevisiae* strains into four populations significantly related to their origin areas. This finding suggests the need to select specific strains from each particular area within Comahue region.

TEC7

Relationship between sensorial, microbial and physicochemical parameters from fermented sausages produced in Argentina.

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Meat industry trends is focalizing in products having high organoleptic standards, specific nutrients to cover special consumer's needs and long storage capacity. Even when local fermented sausage industries have applied starter cultures, the lack of homogeneity on the production, inadequate raw materials as well as the need for quick economical incomes, lead to a lower quality products comparing to European ones. In this sense, quality improvement of Argentinian fermented sausages is a challenge that will allow to successfully competing in the international market. During fermentation, lactic acid bacteria (LAB) as well as Coagulase negative Gram positive Cocci (CGC), as *Staphylococcus* and *Kocuria*, are involved in hygienic and sensorial standards, producing key metabolites related to taste and aroma (peptides, amino acids, alcohols) in the final product. The aim of this work was to carry out a sensory evaluation of local fermented sausages available in the market, to analyze physicochemical and microbial characteristics and to establish relationships between the consumer's attitudes and the analyzed parameters. Ten commercial fermented sausages were subjected to sensorial analysis in two sessions. Two products with the highest (M5, M10) and the lowest (M1, M7) likeness scores were selected for further analyses. When physicochemical results from the first sensorial session were compared, M5 sausage showed higher values for pH, total solids, glucose and free amino acids ($p < 0, 05$) than M1. Fermented sausages from the second session (M10, M7) did not record significant differences with the exception of total solids and soluble proteins. When microbial studies were performed, all analyzed sausages showed to be free of *Staphylococcus aureus*, while *Escherichia coli* and *Listeria monocytogenes* were detected in one sample. The low preferred products showed higher counts of Enterobactericeae as well as enterococci than those with the highest likeness score. All samples showed a satisfactory LAB and CGC counts which were between 10^7 and 10^9 CFU/g. From this study, it could be concluded that, the highest likeness scores were related to a mild acidity, moderate dryness, higher peptides and amino acids content and good hygienic quality. Although, no relationship between determined likeness scores and technological microbial counts could be established, it may be suggested that not only the numbers but, specifically, the strains of LAB and CGC are involved in the sensory quality of fermented sausages. This is the first work from a series of studies on fermented sausages, focused on the characterization of flavor key metabolites arising from bacterial activity which will allow establishing a quality pattern for Argentinian products, not available so far.

TEC8

Oral administration of recombinant *Lactococcus lactis* expressing antigen VP8 rotavirus induce a specific immune response in mice

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Interest in the use of lactic acid bacteria (LAB) as delivery vehicles was focused on the development of mucosal vaccines given that a delivery system is needed to avoid degradation and promote uptake of the antigen in the gastrointestinal tract, and stimulate adaptive immune responses, rather than the tolerogenic immune responses that are seen with soluble antigens. The choice of LAB is based on a number of properties which render them particularly attractive as potential vaccine vehicles. They are considered as GRAS organisms (Generally Recognized As Safe) with a very long record of safe consumption.

Rotavirus is the most cause of severe diarrhea in children younger than five years of age world-wide. The outer layer of the infective particle is made up of VP7 and is decorated with spikes of VP4. To be fully infectious, VP4 must be cleaved by the intestinal trypsin, with the formation of VP5 and VP8. The infection with rotaviruses primarily stimulates an immune response to VP8, therefore this is considered a good candidate for a subunit vaccine. In this context, the use of *L. lactis* for the expression of vp8 would be a valuable technique for vaccination against rotavirus.

As the protective response depends on the location of the antigen, we assessed the potential use of L. lactis strains displaying either cytoplasmic or secreted VP8 protein for immunization trials. For this purpose, we constructed two lactococcal strains, LL(pCYT:vp8) and LL(pSEC:vp8). The production and targeting capacities of such recombinant strains were analyzed by Western blot. Then, four groups of mice (7-9 eight-week-old female Balb/c) were immunized intragastrically, two of them with a bacterial suspension containing $1x10^9$ colony-forming units (CFU) of LL(pCYT:vp8) and LL(pSEC:vp8). The remaining two groups received saline solution (PBS) or *L. lactis* (LL) carrying the empty vector. The specific serum IgG and intestinal IgA induction against VP8 was measured by ELISA. The IgA levels were higher in the group LL(pCYT:vp8) with respect to the group LL (OD; means ± ESM: 2.3 ± 0.10 vs. 1.9 ± 0.06; $p = 0.03$) and PBS group (2.3 ± 0.10 vs. 1.14 ± 0.05; $p = 0.001$), while no response was observed in the group LL(pSEC:vp8). On the other hand, we did not observe a significant increase in the induction of specific serum IgG in the immunized mice in any case ($p \geq 0.05$).

In conclusion, our study allowed us to determine that the strain LL(pCYT:vp8) can be used as delivery vehicles to successfully express VP8 viral protein and to elicit an local immune response (IgA) against the antigen VP8. Therefore this strain will be useful to explore new strategies of vaccination against rotavirus.

TEC9

Inhibition of *Oenococcus oeni* by *Saccharomyces cerevisiae.* Effect of fermentation metabolites.

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In the winemaking process the alcoholic fermentation led by *Saccharomyces cerevisiae*, is occasionally followed by malolactic fermentation (MLF) carried out mainly by *Oenococcus oeni.* The MLF which consists of the enzymatic decarboxylation of L-malic acid into L-lactic acid is required during the vinification of most red wines and certain white wines. Also, this secondary fermentation can improve microbiological stability, taste and flavour of wine. However, the MLF is difficult to accomplish due to the physico-chemical conditions of wine such as a low pH, temperature and nutrient depletion as well as some inhibitory metabolites from yeasts such as ethanol, SO_2, medium chain fatty acids and antibacterial proteins/peptides. We determined by the double-layer plate method that *S. cerevisiae* mc2 wine strain showed a strong inhibitory activity on the growth of *O. oeni* X2L.
The aim of this work was to evaluate the inhibitory effect of *S. cerevisiae* mc2 on *O. oeni* X2L during sequential inoculation in liquid media and to establish the possible correlation between inhibitory activity and the concentrations of ethanol and SO_2 produced by the wine yeast. *S. cerevisiae* was cultured in grape juice medium for 6 days at 25ºC and the supernatant was used as fermented medium for sequential inoculation of *O. oeni*. Unfermented grape juice medium was used as control. To elucidate the possible role of fermentation metabolites in the inhibitory effect on *O. oeni,* ethanol and/or SO_2 were added to the control medium at the concentrations produced in the medium fermented by *S. cerevisiae.* Subsequently, all media were added with MRS components, adjusted at pH 4.5 with citrate-phosphate buffer and sterilized by filtration. The bacterial enumeration was determined by viable plate count. In the medium fermented by *S. cerevisiae*, the growth rate of *O. oeni* decreased 62% and the cell viability declined from 10^8 cfu/ml to 10^5 cfu/ml in regards to control medium. An inhibition of 10, 15 and 24% in the bacterial growth rate was observed by the addition to the unfermented medium of 4% ethanol, 30 mg/l SO_2 or both compounds, respectively. The fermentation compounds did not show a noticeable inhibitory effect on the final cell population of *O. oeni*, only the combined effect of ethanol and SO_2 produced a diminution of half log cycle of viable cells. The results highlight that in the conditions tested neither ethanol nor SO_2 were only factors responsible for the inhibitory activity exerted by *S. cerevisiae* mc2 on the growth of *O. oeni* X2L. Probably a synergistic effect with a proteic factor produced by the yeast could be the responsible of this antagonistic action, since this effect is partially reverted by protease treatment of the yeast fermented medium.

TEC10

Effect of Carnosic Acid on Swimming and Twitching Motilities, Pyocyanin Virulence Factor Production and Antibiotic Susceptibility in *Pseudomonas aeruginosa* PAO1

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Pseudomonas aeruginosa is a Gram-negative, rod-shaped, motile bacterium that contains a single flagellum and type IV pilus, all usually located at the same pole. It is a highly versatile organism that survives in a wide variety of environments, and causes diseases in insects, plants, and animals. In humans, it is an opportunistic pathogen causing a variety of infections in immunocompromised hosts such as patients with cystic fibrosis, burns, cancer, and those requiring extensive stays in intensive care units. Antibiotic resistance of *P. aeruginosa* has been partially attributed to active efflux pumps that remove antimicrobial coumpounds. Furthermore, *P. aeruginosa* is capable of forming biofilms on several surfaces as a survival strategy. Bacterial biofilms are defined as highly structured, surface-attached communities of cells encased within a self-produced extracellular polymeric matrix. *P. aeruginosa* biofilms is a contributing factor in persistent infections because they show a higher degree of resistance to host immune responses and antimicrobial treatments compared with planktonic cells. The initial stages of *P. aeruginosa* biofilm formation require flagellar motility and type IV pili-mediated twitching for surface attachment and microcolony aggregation. *P. aeruginosa* also produces a large number of toxic exoproducts, including proteases, rhamnolipids, and pyocyanin (PCN). PCN is a blue pigment and contributes to the ability of *P. aeruginosa* to persist in the lungs of cystic fibrosis patients and interferes with mammalian cell functions.
The aim of the present study was to evaluate the action of carnosic acid (CA), a natural compound found in plants like *Rosmarinus officinalis* and *Salvia officinalis*, on swimming and twitching motilities and PCN production in the PAO1 strain. The effect of CA alone and in combination with tobramycin on the growth of PAO1 was also evaluated.
We showed that swimming and twitching motilities were strongly affected by sub-MICs of CA. PCN production was inhibited by supplementing the growth medium with different concentration of CA in a dose-dependent manner without affecting bacterial growth rate. The antimicrobial action of tobramycin was increased when CA was added to the culture media, suggesting a synergistic effect. Attenuation of bacterial virulence factor production, enhancement of tobramicyn action and decrease of motility of *P. aeruginosa* by CA may be used to develop a new drug to eradicate *P. aeruginosa* from infections.

TEC11

KINETIC OF XYLITOL FERMENTATION BY *Candida* GROWN UNDER BATCH CULTIVATION

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Xylitol is an economically important product used in the pharmaceutical, chemical and food industries owing to the dietetic and anticariogenic properties.
Traditionally, xylitol has been produced almost exclusively by chemical process, which involves an expensive step of purifications of xylose. In recent years, attention has been focused on the biotechnological process, because it does not require initial xylose purification and is conducted under moderate temperature and atmospheric pressure.
In the present work, we report the kinetic results of xylitol fermentation by Candida grown under batch cultivation. Optimal conditions for xylitol production, including pH value, medium composition and inoculation parameters. *Debaryomyces hansenii* NRRL Y-7426, *Candida parapsilosis* NRRL Y-12969, *Candida guilliermondii* NRRL Y-2075, *Candida boidinii* NRRL Y-2332, *Candida tropicalis* NRRL Y-12968 were used in the fermentation experiments.
The inoculums were prepared from a suspension of cells in shaken flask assay.
Cell concentration was determined by drying washed biomass at 105 ºC to constant weight and number of cells/mL in the Neubauer camera in microscopy and pH evolution. Xylosa and xylitol concentrations were determined by high-performance liquid chromatography (HPLC) using column LiChrosarb-NH2, 5 μm, 200 x 46 mm, detector IR. And xylosa was determined by DNS (reductor sugars) in spectrophometer.
Five strains were used in assays, were determinate fast growth. The high values of dry weight in g/L and cells/ml were valuated in *C. guilliermondii* (20,09; 5,07 x 10^9), *C. tropicalis* (6,27; 1,91 x 10^9) y *C. parapsilosis* (2,85; 2,53 X 10^8) at 40; 20 and 40 hours to the fermentation, respectively. The microscopy observation could see the morphology difference to "*Candida*" with "*Debaryomyces*". The *Candida* was observed bacillary form, when the other, *Debaryomyces*, was esferic. The *D. hansenii* had an small growth biomass production by a maximum values of 1,67 g/L y 7,50 x 10 10 cells/mL at 28 hours. In the pH, were saw that 4 strains to Candida was acid. Finally, the pH were 2,5 - 3,0. For the studies of the different curves of growth were determinate, the specific growth rate (μ),calculated by cero time and where had a most number of micro organisms with values in (h^{-1}): *C. boidinii*, 0,24; *D. hansenii*, 0; *C. parapsilosis*, 0,19; *C. guillermondii*, 0,2 and *C. tropicalis*, 0,27.

TEC12

SELECTION OF BACTERIA CONTAINING 2-DEOXYRIBOSE-5-PHOSPHATE ALDOLASE ACTIVITY

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Aldolases became widely used enzymes due to their ability to catalyze the stereoselective C-C bond formation through a condensation reaction that involves carbonylic compounds, known as aldolic condensation. In particular, these enzymes are useful in the synthesis of carbohydrates since these molecules contain multiple chiral carbon atoms carrying hydroxyl groups of similar reactivity. Among them, 2-deoxyribose-5-phosphate aldolase (DERA) is the only aldolase that accepts two aldehydic substrates. The condensation reaction between D-glyceraldehyde-3-phosphate (G3P) and acetaldehyde catalyzed by DERA generates a chiral carbon with S configuration, producing 2-deoxyribose-5-phosphate (DR5P). As any aldolase, DERA shows high specificity towards the donor substrate, G3P. However, it tolerates other aldehydes as acceptor molecules. Besides acetaldehyde, it has been found that DERA is capable of accepting propanal, acetone and fluoroacetone, enabling the production of substituted 2-deoxyriboses. These compounds are important intermediates in the synthesis of carbohydrates as well as nucleosides. The latter have high comercial interest owing to the fact that they are involved in the preparation of antiviral and antisense drugs and deoxyribonucleotides (dNXPs) for PCR techniques. Both the chemical synthesis of deoxyriboses and the following production of deoxynucleosides (dN) require several and complex steps including those of protection and deprotection. For this reason, the study of simpler, cleaner and economic alternative paths becomes highly interesting.
With the aim of selecting microorganisms containing DERA activity useful to obtain DR5P, our bacteria collection was first screened for finding those ones capable to metabolize 2-deoxyribose as source of carbon and energy. After a secondary screening, microorganisms that tolerated high acetaldehyde concentration were detected. Finally, ten microorganisms belonging to the genera *Bacillus*, *Xanthomona*, *Streptomyces* and *Erwinia* were identified. These bacteria were tested for the DR5P production through the aldolic condensation described above. Among them, four microorganisms generated only 2-deoxyribose but only one produced both 2-deoxyribose and DR5P.
In order to obtain DR5P in a more economical way, glucose was used as substrate to allow the *in situ* generation of G3P. Regarding the necessity of ATP, several cell poration techniques were developed, including not only the use of surfactants like NP-40, Tween-20 and Triton X-100, but also chelant agents as EDTA and solvents like xylene.
Reaction products were analyzed by thin layer chromatography (TLC). 2-deoxyribose and DR5P were quantified by colorimetric methods based on diphenylamine and cysteine chloride reactions as developed by Burton and Stumpf.

TEC13

Genotypic identification of lactic acid bacteria (LAB) from a *Rana catesbeiana* hatchery

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Raniculture has grown substantially due to the increasing demand for frog´s meat and by-products. Farming operations require frogs to be placed in confinement which increases the risk of epizootics, such as red-leg syndrome (RLS). RLS is the main cause of mortality and significant economic losses in raniculture. Currently, the prevention and control of aquaculture diseases have focused on good husbandry practices and the use of vaccines or antibiotics. Treating or feeding frogs with antibiotics may cause the development of resistant bacteria. Thus, the use of probiotics represents an alternative method of prevention. The strains for probiotic use must be exactly identified by different techniques, mainly because aquaculture products are used for human consumption. LAB isolated from the indigenous microbiota of a R. catesbeiana hatchery from Argentina were identified by phenotypic tests and showed some beneficial properties. Therefore, the aim of this work was to apply DNA- and RNA-based methodologies to identify the isolates, namely through a combination of (GTG)5-PCR fingerprinting, Multi Locus Sequence Analysis, and 16S rRNA gene sequencing. Most of the LAB strains in the population were identified as *Pediococcus pentosaceus* and *Lactobacillus plantarum*, although single clusters of *Lb. curvatus*, *Lactococcus lactis*, *Leuconostoc mesenteroides subsp. cremoris*, *E. faecium*, and *E. faecalis* were also found. Moreover, some strains remained unidentified and they could belong to new LAB species. The present work is the first assessment of the genetic identification of this specific group of microorganisms in raniculture. In this field, the use of LAB as biological control agents represents an interesting alternative to avoid the use of antibiotics as it has been reported in fish aquaculture.

TEC14-ORAL

β-lactoglobulin hydrolysis by *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL 656 reduces its allergenicity

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The main whey protein, β-lactoglobulin(BLG), is responsible for producing allergy, especially in children less than three years old. Whey protein concentrates are commonly used to enhance protein content as well as to replace fat in several foods (dairy and bakery products) being a potential risk for patients allergic to milk proteins. Proteolysis is a common method to reduce protein allergenicity. Lactic acid bacteria are well known for their ability to degrade milk proteins. The aim of this work was to evaluate the capacity of *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL 656 to specifically hydrolyze the BLG epitopes (41-60, 102-124, 149-162) thus decreasing its recognition by IgE of allergic patients. β-Lactoglobulin was hydrolyzed using non-proliferating cells incubated at 37°C during 8 h. Bovine BLG degradation was analyzed by Tricine SDS-PAGE and RP-HPLC. Peptides released were identified by LC-MS/MS and the hydrolyzates were assayed for their capacity to inhibit the BLG binding by using a competitive ELISA Test. The results showed that *L. delbrueckii* subsp. *bulgaricus* CRL 656 was able to degrade 32% of BLG after 8 h incubation and to release mainly hydrophilic peptides. Ten peptides with molecular masses between 544.23 and 2541.11 Da were identified by LC-MS/MS. The sequence analysis of these peptides indicated that this strain was able to degrade the main epitopes of BLG. Seventy-one sera of young patients allergic to milk proteins were tested for BLG-IgE recognition. Ten BLG-positive sera were selected for testing BLG binding inhibition using the BLG hydrolyzates by *L. delbrueckii* subsp. *bulgaricus* CRL 656. Eight patient-sera did not recognize the BLG hydrolyzates and the remaining two showed low IgE binding percentages (29.7-45.4%). These promising results indicate that *L. delbrueckii* subsp. *bulgaricus* CRL 656 could be used for developing hypoallergenic dairy products.

TEC15

ABILITY OF *Moraxella bovis* TO FORM BIOFILM ON ABIOTIC SURFACES. PHENOTYPIC CHARACTERIZATION BY FT-IR SPECTROSCOPY

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Moraxella bovis is a causative agent of infectious bovine keratoconjunctivitis (IBK), a disease which produces significant economic losses in cattle industries worldwide. Although *M. bovis* adheres and grows on the epithelial cells of the bovine cornea, these bacteria have traditionally been studied in liquid cultures. Besides, the bacterial biomass included in vaccine formulations is normally produced in stirred bioreactors, where planktonic cells grow at high specific growth rate. It is clear that this culture condition does not represent the mode of life that the bacterium might exhibit in vivo. Biofilm lifestyle is recognised as an important strategy adopted by microorganisms to establish and maintain long term persistent infections on epithelial tissues. Therefore, the aim of this study was to evaluate the capacity of *M. bovis* to grow adhered to surfaces as biofilm, and to compare the phenotypic expression of such sessile cells with their planktonic counterparts.
The biofilms were grown on polypropylene beads in column bioreactors incubated at 37oC in tryptic soy broth (TSB). The biomass was monitored for up to 96 h using crystal violet (CV) staining and viable cell count. The biofilm biomass increased up to 72 h and then started to decline. Phenotypical traits of cells harvested from biofilm, liquid and solid cultures were comparatively analyzed by Fourier transform infrared (FT-IR) spectroscopy and chemical methods. IR spectra showed that macromolecular composition of sessile cells differed significantly from that of planktonic and solid medium cultured cells. Spectral differences were essentially associated to a marked increased of IR bands assigned to carbohydrates functional groups (1200 - 900 cm^{-1} region). Likewise, differential spectral bands corresponding to characteristic biofilm markers (carboxylate group bands at 1376 and near 1400 cm^{-1}, the presence of N-acetyl groups by the bands at 1733, 1260 cm^{-1} and 839 and 800 cm^{-1} bands due to carbohydrates anomeric configuration), were detected in sessile cell spectra, as previously reported for others organisms. Semi-quantitative analysis of spectral data revealed a 4.5 and 7-fold higher carbohydrate/protein ratio for biofilm cells compared to planktonic and solid medium cells, respectively, at the time point of higher carbohydrate production. These results were associated to matrix formation by adhered cells. Chemical analysis supported these results.
In conclusion, we demonstrated the ability of *M. bovis* to grow as biofilm on abiotic surfaces, and differential phenotypic characteristics of sessile cells which were principally associated to the over-expression of extracellular polysaccharides. These are important components for biofilm formation and could play a significant role in the pathogenesis of *M. bovis.*

TEC16

MICROHYDROGELS SUPPORTED ONTO MACROPOROUS (SCAFFOLDS) FOR BACTERIAL IMMOBILIZATION: NEW ALTERNATIVES FOR BIOCATALYSIS PROCESS

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The enzyme biocatalysis provides several advantages over traditional chemical routes, such as regio and stereoselectivity and environmentally clean production process. Moreover, microbial whole cells can be directly used as biocatalysts, what provides a simpler and cheaper methodology since enzyme isolation and purification are avoided. Very few reports have so far dealt with the use of immobilized microorganisms and most of them involved entrapment techniques that use materials with poor mechanical strength and durability (agar, agarose, alginate, chitosane) or toxicity to microorganisms (polyacrylamide, polyurethane). Therefore, the search of alternative techniques such as adsorption may provide new materials for whole cells supports. In many surface modification methods that promote or reduced bacterial interactions, radiation induced graft polymerization (RIGP) has an advantage in that enables the introduction of graft-chain-containing interfaces bearing functional groups into various polymeric backbones, providing the possibility of use materials with better mechanical features. It is well known that bacteria cells have predominantly negative charges on their surfaces at neutral pH, therefore they can be efficiently adsorbed on a polymeric material carrying cationic groups. The aim of this work was the development of polymeric supports with new properties for whole cell inmobilization.
A novel brush-type copolymeric interface was prepared onto macroporous sheets. As a result, a strategy for tailoring new supports for microbial cell immobilization has been explored. The prepared *scaffolds* were examined for their immobilization ability by contacting with cells suspensions in batch mode of a Gram-negative and Gram-positive bacteria strain. A high biocatalityc activity was observed in all the immobilization systems, with values that outperformed previously reports with *Escherichia coli* immobilized in a macroporous support grafted with pGMA modified by EDA. The results reflect the bacterial viability and retention capacity on the new polymeric support, with an increase of the biocatalytic activity, providing a new system for the development of immobilized biocatalyst.

TEC17

Rapid determination of aromatic ring hydroxylating dioxygenases (ARHDs) activity in J26 strain whole-cell

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ARHDs catalyzes the initial oxidation step of such polycyclic aromatic hydrocarbons (PAHs) and are recognized as the major pathway by which these compounds are mineralized in the environment.
Some methods for activity determination of cells expressing these enzymes are based on the UV spectrometric determination of the concentration of the substrate or reaction products, on measuring the oxygen consumption and on the radiometric determination of the non-volatile metabolites of [14C]substrates after their separation by thin-layer chromatography.
Cells containing the ARHDs enzymes are capable of producing indigo when cultured in the presence of indole. The reaction sequence involves cis-dihydroxylation of the heterocyclic ring followed by dehydration to yield indoxyl, which autooxidizes to indigo, a blue color measured spectrophotometrically by absorbance at 600 nm.
Recently, a colorimetric method was used by Hae-jin Woo et al, where cells are washed with phosphate buffer solution pH 7.2 and reaction was initiated by addition of indole in N,N-dimethlylformamide. However, they quantified indoxyl by fluorescence at 470 nm instead of indigo formation (600 nm) because of interference by the big amount of suspended biomass used.
J26 strain is a marine PAH-degrading bacterium harboring type-nahAc naphthalene dioxygenase genes. It is capable of grow in naphthalene and phenanthrene as sole carbon and energy sources. It was isolated based on its capability of producing a blue color after 24 hs when indole crystals were placed on the lid of petri dish after growing in minimal seawater medium SWYE.
In this work, we developed a sensitive and rapid technique for measuring naphthalene dioxygenase activity in J26 strain whole-cell.
The initial rate of indigo formation was determined by plotting the increase in indigo absorbance as a function of time when cells in late exponential phase grew (DO_{600nm}= 0.8) in presence of naphthalene were exposed to indole. Assays were performed in a 96-wells microarray multiplate by duplicate. Indigo formation was monitored spectrophotometrically at 600 nm over 3 hours against a control (resuspended cells without indole) and different indole (2.5 and 5 mM), and biomass amounts were tested in order to optimize the response.
The best indigo production was reached when the biggest amount of biomass tested was treated with indole 2.5 mM. Nevertheless, when indole in the same concentration was added to not-concentrated cells we obtained little less sensitive but operationally easier and more reproducible responses.
We conclude that this work offer a fast and sensitive spectrophotometric method for determining ARHD activity by whole-cells based on the biotransformation of indole to indigo.

TEC18

Trans-activation of recombinant haloalkaliphilic protease produced in *E. coli*

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Optimization of heterologous production of enzymes with potential use in Biotechnology -such as proteases- is highly desirable. Enzymes from extremophilic organisms are active in conditions where their mesophilic counterparts are denatured or inactivated, which makes them a very interesting tool for biotechnological processes. The haloalkaliphilic archaeon *Natrialba* magadii produces an extracellular serine protease (Nep) that has been characterized in our laboratory. Nep is closely related to several proteases of the subtilisin family. Enzymes of this family are synthesized as inactive precursors with a signal peptide followed by a propeptide at the N-terminus. Once translocated, the pre-peptide is cleaved by a signal peptidase and then the propeptide is removed autoproteolitically. The *nep* gene was cloned and expressed in two different organisms: the haloneutrophilic archaeon *Haloferax volcanii* and *Escherichia coli*. The recombinant enzyme produced in *Hfx. volcanii* was identified as Nep (*Hv*Nep) by mass spectrometry and had similar biochemical properties as the native protease. However, purification of the recombinant enzyme by bacitracine or nickel affinity chromatography was not successful.
The recombinant protease produced in *E. coli cells* (*Ec*Nep) was identified as the precursor of Nep by mass spectrometry and these cell extracts showed very low activity. Unprocessing of pre-pro-Nep in *E. coli* may be due to the low salt conditions preventing proper folding and thus further processing/translocation of the protease; lack of the components necessary for these processes or a combination of both factors.
In this work we show that adding small amounts of native mature Nep to cell extracts of recombinant *E. coli* expressing pre-pro-Nep-His6 triggers protease activation. This process is dependent on time, temperature and amount of protease added in trans. Moreover, pre-proNep-His6 was purified by nickel affinity chromatography and activated by addition of mature Nep. The affinity purified polypeptides had similar electrophoretic mobility as the protein species identified as pre-pro-Nep in *E. coli* cell extracts. Preliminary results show that trans -activated *Ec*Nep cannot be purified by nickel affinity chromatography suggesting that the His6 tag may be removed by autoproteolysis. This could be a possible explanation for our previous results with *Hv*Nep. Taken together, these results suggest that the processing of Nep is autoproteolytic and that the factors and/or conditions necessary for triggering protease activation are absent in *E. coli* cells. In addition, *trans*-activation of *Ec*Nep opens the possibility of large scale production of active recombinant enzyme for future applications.

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TEC19

Comparative analysis of genetic diversity and killer sensitivity of *Brettanomyces bruxellensis* and *Pichia guilliermondii* from Patagonian grapes and wines

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The yeasts of the genus *Dekkera/Brettanomyces* are known because of their volatile phenols production that lead off flavours wine defects. Recently, it has been demonstrated that the specie *Pichia guilliermondii* is also able to produce these compounds. *P. guilliermondii* is a common yeast of grapes, fermentation musts and surfaces of Patagonian wineries, although any isolation of *B. bruxellensis* had been found until this moment in the same substrates. In this work, *B. bruxellensis* indigenous isolates were detected for the first time in Patagonian disturbed wines. The intraspecific diversity of this specie as well as the diversity among *P. guilliermondii* indigenous isolates kept in the culture collection of the laboratory were also analyzed. Finally, the sensibility of both species to killer toxins produced by reference and indigenous yeasts was evaluated as a first step in the development of an efficient biocontrol strategy against these spoilage yeasts.
The taxonomic identification of the yeasts was carried out by ITS1-5.8S-ITS2 PCR-RFLP and sequence analysis of the D1/D2 26S-ADNr region. The intraspecific characterization was carried out by RAPD-PCR and mtDNARFLP with the endonuclease HinfI. Killer sensitivity was evaluated against 20 indigenous and three reference yeasts belonging to the species *Metschnikowia pulcherrima*, *Pichia anomala* and *Torulaspora delbrueckii* using the grown inhibition method in YPD-MB agar plates.
From a total of 10 different primers tested, only OPA 3 and OPA 10 evidenced polymorphism among indigenous *B. bruxellensis* and *P. guilliermondii* indigenous isolates respectively. Five different patterns were detected among *B. bruxellensis* and six among *P. guilliermondii*. On the other hand, the mtDNA-RFLP analysis evidenced eight different patterns among *B. bruxellensis*, and a common pattern for all the *P. guilliermondii* isolates. The combined use of both RAPD-PCR and mtDNA-RFLP characterization methods allowed us to discriminate a higher number of different profiles (different strains). The intraspecific diversity detected among *B. bruxellensis* isolates was larger than that observed among *P. guilliermondii* isolates, even though the former were obtained from the same cellar and the latter from different cellars and vintages.
A differential sensitivity against the three killer species tested was detected among the spoilage species analyzed; evidenced a high sensitivity against *P. anomala* killer yeasts but the isolates belonging to the *B. bruxellensis* species also showed a high sensitivity against *M. pulcherrima* killer yeasts. Only one isolate belonging to *T. delbrueckii* species was able to kill, in a high percentage, the *B. bruxellensis* indigenous isolates. Some of these indigenous killer isolates could be taken into account as future wine spoilage yeasts biocontrol tools.

TEC20

Comparative analysis of stress response patterns of *Brettanomyces bruxellensis* and *Pichia guilliermondii* from Patagonian grapes and wines.

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Brettanomyces bruxellensis and *Pichia guilliermondii* yeast species are associated with wine spoilage. *B. bruxellensis* contaminations are related to finished wines, while *P.guilliermondii* ones occur during the first stages of fermentation. Consequently, these species are put under different environmental stress conditions that influence appreciably their growth and, consequently, their spoilage potential capacities. Biological interactions as the killer-sensitive ones are also an important factor affecting yeast viability during fermentation process.
In this work, the response patterns of both *B.bruxellensis* and *P.guilliermondii* spoilage yeasts to physical-chemical and biological stress conditions occurring during wine fermentation was analyzed. Yeast isolates bearing different molecular patterns were tested in their tolerance to stress factors, by colony growth of different dilutions on agar plates. Killer sensitivity was evaluated against 20 indigenous yeasts usually detected in wine fermentations by means of the growth inhibition method. A matrix value of 1 or 0 was assigned to denote the presence or absence of colony growth respectively, as well as for the sensitivity or resistance to each killer strain respectively. Relationships among the strains were evaluated by Principal Coordinates Analysis (PCoA).
PCoA (50% variability) obtained for *P.guilliermondii* using stress response patterns grouped the isolates into two clusters. Cluster I isolates exhibited low ethanol and high sugar tolerances, and they became from grapes or unfermented grape juice. Cluster II joined isolates showing high ethanol tolerance and average tolerance to high sugar concentrations; these isolates were mainly obtained from fermenting musts. In general, the strains showing high ethanol tolerance also evidenced a high resistance to killer yeast according to PCoA of killer response patterns (60% variability).
PCoA (51% variability) using *B.bruxellensis* stress response patterns showed three clusters significantly related to the isolates origin. Isolates from fermentation vat 56 (grouped in cluster I), exhibited lower ethanol tolerance than the isolates from vat 46 (cluster II). Cluster III showed only one isolate from vat 56 characterized by its high ethanol tolerance. The same clustering pattern according to vat origin was also evidenced when killer sensitivity patterns were analyzed by PCoA (57% variability).
According to our results, the different wines stored in the vats could have selected different *B.bruxellensis* strains. EP.56.35 could represent the more dangerous isolate because of its high tolerance to ethanol and resistance against indigenous killer yeasts.
We observed both a species-specific and an isolate-specific stress response among studied spoilage yeasts. Stress response patterns could be interesting in the identification of those potentially more dangerous isolates for the wine industry.

TEC21

Effects of prebiotic and pharmaceutical substances on the viability of freeze-dried vaginal lactobacilli during storage

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Lactobacilli are widely applied as probiotic microorganisms to restore the ecological balance of different animal or human tracts, and to produce a physiological effect in the host. They contribute to the reestablishment of the indigenous vaginal microflora preventing the adhesion or the growth of uropathogenic microorganisms, or stimulating the immune system. To design a probiotic product to restore and maintain the indigenous vaginal flora, both functional and technological properties of the potentially probiotic strains must be well determined. Maximum survival of probiotic microorganisms during the industrial processes of biomass production and storage is of vital importance. The objective of the present study was to determine the effects of lyoprotectors on the survival of four potentially probiotic vaginal lactobacilli during the freeze-drying process and their subsequent storage in presence of prebiotic or pharmaceutical substances. *Lactobacillus* spp. CRL 1263, *Lactobacillus* spp. CRL 1251, *Lactobacillus* spp. CRL 1294 and *L. salivarius* CRL 1328 were subcultured under optimal growth conditions at 37°C three times, and the last culture harvested in stationary phase. The cell pellets were washed twice with distilled water, and re-suspended in each one of the following protective medium: 10% whey protein concentrate (WPC) or 6% reconstituted skim milk added with 12% lactose (RSM/lactose). Bacterial suspensions were freeze-dried, the lyophilized powders were combined with inulin, ascorbic acid or asiatic centella extract, the resulting mixtures were placed into gelatin capsules and stored at 7°C. Viable counts of vaginal lactobacilli were determined before and after freeze-drying, and at different times throughout 60 days of storage, by employing the plate dilution method. The degree of survival was expressed in log CFU/g (colony forming units per g of freeze-dried powder). For most of the microorganisms, losses of viability during freeze-drying were not significant in presence of the two lyoprotectors evaluated, except for *L. salivarius* CRL 1328 (2 log down in RSM/lactose). Viability of microorganisms during storage was affected at different extents depending on lyoprotectors, storage time and substances added to the freeze-dried powders. As a general result, maximum survival up to 60 days was observed with WPC and RSM/lactose, supplemented with either inulin or asiatic centella for all the strains. The results of this work indicate that both lyoprotectors tested were effective in protecting the microorganism during the freeze-drying process and subsequent storage. Among the substances added after lyophlization process, only ascorbic acid not favored the survival of the cells during storage in gelatin capsules. These findings help in the design of a pharmaceutical product containing probiotic microorganisms for the prevention of urogenital infections, which is the final objective of our research group.

TEC22 - ORAL

Evidence for lectinic factor involved in self-flocculation induction of *K. apiculata* mc1 by glucose.

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Yeast flocculation is the phenomenon of cellular aggregation when cells adhere, reversibly, to one another to form macroscopic flocs. Cell–cell adhesion of brewing and wine yeasts is often exploited as a convenient and cost-effective way to separate biomass. The lectin-like theory suggests that yeast flocculation is mediated by an aggregating lectinic factor. *Kloeckera apiculata* mc1, a non-*Saccharomyces* yeast isolated from grape berries, expresses a flocculent phenotype mediated by galactose-specific lectins and stabilized by Ca^{2+}. Glucose is an inductor of the flocculation of *K. apiculata* mc1. This strain was strongly flocculent in presence of high glucose concentration (20 g/l and 50 g/l). The purpose of this study was to evaluate the reflocculating capacity of the proteic extract obtained from whole flocculent cells of *K. apiculata* mc1 growing in different glucose concentrations. Yeast cells growing in YMP medium plus 2 g/l, and induced by 20 and 50 g/l glucose, were harvested after 12 h of incubation and was extensively washed with a PBS buffer pH 7.4. The proteic extract was obtained suspending yeast cells at a concentration of 4% (w/v) in the same buffer supplemented with 10 mmol/l EDTA and incubated under slight stirring at 37°C for 5 h. After centrifugation at 3000g for 10 min, the supernatant was dialyzed at 4°C against distilled water for at least 48 h then lyophilized. Reflocculating activity of EDTA extract was assayed by mixing 100 μl twofold serial dilutions of 0.5% crude extract solution in Helm´s buffer and 100 μl1% K. apiculata mc1 deflocculated cell suspension (2x108 cells/ml) in the same buffer. The mixtures were incubated for 2 h at room temperature. A positive reaction resulted in formation of flocs, which was estimated by visual observation and by optical microscopy. The reflocculating titre corresponded to the reciprocal of the highest dilution of the EDTA crude extract giving detectable aggregation of deflocculated yeasts. After EDTA treatment, the *K. apiculata* mc1 cells were no longer flocculent in Helm´s acetate buffer (reflocculating buffer), while the EDTA extract was able to reflocculate *K. apiculata* deflocculated cells. The extraction yield and quantity of protein achieved with the cation chelating agent were higher as the concentration of glucose pulse increases in the culture medium. Also, the reflocculating capacity of *K. apiculata* crude extracts on the deflocculated yeast cells was correlated with the sugar concentrations (from titre 2 for cells obtained from YMP with 2 g/l to 32 and 64 for cells induced with 20 and 50 g/l glucose, respectively). The reflocculating activities were irreversibly abolished when the EDTA extracts were previously heating at 100°C for 10 min. These results indicated that lectinic proteins are directly involved in the flocculation induction of *K. apiculata* mc1 by glucose.

TEC23

APPLICATION OF *in vitro* METHODS FOR SELECTION OF BOVINE ORIGEN *Lactobacillus salivarius* STRAINS AS PROBIOTICS

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The rearing of calves in intensive systems that separates them from their mothers at an early age, hindering the colonization of the indigenous intestinal microbiota and facilitates the activity of pathogenic microorganisms causing economic losses. Probiotics have been defined as “living microorganisms, which upon ingestion in certain numbers, exert health affects beyond inherent basic nutrition”. The inoculation of probiotics during the first days of life of the animal allow intestinal colonization with bacteria that exert a protective role against infections. The selection of probiotics before incorporation in diet requires close scrutiny in the form of *in vitro* as well as *in vivo* tests. The present study was undertaken to check different *in vitro* characteristics of nine *Lactobacillus salivarius* strains of bovine origen. The characteristics studied include aggregation, coaggregation and inhibition tests with *Escherichia coli* and *Salmonella dublin* DSPV 595T. *Lactobacillus* were grown in MRS broth, then were centrifugated and the cells were separated from the supernatant. The cells were resuspended in 1 ml of Ringer 1/4 solution to which was added 0.1 ml of the supernatant for the aggregation test. Pathogen strains were grown in BHI broth and once centrifugated, the cells were resuspended in Ringer 1/4 solution. For the coaggregation study, 0.45 ml de Lactobacillus suspensions were added with 0.45 ml of the pathogen suspensions and 0.1 ml of *Lactobacillus* supernatant. In both studies, the presence of precipitate was indicating the aggregation or coagregación capacity of the *Lactobacillus* strain. The inhibition was assessed by inoculating of pathogenic microorganism in agar BHI. Before the incubation of the plates, holes were made in the agar and the supernatants were placed in them. The inhibition of bacterial growth was verified by the presence of areas without cell growth around the hole. Six strains resulted positive to the aggregation test, of which five coaggregated with both pathogens and the remaining strain aggregated only with *S. dublin.* The inhibition test was positive for all strains evaluated when they were confronted with *S. dublin*, but only five of them inhibited the growth of *E. coli*. The ability to antagonize these pathogens was attributed to production of organic acids and no specific compound caused the inhibitory effect. Only three strains were able to aggregate, coaggregate and inhibit the growth of both pathogens. This study shows the variations between different strains of *L. salivarius*, isolated in the same ecosystem, on some probiotic properties and that is why it is advisable the screening of the best microbial exponents for the development of a probiotic inoculum.

TEC24

EFFECT OF PROTEASES ON ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA AGAINST *Listeria monocytogenes.*

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Microorganisms have developed different strategies along evolution to compete for environmental nutrients. Some of them have elaborated antimicrobial compounds to inhibit the growing of other microorganisms. Peptides with antimicrobial activity produced by lactic acid bacteria (LAB) are used in the food industry because of their inhibitory activity against food spoilage and/or pathogenic microorganisms. These peptides must preserve their activity during food processing and conservation, do not alter the food organoleptic characteristics, and be degraded in the human gastrointestinal tract. Researchers´ attention has been paid to the inhibition of *Listeria monocytogenes*, the causal agent of listeriosis, due to an increase in its frequency of occurring given the strain natural resistance. Our goal was to determine the chemical nature of antimicrobial compounds produced by LAB regional strains. We used three LAB strains isolated from raw goat milk provided by a goat milk products manufacturer located in San Luis, Argentina. The strains were typified as *Lactococcus lactis ssp lactis 1, Lactococcus lactis ssp lactis 2* and *Lactobacillus paracasei ssp paracasei 1,* using API CH 50 commercial kit. The strains were grown in MRS broth during 48 h at 30ºC in anaerobiosis and showed inhibitory activity against *Listeria monocytogenes 74902.* Cells-free supernatants were obtained by centrifugation at 45000 rpm during 20 min at 20ºC, filtered through 0.2 μm pore filters, and treated with 1mg/ml of Pepsin at pH 2, or 1mg/ml of Chymotrypsin at pH 6.5, for 2 h at 37ºC. Antimicrobial activity was measured by the agar-diffusion assay. Briefly, 20 ml of TSA plated in 10cm Petri dishes were inoculated with a *Listeria monocytogenes* suspension corresponding to 0.5 of Mc Farland. Wells of 5 mm diameter were cut and 30 ul of protease-treated supernatant were added to them and allowed to diffuse during 48 h at 30 ºC in aerobiosis. Plates inoculated with the same protease-free supernatants showed 7 and 9 mm diameter *Listeria monocytogenes* growing-inhibition zones and were used as positive controls. No inhibition zone was observed in the plates inoculated either with Pepsin-or Chymotrypsin-treated supernatants from any of the assayed LAB strains. These results would indicate the peptide nature of the antimicrobial substance/s produced by the isolated regional LAB strains. Further studies will be carried out in order to characterize these antimicrobial peptides as bacteriocins, with a potential application as biopreservant in the food industry.

TEC25

Screening of common herbs for antibacterial activity and phenolic compounds contents

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Phenolic compounds are secondary metabolites which are synthesized by plants and play important structural roles in the cell wall, act as a defense against UV light, protect against pathogen ingress and are involved in repair of injury. Many foods and beverages contain high levels of phenolic compounds, which often provide colour, taste, astringency and other sensory characteristics. The most common flavonoids in tea are the flavan-3-ols. Tea phenolic compounds are regarded as the biologically important active compounds of tea and that provide health benefits. Epidemiological studies associate phenolic consumption with lower mortality, especially caused by coronary diseases. The aim of the present study was to investigate the phenolic content and the antibacterial activity of 6 herb infusions prepared in common way in which teas are prepared for human consumption, *Lippia integrifolia (Incayuyo), Mentha piperita L. (Menta), Wendita Calysina (Té de Burro), Peumus boldus (Boldo), Aloysia triphylla L´Herit (Cedrón) and Ilex paraguayensis (yerba mate).* Boiling water (250 ml) was added to 2 g leaf tea in a conical flask and stirred by a magnetic bar on a hot plate at 90°C for 10 min. Then, the solution was filtered. Colorimetric determination of total phenolics was based on the procedure of Singleton and Rossi. The infusions were added to the nutrient broth medium. The medium were inoculated with an overnight culture of *Escherichia coli* ATCC 35218. Bacterial survival was followed by taking samples from the cultures at the end incubation. Samples were diluted with physiological solution and the proper dilutions were plated, incubated and bacterial counts were recorded. Clarified infusions were used as control without phenolic compounds. The total phenolic content ranged from 409.42 to 925.02 mg of gallic acid equivalents (GAE)/ml. The lowest value was found in *Peumus boldus* and the highest in infusions. In control medium, the number of viable cells increased from 2.7 x 107 to 2.0 x 109 cfu/ml. The addition of *Lippia integrifolia (Incayuyo), Mentha piperita L. (Menta), Wendita Calysina (Té de Burro), Peumus boldus (Boldo), Aloysia triphylla L´Herit (Cedrón) and Ilex paraguayensis (yerba mate)* infusions reduced viable counts by 0.64, 0.7, 0.596, 0.392, 0.12 and 0.76 log cycles with respect with their corresponding clarified infusion, respectively. The highest antimicrobial activity was found in the infusion with the higher phenolic compound concentration (*Ilex paraguayensi*s), so we suggested that the antimicrobial effect was related with phenolic compound concentration.

TEC26

DEVELOPMENT AND FIELD EVALUATION OF A LIQUID PEANUT INOCULANT WITH NATIVE RHIZOBIAL ISOLATES FROM THE PRODUCING AREA OF CORDOBA

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A critical process for yield increase of leguminous crops is the Biological Nitrogen Fixation (BNF). Inoculant application with nitrogen fixing bacteria is a practice more economic and environmentally friendly compared to chemical fertilization. The aim of this study was to obtain a high quality and competitive liquid peanut inoculant formulated with native rhizobia. From 220 native isolates obtained from peanut nodules of the producing area of Cordoba, we selected two, named J-81 and J-237, considering their high effectiveness and competitiveness. Analysis of their 16S RNAr gene sequences showed that both isolates belong to *Bradyrhizobium* genera. Optimal bacterial growth medium and stabilizing solution were determined in order to obtain highest bacterial concentration and viability, respectively. Reference strains *Bradyrhizobium* sp. SEMIA 6144 and C-145 were used as control treatments. Assays in microcosm demonstrated higher symbiotic parameters when plants were inoculated with *Bradyrhizobium* sp. J-81 and J-237 than reference strains. For both, the number of viable cell reached in the balanced medium L1 (this work) was 1.10^{10} cfu/ml while in YEM (Vincent, 1970) L2, L3 and L4 (this work) mediums it was 1.10^{9} cfu/ml. Highest viability was reached with the stabilizing solution B obtaining more than $2x10^{8}$ cfu/ml after two (J-237) and six (J-81) month of storage. Two inoculants were then produced and assayed in a field situated in south-southwest of Cordoba province during 2007/08 production season. Field experiment indicated that inoculation with both inoculants obtained in this work increased field yields compared to noninoculated treatment although no significant differences were found respect to commercial inoculants treatments. The results of this study demonstrate that inoculants produced are high quality and competitive products.

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TEC27

Influence of nitrogen availability on fructans production by *Gluconacetobacter diazotrophicus*

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Food industry is showing an interesting development in the production of the so-called "functional food" (food products that confer health benefit). This concept includes prebiotics and probiotics. The former refers to nondigestable food fibers which stimulate the growth of certain bacteria in the intestines. Fructans (polymers of fructose residues), either of vegetable origin (inulin) or produced by different microorganisms, are one of the most reported prebiotic food additives. *Gluconacetobacter diazotrophicus* synthesizes constitutively an extracellular levansucrase (LsdA) which is responsible for the production of fructans in cultures of this organism. For this reason LsdA of *G. diazotrophicus* appears to be a promising bacterial enzyme for use in the commercial production of this prebiotic. In the present work we have studied how variations in the nitrogen source availability (with a constant offer of carbon source) influence LsdA synthesis and fructan production in cultures of this organism. *G. diazotrophicus* PAL5 was grown in a LH fermentor (2.0 liter total volume with 1.7 liter of culture) at 30 ºC and pH controlled at 6.00. Sucrose (20 $g.l^{-1}$) was used as carbon and energy source and nitrogen source was varied as follows: i) 0.132 $g.l^{-1}$ de $(NH_4)_2SO_4$, condition which allows expression of biological nitrogen fixation (BNF); ii) 3.0 $g.l^{-1}$ de$(NH_4)_2SO_4$, standard medium; iii) 6.0 $g.l^{-1}$ de $(NH_4)_2SO_4$, excess of nitrogen source and iv) 6.0 $g.l^{-1}$ de $(NH_4)_2SO_4$, excess of nitrogen source supplemented with aminoacids. Results showed that biomass concentration in cultures was not significantly affected by the availability of nitrogen source but decreased significantly under BNF, likely because of the high energetic demand of N_2 fixation. Levansucrase activity was much higher under BNF conditions wich was accompanied by the higher conversion of sucrose in fructans (17% w/w).

TEC28

EFFECT OF OXYGEN AVAILABILITY ON POLY(3-HYDROXYBUTYRATE) ACCUMULATION FROM GLYCEROL AND GLUCOSE BY RECOMBINANT *Escherichia coli*

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Polyhydroxyalkanoates (PHAs) are thermoplastic biopolyesters accumulated by numerous microorganisms under unfavorable growth conditions. These polymers, that act as energy reserve and electron sink in the cells, have drawn much attention as environmentally-friendly plastics because they are completely biodegradable. Poly(3-hydroxybutyrate) (PHB) is the best characterized PHA. Accumulation of PHB in recombinant *E. coli* has many advantages, including the capability to synthesize it from several carbon sources. In the last years, there has been an important increase in the production of glycerol, a by-product in the synthesis of biodiesel. For this reason, glycerol has become an attractive substrate for bacterial fermentations. PHA granules are accumulated in the cytoplasm, and in natural producers they are surrounded by a protein layer. The most abundant proteins in the granules are small hydrophobic proteins called phasins, which act as a barrier avoiding interactions between PHAs and other cellular components. PHB-biosynthetic genes from *Azotobacter* sp. strain FA8, as well as *phaP*, coding for a phasin, were cloned in our laboratory and expressed in *E. coli* resulting in strain K24KP, which efficiently accumulates PHB from glucose and glycerol.
PHB accumulation from glycerol in *E. coli* is poorly characterized. Considering that glycerol is a highly reduced substrate when compared to glucose, it is possible to assume that oxygen availability would have a different impact on PHB synthesis (a process which consumes NADPH) depending on the carbon source. The aim of this work was to compare the effect of oxygen availability on PHB synthesis from glucose and glycerol by *E. coli* K24KP. Shaken flask experiments were set by varying the medium-to-flask volume ratio and rotational agitation, giving rise to three levels of aerobiosis. Biomass followed a linear correlation with increased oxygen availability, being higher when glycerol was used as carbon source for all conditions. PHB accumulation (expressed as a percentage of biomass) also followed a linear correlation for the three levels of aerobiosis tested, but with opposite trends for each carbon source. Highest PHB accumulation from glucose was 33.3 ± 2.9% while that from glycerol was 32.1 ± 1.9%, and were obtained under maximal and minimal aeration, respectively. Under low oxygen availability conditions cells shift down the production of reducing equivalents. Since glycerol is more reduced than glucose, the optimal amount of reducing equivalents for growth and PHB synthesis from this substrate is probably achieved at lower oxygen concentrations. Because of this, aeration conditions for PHB production from substrates with different oxidation states must be carefully assessed in order to optimize polymer accumulation.