XII CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL

2 al 4 de agosto de 2017

San Miguel de Tucumán | ARGENTINA

SAMIGE

Asociación Civil de Microbiología General

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La Comisión Organizadora Local agradece muy especialmente la colaboración, trabajo ypermanente disposición de Daniela Russo y Diana Vullo.

DISEÑO Y DIGRAMACION DE LIBRO

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PROGRAMA

XII CONGRESO DE MICROBIOLOGÍA GENERAL

Miércoles 2 de Agosto	Jueves 3 de Agosto	Viernes 4 de Agosto
08:30 - 09:00 Recepción y bienvenida		
09:00 - 10:30 Mesa redonda: Biocatálisis Julia Pettinari. UBA-CONICET (B.A.) Rosana De Castro. IIB-UNMdP (M. d.Plata) Javier Breccia, INCITAP- UNLaPam (L.Pampa)	**09:00 - 10:30 Mesa redonda: Patogénesis microbiana** Juan E. Ugalde. IIB-INTECH-UNSAM (B.A.) Mónica Delgado. INSIBIO -UNT (Tuc) Patricio Diosque. UEM-UNSa (Salta)	**09:00 - 10:30 Mesa redonda: Microbiología de Suelo** Sonia Fisher.UNRC (Córdoba) Diego Sauka. INTA Castelar (B.A.) Aníbal Lodeiro. IBBM- UNLP (B.A.)
10:30 - 11:30 Posters y café	**10:30 - 11:30 Posters y café**	**10:30 - 11:30 Posters y café**
11:30 - 12:30 Comunicaciones orales	**11:30 - 12:30 Comunicaciones orales**	**11:30 - 12:30 Conferencia Plenaria** Peter Kämpfer Institute of Applied Microbiology. Justus-Liebig Giessen University, Germany
12:30 - 14: 00 Almuerzo	**12:30 - 14: 00 Almuerzo** **Charla para jóvenes investigadores. ASM**	**12:30 - 14: 00 Almuerzo**
14:00 - 15:00 **Videoconferencia. ASM** Luciano Marraffini The Rockefeller University, USA	**14:00 -15:00 Mesa redonda: Nuevas biotecnologías** Alejandro Nadra. UBA, IQUIBICEN-(B.A.) Leonardo Erijman. INGEBI (B.A.)	**14:00 - 15:30 Comunicaciones orales**
15:00 -16:00 Posters y café	**15:00 - 16:00 Posters y café**	**15:30 - 16:30 Posters y café**
16:00 -17:30 Comunicaciones orales	**16:00 - 17:30 Comunicaciones orales**	**16:30 - 17:30 Conferencia de Clausura. ISME** James M. Tiedje Center for Microbial Ecology Michigan State University, USA
17:30 - 18:30 Conferencia plenaria Thamy Ribeiro Corrêa Universidade Estadual de Campinas, Brasil	**17:30 - 18:30 Conferencia plenaria** Matthew R Parsek U. of Washington, Seattle, USA	
19:00 - 21:00 Vino de honor	**18:30 - 20:00 Asamblea general SAMIGE**	**18:00 Despedida**

PROGRAMA GENERAL

Miércoles 2 de agosto de 2017	
08:30 - 09:00	Recepción y Bienvenida
09:00- 10:30	Mesa redonda: BIOCATÁLISIS Moderador: Osvaldo Yantorno Julia Pettinari, FCEyN, UBA-CONICET, Buenos Aires "The amazing phasin: properties and applications of a multifunctional protein" Rosana De Castro Instituto de Investigaciones Biológicas, IIB-CONICET-UNMdP "Archaeal Proteolysis: Functional characterization and identification of natural targets of regulatory proteases" Javier Breccia, INCITAP, La Pampa "Uncommon glycoside hydrolases: biotransformations in food and bioprocesses"
10:30 -11:30	Café y Posters BIOTECNOLOGÍA Y FERMENTACIONES
11:30-12:30	Comunicaciones orales: BIOTECNOLOGÍA Y FERMENTACIONES/ FISIOLOGÍA MICROBIANA Moderador: Nancy López EFFECT OF NANO-MICROMETRIC TOPOGRAPHIES ON EARLY STEPS OF BIOFILM FORMATION. María A Colonnella 1, Gastón Paris 1, Leonardo Lizárraga 1. 1 Centro de Investigaciones en Bionanociencias (CIBION)-CONICET. METABOLIC ENGINEERING OF A DIAZOTROPHIC BACTERIUM IMPROVES AMMONIUM RELEASE AND BIOFERTILIZATION OF PLANTS AND MICROALGAE. Rafael Ambrosio 1 2, Juan C Ortiz-Márquez 1 2, Leonardo Curatti 1 2. 1 Instituto de Investigaciones en Biodiversidad y Biotecnología Consejo Nacional de Investigaciones. 2 Fundación para Investigaciones Biológicas Aplicadas. THERMOTOLERANCE OF THE IMMUNOBIOTIC Lactobacillus rhamnosus CRL 1505: EFFECTOF INTRACELLULAR POLYPHOSPHATE INCLUSIONS Maria A. Correa Deza 1, Mariana Grillo Puertas 2, Susana Salva 1, Gladys I. Martos 1 4, Viviana A. Rapisarda 2, Carla L. Gerez 1, Graciela M. Font 1 3. 1 Centro de Referencia para Lactobacilos (CERELA CONICET). 2 Instituto Superior de Investigaciones Biológicas (INSIBIO), CONICET-UNT. 3 Facultad de Bioquímica, Química y Farmacia. UNT. 4 Facultad de Medicina, UNT. PARTIAL ANTAGONISM BETWEEN BIOFILM-FORMING Escherichia coli AND Klebsiella pneumoniae UROPATHOGENIC STRAINS ISOLATED FROM A CATHETER-ASSOCIATED BACTERIURIA: DETRIMENTAL EFFECT OVER E. coli. Guillermo E Juárez 1 2, Estela M Galván 1 2. 1 IIBBA (CONICET) - Fundación Instituto Leloir. 2 CEBBAD (CONICET) - Carreras de Farmacia y Bioquímica, Universidad Maimónides.
12:30 - 14:00	Almuerzo
14:00 – 15:00	Videoconferencia ASM: "CRISPR-Cas: the acquired bacterial immune system" Luciano Marraffini - The Rockefeller University, USA. Moderador: Raúl Raya / Diana Vullo
15:00- 16:00	Café y Posters FISIOLOGÍA MICROBIANA

PROGRAMA GENERAL

16:00-16:45	Comunicaciones orales: FISIOLOGIA MICROBIANA/INTERACCIONES PROCARIOTA-EUCARIOTA Moderador: Néstor Cortéz MECHANISMS INVOLVED IN THE ADAPTATION OF ESCHERICHIA COLI O157:H7 TO THE INTESTINAL MICROENVIRONMENT. Romina Fernández-Brando 1, Martin Gómez 1, Andrea Bruballa 1, Gonzalo Pineda 1, María Victoria Ramos 1, Cristina Ibarra 2, Sean McAteer 3, David Gally 3, Marina Palermo 1. 1 Laboratorio de Patogénesis e Inmunología de Procesos Infecciosos, Instituto de Medicina Experimental. 2 Laboratorio de Fisiopatogenia, Facultad de Medicina, UBA. 3 Division of Infection and Immunity, The Roslin Institute, University of Edinburgh. ANTAGONIC EFFECT OF LACTIC ACID BACTERIA ISOLATED FROM MINIMALLY PROCESSED FRUITS AGAINST Salmonella typhimurim. Luciana del valle Rivero 1, Maria José Rodríguez Vaquero 1, Fabiana María Saguir 1. 1 Universidad nacional de Tucumán. BACTERIAL QUORUM SENSING MOLECULES MODIFY THE OXIDATIVE STRESS RESPONSEIN THE ENDOPHYTIC YEAST Meyerozyma guilliermondii 6N. Elisa V Bertini 1, Ana C Leguina 1, Andrea C Barrios 1, Lucía I Castellanos de Figueroa 1 2, Jean-Michel Camadro 3, Carlos G Nieto Peñalver 1 2. 1 Planta Piloto de Procesos Industriales Microbiológicos (PROIMI - CONICET). 2 Inst. de Microbiología, Fac. de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. 3 Institut Jacques Monod (Université Paris Diderot/CNRS, París, Francia).
16:45-17:30	Comunicaciones orales: EDUCACIÓN EN MICROBIOLOGÍA/ BIORREMEDIACIÓN Y BIOCONTROL Moderador: Diana Vullo LEARNING EXPERIENCE WITH VIRTUAL ACTIVITIES FOR TEACHING MICROBIAL BIOTECHNOLOGY. Ivana D Galera 1 2, Edgardo Oberti 1, Marcelo Suárez 1, Mariana Peralta 1 3, Paulina L Páez 3, José L Baronetti 1 2, María G Paraje 1 2. 1 Cátedra de Microbiología, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba. 2 Instituto Multidisciplinario de Biología Vegetal (IMBIV CONICET). 3 Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. CIENCIA (RE) CREATIVA: SCIENCE IS LEARNED BY PLAY. ME Danti 1 2, R Diaz Peña 1, E Petrera 1, R Pozner 3, MM Ricardi 4 5, LJ Raiger Iustman 1 2, EC Solar Venero 1, PM Tribelli 1 2. 1 Dpto. de Química Biológica. FCEyN-UBA. Buenos Aires, Argentina. 2 IQUIBICEN-CONICET-UBA.Buenos Aires, Argentina. 3 Instituto de Medicina Experimental (CONICET-Academia Nacional de Medicina). Buenos Aires, Argentina. 4 IFIBYNE-CONICET, FCEyN, UBA. Buenos Aires, Argentina. 5 Dpto de Fisiología, Biología Molecular y Celular. FCEyN-UBA. Buenos Aires, Argentina. MICROFLUIDIC DEVICES FOR THE ASSESSMENT OF THE PAHs REMOVAL CAPACITY BY BACTERIAL BIOFILMS. Natalia Bourguignon 1 5, Mauricio Alessandrello 2 5, Betiana Lerner 1 3, Maximiliano S Pérez 1 3, Marcela A Ferrero 2 4. 1 Universidad Tecnológica Nacional (UTN), Facultad Regional Haedo, Buenos Aires. 2 PROIMI-CCT Tucumán-CONICET, Tucumán. 3 Universidad de Buenos Aires (UBA), Facultad de Ingeniería, Instituto de Ingeniería Biomédica, Buenos. 4 Universidad Nacional de Tucumán-Facultad de Bioquímica, Química y Farmacia (UNT).
17:30 – 18:30	Conferencia plenaria: Thamy L. Ribeiro Corrêa - Universidade Estadual de Campinas -UNICAMP, Brasil "CAZymes: structure, diversity and novelties aiming the production of 2nd generation ethanol" Moderador: María Alejandra Martínez
19:00-21:00	Vino de honor

Jueves 3 de agosto de 2017	
09:00 - 10:30	Mesa redonda : PATOGÉNESIS MICROBIANA Moderador: Angeles Zorreguieta Juan E. Ugalde Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde", IIB-INTECH-UNSAM "Breaching barriers: the interaction of Brucella with epithelial cells" Mónica Delgado INSIBIO-CONICET-UNT "Strategies for virulence genes regulation in Salmonella Typhimurium: development of attenuated vaccines" Patricio Diosque Unidad de Epidemiología Molecular (UEM), Instituto de Patología Experimental, Universidad Nacional de Salta-CONICET "Intraspecific phylogeny of Trypanosoma cruzi and Chagas disease pathogeny"
10:30 – 11:30	Café y Posters MICROBIOLOGÍA MOLECULAR
11:30 – 12:30	Comunicaciones orales: MICROBIOLOGÍA MOLECULAR Moderador: Eleonora García-Véscovi IDENTIFICATION OF SECRETED CELLULASES AND HEMICELLULASES FROM A NATIVE ISOLATE OF Cellulomonas sp. AND RECOMBINANT EXPRESSION OF A GH10 ENDOXYLANASE. Florencia E Piccinni 1, Ornella M Ontañon 1, Silvina Ghi o1, Paola Talia 1, Eleonora Campos 1. 1 Instituto de Biotecnología, CICV y A., Instituto Nacional de Tecnología Agropecuaria (INTA). Stenotrophomonas maltophilia ISOLATES FROM CYSTIC FIBROSIS PATIENTS IN ARGENTINA: GENOTYPIC AND PHENOTYPIC CHARACTERIZATION Eliana S Alcaraz 1, Agostina Schinero 1, Carlos A García 1, Laura E Friedman 1, José A Di Conza 1 2, Daniela Centrón 3, Beatriz N Passerini de Rossi 1. 1 Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, UBA. 2 Cátedra de Microbiología General, Facultad de Bioquímica y Ciencias Biológicas, UNL. 3 Instituto de Investigaciones en Microbiología y Parasitología Médica, IMPaM, UBA-CONICET. THE ACINETOBACTER BAUMANNII XERC/D SITE-SPECIFIC RECOMBINATION SYSTEM MODULATES PLASTICITY OF PLASMIDS CARRYING GENETIC ELEMENTS CONFERRING BLAOXA-58 -MEDIATED CARBAPENEM RESISTANCE. María M Cameranesi 1, Jorgelina Moran-Barrio 1, Guillermo D Repizo 1, Adriana S Limansky 1, Alejandro M Viale 1. 1 Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET). Dpto. Microbiología, FCByF,UNR. WHERE, WHEN, HOW AND WHY?: STUDY OF THE INTERACTION BETWEEN THE CYTOPLASMIC MEMBRANE AND PLSX, A KEY PHOSPHOLIPID SYNTHESIS ENZYME FROM Bacillus subtilis. Diego E Sastre 1 2, André A Pulschen 1, Luis B Mansor 2, Caterina G Netto 3, Marcos Navarro 2, Diego De Mendoza 4, Frederico Gueiros-Filho 1. 1 Instituto de Química, Depto. Bioquimica, Universidade de São Paulo, Sao Paulo, SP, Brasil. 2 Intituto de Física, Universidade de Sao Paulo, São Carlos, SP, Brasil. 3 Departamento de Química, Universidade Federal de São Carlos, SP, Brasil. 4 Instituto de Bioquímica y Biología Molecular y Celular de Rosario, IBR, Rosario, Argentina.
12:30 – 14:00	Almuerzo – Charla para jóvenes investigadores: "Microbes: Behind the Science". Alejandro Nadra, James Tiedje, Rosana de Castro. Actividad auspiciada por ASM (American Society for Microbiology) Moderador: María Laura Ferreira
14:00-15:00	Mesa redonda: NUEVAS BIOTECNOLOGÍAS Moderador: Marcela Ferrero Leonardo Erijman, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr Héctor N. Torres" (INGEBI-CONICET) "Genome-resolved metagenomics reveals bacterial life history strategies in environmental biotechnology processes" Alejandro Nadra, FCEN, UBA, IQUIBICEN-CONICET "Interdisciplinary designed biosensor for arsenic detection in drinking water"

PROGRAMA GENERAL

15:00 – 16:00	Café y Posters BIORREMEDIACIÓN Y BIOCONTROL
16:00 – 16:45	Charla: Alejandro Nadra, FCEN, UBA, IQUIBICEN-CONICET "TECNOx and the emergence of a technological community in Latin America" Moderador: Marcela Ferrero
16:45 – 17:30	Comunicaciones orales: MICROBIOLOGÍA MOLECULAR Moderador: Liliana Villegas EMPLOY OF ATOMIC FORCE MICROSCOPY FOR THE ANALYSIS OF THE METABOLIC RESPONSE OF BACTERIA TO STRESS CONDITIONS IMPOSED BY ANTIBIOTICS. M I Villalba 1, P Stupar 2, L Arnal 1, N Cattelan 1, G Guillén 3, M E Vela 4, S Kasas 2 5, O Yantorno 1. 1 Centro de Investigación y Desarrollo de Fermentaciones Industriales (CINDEFI-CONICET-CCT La Plata). 2 Laboratoire de Physique de la Matière Vivante, EPFL, Lausanne, Switzerland. 3 Centro de Ingeniería Genética y Biotecnología, La Habana, Cuba. 4 Laboratorio de Nanoscopías y Fisicoquímica de Superficies (INIFTA, CONICET). 5 Plataforme de Morphologie, Université de Lausanne, Lausanne, Switzerland. CATALASES IN Acinetobacter: KATG SIGNAL PEPTIDE LEADS FUNCTIONAL FOLDING ANDPERIPLASMIC LOCALIZATION. Mariana G Sartorio 1, Marcelo A Palavecino 1, Néstor Cortez 1. 1 IBR, Instituto de Biología Molecular y Celular de Rosario (UNR&CONICET). CHARACTERIZATION OF A TYPE I-F CRISPR-CAS SYSTEM FROM THE CLINICAL ISOLATE Shewanella sp. Sh95. Gisela Parmeciano Di Noto 1, Daniela Centrón 1, Cecilia Quiroga 1. 1 Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM, UBA-CONICET), BA.
17:30 – 18:30	Conferencia Plenaria: Matthew R Parsek University of Washington, Seattle, School of Medicine USA "Pseudomonas aeruginosa hedges its bets on a surface" Moderador: Eleonora García-Véscovi
18:30 – 20:00	Asamblea general SAMIGE
Viernes 4 de agosto de 2017	
09:00 - 10:30	Mesa redonda: Microbiología de Suelo-DIMAYA (AAM) Moderador: Cecilia Quiroga Sonia Fisher, Universidad Nacional de Rio Cuarto, FCEFQyN, Dpto Cs Naturales "Bacteriocinas tipo cola fago: una nueva estrategia para el biocontrol de bacterias fitopatogenas" Diego Sauka, Instituto de Microbiología y Zoología Agrícola. INTA, Castelar "Distintos enfoques para la identificación de factores de virulencia de la bacteria entomopatógena Bacillus thuringiensis" Anibal Lodeiro, IBBM-Facultad de Ciencias Exactas, UNLP-CONICET. "Comunidades microbianas de la rizósfera y su posible aprovechamiento agronómico"
10:30 – 11:30	Café y Posters MICROBIOLOGÍA AMBIENTAL Y DE SUELOS
11:30 – 12:30	Conferencia Plenaria: Prof. Peter Kämpfer, Institute of Applied Microbiology- Justus-Liebig Giessen University, Germany. "Genotype versus phenotype in the taxonomy of Prokaryotes - Some examples from the genus Rhizobium" Moderador: María Alejandra Martínez
12:30 – 14: 00	Almuerzo
14:00 – 15:30	Comunicaciones orales: MICROBIOLOGÍA AMBIENTAL Y DEL SUELO/ BIODIVERSIDAD Moderador: Claudio Valverde IDENTIFICATION OF 29 COMPLETE BACTERIAL GENOMES FROM TWO SMALL DAIRY INDUSTRY WASTEWATER STABILIZATION PONDS. José Matías Irazoqui 1 2, Ariel F Amadio 1 2. 1 Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Rafaela. 2 CONICET. DETECTION OF CO-OCURRENCE PATTERNS IN SOIL MICROBIOTA USING GRAPH THEORY ON AMPLICON BASED METAGENOMIC DATA. Juan Félix O Orlowski 1 2, Marcelo Abel S Soria 1. 1 Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (INBA) - CONICET. 2 Facultad de Agronomía - Universidad de Buenos Aires. ANALYSIS OF SOIL BACTERIAL COMMUNITIES ASSOCIATED TO GENETICALLY MODIFIED DROUGHT TOLERANT CORN PLANTS. Jose Ibarra 1 2, Roxana Colombo 3 4, Alicia Godeas 3 4, Nancy López 1 2. 1 IQUIBICEN-CONICET. 2 Departamento de Química biológica, FCEyN-UBA. 3 IBBEA-CONICET. 4 Departamento de Biodiversidad y Biología Experimental, FCEN-UBA. ADVANCES IN THE SEARCH FOR ELECTROGENIC HALOPHILIC MICROORGANISMS Juan I Solchaga 1, Juan P Busalmen 2, Débora Nercessian 1. 1 Instituto de Investigaciones Biológicas, UNMdP- CONICET. 2 Instituto de Investigaciones en Ciencia y Tecnología de Materiales, UNMdP- CONICET. METAGENOMIC APPLIED TO MICROBIAL DIVERSITY STUDY IN A ZONE AFFECTED BY AN ACID MINE DRAINAGE: RELATION BETWEEN PHYSICOCHEMICAL PARAMETERS AND TAXONOMIC GROUPS José O Bonilla 1 2, Daniel G Kurth 3, Raúl A Gil 1 2, Liliana B Villegas1 2. 1 INQUISAL-CONICET. San Luis, Argentina. 2 Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. San Luis, Argentina. 3 PROIMI-CONICET. Tucumán, Argentina.
15:30 – 16:30	Café y Posters INTERACCIONES PROCARIOTA-EUCARIOTA-BIODIVERSIDAD-EDUCACION EN MICROBIOLOGÍA
16:30 – 17:30	Conferencia de Clausura: James M. Tiedje, Center for Microbial Ecology and Departments of Plant, Soil and Microbial Sciences and of Microbiology and Molecular Genetics, Michigan State University "Metagenomics for Function: From the Rhizosphere to Antibiotic Resistance" Moderador: María Alejandra Martínez
18:00	Despedida

CONFERENCIAS PLENARIAS Y MESAS REDONDAS

001

THE AMAZING PHASIN: PROPERTIES AND APPLICATIONS OF A MULTIFUNCTIONAL PROTEIN

María Julia Pettinari

FCEyN, UBA-CONICET, Buenos Aires

Phasins are proteins associated to intracellular polyhydroxyalkanoate (PHA) granules, a biodegradable polymer accumulated by many bacteria as a carbon and energy reserve. Phasins play an important role in PHA granule formation, enhancing polymer accumulation. In the non-natural PHA producer *Escherichia coli* PHA accumulation causes a stress that is alleviated by the presence of phasins (PhaP). Surprisingly, expression of PhaP in non-PHA-producing *E. coli* strains decreases the expression of heat shock proteins and protects cells against heat shock and superoxide stress. Analysis of the properties of PhaP revealed that it has chaperone-like functions, as it promotes protein folding and prevents protein unfolding and inclusion body formation.
One of the limitations for the microbial production of biofuels and other chemicals is their toxicity. The capability of PhaP to enhance tolerance to several solvents and chemicals was tested and compared to the known chaperone GroELS. Additionally, overexpression of *groEL* or *phaP* in ethanol or 1,3-propanediol producing recombinant strains resulted in an increase in final biomass, resulting in higher bioproduct titers. These results further expand the variety of applications for the multifaceted phasin family, and opens the road for the development of new strategies for microbial cell factory optimization, by enhancing the fitness of producing strains.

002

ARCHAEAL PROTEOLYSIS: FUNCTIONAL CHARACTERIZATION AND IDENTIFICATION OF NATURAL TARGETS OF REGULATORY PROTEASES

Rosana De Castro

Instituto de investigaciones Biológicas, IIB-CONICET-UNMdP, Funes 3250 4to Nivel, Mar del Plata (7600) Argentina.

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Proteolysis is a key process in cell physiology as it controls protein homeostasis, regulation of gene expression, precursor processing among other cellular events. The energy-dependent Lon protease is conserved across domains of life (*Eukarya, Bacteria and Archaea*) and is involved in housekeeping as well as pathological processes in bacteria and eukaryotic organisms. In *Archaea* Lon is unusually associated to the cell membrane and its biological role and natural targets remain unknown. *Archaea* mostly thrive in extreme habitats and insights on protease biology/function may help to discover the molecular mechanisms of their astonishing adaptation capability to the adverse environmental conditions. To address these questions, we constructed mutant strains in the model haloarchaeon *Haloferax volcanii* (growth conditions 1.5-3.5 M NaCl) with reduced Lon expression and characterized these strains phenotypically and at the proteome level. We demonstrated that Lon is essential for viability of this archaeon and suboptimal expression affected the cellular content of proteins involved in metabolism, cell shape, genetic processes and unknown function. Carotenogenesis was notably affected, rendering cells with increased bacterioruberin content due to the stabilization of key enzymes of this pathway.

005

CAZYMES: STRUCTURE, DIVERSITY AND NOVELTIES AIMING THE PRODUCTION OF 2ND GENERATION ETHANOL

Thamy Lívia Ribeiro Corrêa

Brazilian Bioethanol Science and Technology Laboratory – Universidade Estadual de Campinas

The lignocellulosic biomass emerges as an alternative to fossil fuels to obtain energy given its low cost and renewable nature. The production of second generation (2G) ethanol includes three steps: 1, pretreatment of lignocellulosic biomass; 2, enzymatic saccharification; and 3, fermentation. Due to its recalcitrant nature, an arsenal of enzymes is required to deconstruct the lignocellulosic biomass into fermentable sugars. Regarding the enzymatic saccharification, some aspects of CAZymes (Carbohydrate active enzymes) will be discussed in this conference: a mechanism displayed by a thermophilic β -glucosidase to tolerate high concentration of monosaccharides, mainly glucose and xylose; the dynamics of (hemi) cellulose deconstruction by *Penicillium* sp. and *Trichoderma* sp. grown on sugarcane straw and energy cane bagasse; and the importance of Lytic Polysaccharide Monooxygenases (LPMOs) as additives in enzymatic cocktails by boosting the activity of canonical enzymes, such as celulases and hemicellulases.

007

STRATEGIES FOR VIRULENCE GENES REGULATION IN *Salmonella* TYPHIMURIUM: DEVELOPMENT OF ATTENUATED VACCINES

Mónica A. Delgado

Instituto Superior de Investigaciones Biológicas- Universidad Nacional de Tucumán (INSIBIO-CONICET- UNT)

Foodborne diseases (FBD) affect the worldwide human health and are among the top five causes of death in children under 5 years. When are caused by bacteria, are usually presented as gastroenteritis with inflammatory or no inflammatory diarrheas, and in more severe cases produces the individual death. The currently strategies used to solve and prevent its diseases acquisition is the vaccine's treatment, developed with genetically modified microorganisms attenuated in their virulence. In my lab, several *S*. Typhimurium virulence-attenuated strains have been obtained, some of which are capable to replication into dendritic cells, producing the host immunogenic response, but that are unable to replicate within macrophages, by which are gradually removed from the host. This makes them excellent candidates for new vaccines development. My lab's goals are: i- to conduct a deep and controlled survey of pathogens causing FBDs in the NOA region (*Shigella*, *Salmonella* and *E*. *coli*); ii- characterize the main immunogenic components present in these isolates, and iii- to develop attenuated and vectors vaccines for the heterologous antigens expression, as an effective treatment to prevent and combat such diseases.

012

PHAGE TAIL-LIKE BACTERIOCINS: A NEW STRATEGY FOR THE BIOCONTROL OF PHYTOPATHOGENIC BACTERIA

Fischer, Sonia; Fernandez, Maricruz; Príncipe, Analía

Dpto. de Cs. Naturales, FCEFQyN. Universidad Nacional de Río Cuarto.

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Phage tail-like bacteriocins, called tailocins, represent a class of protein complexes synthesized by different bacterial species. The tailocins most known are R-type and F-type pyocins from *Pseudomonas aeruginosa*. In the last years, R-type pyocins have attracted attention as a strategy to replace antibiotics. Analysis of *Pseudomonas* spp. genomes showed that tailocins are also abundant in species other than *P. aeruginosa.* In our lab, we characterized the first tailocin in *Pseudomonas fluorescens* (strain SF4c). Phage tail-like structures were observed using Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM). These tailocins have antimicrobial activity against phytopathogenic bacteria, such as *Xanthomonas axonopodis* pv. *vesicatoria,* the causal agent of bacterial spot on tomato. An analysis through AFM demonstrated that bacteriocins adhere and cause damage to the cell envelope of *X. axonopodis* pv. *vesicatoria* Xcv Bv5–4a. This results in a rapid leakage of intracellular materials, with the subsequent decrease of cell volume. Under greenhouse conditions, the foliar application of tailocins significantly reduced of symptoms of bacterial leaf spot on tomato plants. Moreover, the bacteriocins were also effective in decreasing the severity of disease in fruit. Therefore, SF4c tailocins represent an alternative to antibiotics for their application in the biocontrol of bacterial diseases.

015

GENOTYPE VERSUS PHENOTYPE IN THE TAXONOMY OF PROKARYOTES - SOME EXAMPLES FROM THE GENUS *Rhizobium*

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Modern taxonomic approaches in microbiology aim at the establishment of a system that mirrors the evolution with the ultimate goal to describe the whole evolutionary order back to the origin of life. With the recognition of molecular markers present in all organisms (e.g. rRNAs,ssRNSs), this possibility has become more and more feasible and the generation of gene and increasing numbers of genome sequences allow now the generation of large amounts of data and a very detailed insight into the genetic potential of prokaryotes. The possibility to generate whole genome sequences leads to a strong tendency to base the taxonomic system more and more on sequence data, the genotype.
Some examples for the genera *Pseudomonas* and *Rhizobium* for which genotypic methods are now key elements in the differentiation of species will be given. In addition, two examples for the importance of intraspecies diversity analysis at the genotypic and especially phenotypic level for will be presented. (i) Results of two *Rhizobium/Agrobacterium radiobacter* strains showing 99.8% average nucleotide identity with strong different ecological functions – and (ii) A specific cluster of phyllosphere colonizing strains of a novel *Rhizobium* clade, which shows a pronounced intra-species diversity at the genotypic and phenotypic level. These examples show, that a comprehensive understanding of all the information behind sequence data is lagging far behind their accumulation. Genes and genomes may (or may not) function only in a given "environment", with the cell as basic entity for the display of this potential.

016

METAGENOMICS FOR FUNCTION: FROM THE RHIZOSPHERE TO ANTIBIOTIC RESISTANCE

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Metagenomics and its namesake, the microbiome, has become a core of new age microbial ecology. Metagenomics has advanced rapidly and taken many directions - those determined by the various methods employed, the complexity of the different communities, the resources available and of course the compelling questions. My lab has focused recently on ecofunctional genes, i.e. those genes that directly control important ecological function. I will illustrate with two examples: one of soil and its rhizosphere microbiome, and the other of understanding the ecology of antibiotic resistance genes in the environment. For soil I will show how new computational tools, especially gene–targeted assembly using Xander, helps quantify and categorize N cycle and other genes, and how we can assimilate metagenomic, metatranscriptomic and metaproteomic data from field soil to gain some insight into rhizosphere activities. For antibiotic resistance we used highly parallel qPCR, with over 300 primer pairs for antibiotic resistances and mobile genetic elements, to assess quantity, type and fate of these pollutants. These are just two examples of metagenomics and microbiome science, a field with big opportunities since there are many habitats, directions and needs, especially for creative ways to interrogate data to uncover new knowledge, including better insight into function. It is a great opportunity area for students.

FISIOLOGÍA MICROBIANA

MODALIDAD ORAL

FM-001

PARTIAL ANTAGONISM BETWEEN BIOFILM-FORMING *Escherichia coli* AND *Klebsiella pneumoniae* UROPATHOGENIC STRAINS ISOLATED FROM A CATHETER-ASSOCIATED BACTERIURIA: DETRIMENTAL EFFECT OVER *E. coli*

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Development of polymicrobial biofilms on catheter surfaces by microorganisms causing catheter-associated urinary tract infections (CAUTI) is frequent in patients with prolonged catheterization. However, the knowledge on interspecies interactions between uropathogens forming biofilms is limited. In our laboratory, it was reported a growth inhibition of *Escherichia coli* (Ec) when it was co-cultivated in dual-species biofilms with *Klebsiella pneumoniae* (Kp), being both bacterial strains co-isolated from a patient with CAUTI. The aim of this work was to characterize the nature of this detrimental effect. For this purpose, Kp-Ec dual-species (1:1 ratio) and single-species biofilms were performed in artificial urine medium (AUM) or M9 medium. For biofilm formation, bacteria were allowed to attach for 3 h and then culture media was either replaced every day for batch cultures or renewed at a flow rate of 35 ml/h for continuous cultures. Biofilm formation over time was monitored by colony forming unit counts of mechanically disrupted biofilms plated on selective antibiotics to differentiate each bacterial species. Additionally, bacterial biomass was assessed by confocal microscopy over biofilms formed by Kp and GFP-expressing Ec, also employing propidium iodide for total staining of fixed biofilms. On the other hand, biofilm cell-free supernatants were investigated for planktonic, solid, and biofilm growth-inhibitory activity. Results of batch experiments in AUM showed that Ec growth was inhibited in dual-species biofilms (around 15- and 100-fold at 3 and 7 days of incubation, respectively), related to single Ec biofilms. Similar results were obtained when batch biofilms were performed in M9 medium. Confocal microscopy analysis showed that Ec biofilm biomass was significantly reduced in dual-species biofilms, compared to single Ec biofilms (3-, 12- and 58-fold at 1, 3 and 5 days of incubation, respectively). Conversely, Kp growth in dual-species biofilm was not affected in comparison to single cultures. Biofilm development under an AUM continuous flow showed higher growth of Ec and Kp in single biofilms respect to batch biofilms (5- and 39-fold increase, respectively). Noticeably, not significant differences were observed between Ec and Kp growth in single and dual-species cultures. On the other hand, supernatants obtained from Kp and Kp-Ec batch biofilms were not able to inhibit neither planktonic growth in AUM nor growth in solid LB medium of Ec. Moreover, these supernatants did not affect Ec biofilm development. Our findings suggest that the partial inhibition of Ec in Kp-Ec dual-species biofilm is not due to a soluble antimicrobial compound secreted by Kp, but it may occur when nutrient supply is limited, since this effect was prevented in continuous cultures. Studies are currently in progress to further define the role of nutrient competition in the interaction between these bacterial species in biofilm establishment.

FM-002

MECHANISMS INVOLVED IN THE ADAPTATION OF *Escherichia coli* O157:H7 TO THE INTESTINAL MICROENVIRONMENT

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Although the production of Shiga toxin by enterohemorragic *Escherichia coli* (EHEC) determines Hemolytic Uremic Syndrome (HUS) onset, factors that modulate intestinal colonization are key components in pathogenesis and host mucosal immune response. We showed previously that the passage of a clinically isolated EHEC strain (125/99) through the gastrointestinal tract of mice increases its pathogenicity in mice, and that stool-recovered strains (125R and 125RR) induce a more generalized and persistent colonization than the parent strain (Fernandez-Brando et al, 2012). We aimed at elucidating the underlying mechanism involved in the pathogenesis and bacterial adaptation to the intestinal environment of mice. We assessed the global transcription profile by microarray and found more than 100 differentially expressed genes in 125RR strain: small RNAs (sRNA), proteins from the type three secretion system, several enzymes, membrane transporters and receptors and several putative transcripts. We confirmed the augmented expression of EspB and fliC ($p<0.05$) and the diminished expression of ECs1537/1561 ($p<0.05$) by qPCR. We also demonstrated the augmented expression of EspD by western blot, which could explain the greater colonization of stool-recovered strains. In an attempt to elucidate targets for sRNA regulation we studied acid resistance mechanisms, since arcZ, rprA, and ryhB are involved in that mechanism. The 125RR strain showed an increased survival at pH 2.5 for 1 h ($p<0.05$), which could determine a lower infectious dose. Given the importance of motility in surpassing the mucus barrier in the mucosal environment and the finding of the augmented expression of flic, we tested the motility phenotype in semisolid agar. The 125RR strain showed an increased motility compared to 125/99 and 125R ($p<0.01$). These results suggest that the stool-recovered strain is more proficient to deal with the murine mucosal barrier thus leading to the onset of HUS characteristic symptoms in mice.

FM-003

ANTAGONIC EFFECT OF LACTIC ACID BACTERIA ISOLATED FROM MINIMALLY PROCESSED FRUITS AGAINST *Salmonella typhimurim*

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Demand for fresh fruits and minimally processed products (MP) is constantly growing among the world's population. The use of bio-preservation is a promising technique to ensure the microbial safety of MP fruits. Lactic bacteria (LAB) could prevent the development of pathogenic microorganisms present in these foods. However, information about this is scare yet. LAB strains with antimicrobial activity against various pathogens including *Salmonella typhimurim* in culture medium (BHI) isolated from fruits were selected in a previous study. The aim of this study was to determine the antimicrobial activity of three selected strains of LAB against *S. typhimurium* when cultivated in a commercial fruit juice stored at 30 °C. Flasks containing 30 mL of commercial juice were co-inoculated with *S. typhimurium* (3% v/v) previously grown in BHI medium and with each LAB strain (*Lactobacillus plantarum* N4 from orange and *Lactobacillus* sp. FEV3 and JES1 from salad fruits) grown in MRS medium, pH 6.5 and incubated at 30°C for 72 h. Before inoculation fruit juice was clarified by centrifugation (7500 rmp, 15 min) and separated in two group: without or with adjustment of pH (3.43 and 6.5 respectively). At the same time uninoculated juice was tested at both pH. Bacterial growth was determined by measuring cfu/mL in BHI medium (*S. tiphymurium*). The initial concentration of pathogen and LAB strains was in order of 106 cfu/ mL for all treatments (single and co-inoculated cultures). In juice, pH 3.43 no growth of *S. typhimurium* was observed regardless LAB presence. At pH 6.5, *S. typhimurium* alone grew 2.49 log units at 24 h, then decreased of about 4 units until 72 h inoculation. The *S. typhimurim* growth was totally and almost 50% inhibited in co-cultures with FEV3 and N4 strain while no effect was observed for JES1 at 24 h. Moreover, no growth was detected at 48 h for N4 and FEV3 while this fact was observed later for strain JES1 (72 h). In this condition, the initial pH of 6.5 decreased to final values of 4.95 (in 24 h) and ̴ 3.40 (in 48 h) in presence of pathogen alone and co-cultures with each LAB respectively. Thus, result demonstrated that *S. hyphimurim* was totally inactivated in commercial fruit juice at 24 h at 30 °C due to acid pH, as it rapidly grew in juice adjusted to pH 6.5. In this condition, the LAB strains tested significantly reduced counts of *S. typhimurium* until levels below detection limit. However, the effectiveness of their effect antagonist significantly varied among them, being FEV3 the most effective. This difference appears have no relation with the fruit from which was isolated as well as with its acidification rate which was similar respect to other test bacteria.

FISIOLOGÍA MICROBIANA

MODALIDAD POSTER

FM-004

EVALUATION OF ANTIRADICAL CAPACITY OF FERULOYL ESTERASE- PRODUCING *Lactobacillus* STRAINS AND MAINTENANCE OF ENZYMATIC ACTIVITY AFTER EXPOSURE TO GASTROINTESTINAL TRACT CONDITIONS

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Feruloyl esterases (FE) are enzymes that catalyze the hydrolytic release of ferulic acid (FA), present in vegetable foods. FA is a phenolic acid with proven antioxidant properties. *Lactobacillus fermentum* CRL1446 (Lf), *Lactobacillus johnsonii* CRL1231 (Lj) and *Lactobacillus acidophilus* CRL1014 (La) are potential probiotic strains selected for their FE activity capable of releasing FA *in vitro*. The aim of the present work was to evaluate the antioxidant capacity of these FE-producing strains and the maintenance of FE activity after exposure to gastrointestinal tract (GIT) conditions. The antioxidant capacity was determined based on the antiradical activity against the synthetic radical DPPH (1, 1-diphenyl-2-picrylhydrazyl), which was evidenced by decrease of the absorbance at 517 nm. Supernatants and cell suspensions obtained from cultures in MRS and MRS medium supplemented with ethyl ferulate (EF) were incubated in presence of methanolic solution of DPPH (0.1 mM). After 30 minutes of incubation (darkness, room temperature), samples were centrifuged (6000 rpm, 10 min) and then the absorbance was measured at 517 nm. To evaluate the maintenance of FE activity after exposure to GIT conditions, cell suspensions were transferred to simulated gastric juices at pH 3 and 4. Samples were incubated at 37°C and harvested by centrifugation after 2h. Subsequently, cells were resuspended in simulated intestinal juice and further incubated 2h at 37°C. The samples were then centrifuged and the pellets were washed twice and resuspended in PBS pH 7. FE activity was determined using 1 mM methyl ferulate as substrate. Released FA was detected by HPLC. Supernatants showed radical capture percentages between 82-89%, being slightly higher in samples from MRS supplemented with EF, indicating that the presence of free FA as a product of hydrolysis of EF is partly responsible for the higher antioxidant capacity of these samples. With regard to cell suspensions, no significant differences were observed between the antiradical activity of cells grown in the absence and presence of EF. In both cases, the antiradical activity showed the following order: Lf> Lj> La. Among the three strains evaluated, Lf showed the highest maintenance of FE activity after GIT exposure (̴ 65 and 50% at pH 3 and 4, respectively). These *in vitro* results validate the use of *L. fermentum* CRL1446 as an oral probiotic with antioxidant properties that could be able to protect against oxidative stress.

FM-005

CAN CO2 CHANGE THE MECHANISM OF ACTION OF CIPROFLOXACIN IN *Escherichia coli*?

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Keywords: Oxidative stress, ciprofloxacin, CO2, *Escherichia coli*.

CO2 has become in an importan poin to study due to the rapid increase in atmospheric concentrations at the recent years. This has generated not only climate changes but also the adaptation of macro and microscopic living organisms. CO2 is a major by-product of cellular metabolism and it has been shown that it can react with the hydroxyl radical (HO·) and hydrogen peroxide (H2O2) that increase their toxicity in a dose dependent manner. The oxidative action of these radical species, generating oxidative stress, leads to oxidation of macromolecules like proteins, lipids and DNA producing the cell death. It's well-known that ciprofloxacin (CIP) is a bactericidal antibiotic capable of stimulating production of HO· in Gram positive and Gram negative bacteria. In previous studies with CIP at different concentrations (0; 0.5 y 50 µg/mL) in atmospheric conditions and CO2 controlled atmosphere (50 ppm y 5%) in *E. coli* ATCC 25922, we determinate that CO2 modifies the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) mediated by CIP which affect its antimicrobial activity in *E. coli*. Following to our previous results, we wanted to delve into the mechanism of action of CIP and CO2 against *E. coli* ATCC 25922. We studied the oxidative stress markers, such as protein oxidation (AOPP), lipids, DNA and changes in the membrane potential. It was found, under atmospheric conditions, that the maximum oxidation for AOPP is given at 4h in both CIP concentrations, while for the controlled atmospheres of CO2 the protein oxidation was favored at shorter times for CO2 50 ppm and CIP 50 mg/mL. For CO2 5% and CIP 0.5 mg/mL it was given at a time of 4h. Most malondialdehyde (MDA) formation occurs under atmospheric conditions for both concentrations of CIP at 4 h of reaction, while the CO2 does no bring on lipid peroxidation mediated by CIP. Both in atmospheric conditions and in controlled atmospheres of CO2 it was evident an alteration in the bacterial membrane potential generated by the highest concentration of CIP. In controlled atmospheres of CO2, the oxidation of DNA was lower than in atmospheric conditions. In conclusion, we could say that CO2 modifies the bactericidal effect of CIP against *E. coli*, reducing the damage on the different macromolecules.

FM-006

EFFECT OF LUTEOLIN ON CIPROFLOXACIN AND CHLORAMPHENICOL ANTIMICROBIAL ACTIVITY IN *Escherichia coli* AND *Staphylococcus aureus*

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Luteolin (LT) a flavonoid isolated from fruits of *Prosopis strombulifera* var. *strombulifera* with antioxidant properties and scavenger capacity of free radicals, has demonstrated in previous studies of our group, a marked protective effect against the production of reactive oxygen species (ROS) induced by gentamicin (GEN), ciprofloxacin (CIP) and chloramphenicol (CMP) in human leukocytes showing besides synergistic effect on GEN antimicrobial activity. These results motivated us to study the effect of LT on the activity of CIP and CMP, in order to determine if its protective action on human cells can modify the antibacterial activity of these antibiotics in different strains of *Escherichia coli* and *Staphylococcus aureus*. A reference strain of *E. coli* ATCC 25922 and a clinical strain of *E. coli* resistant to GEN and CIP, a reference strain of *S. aureus* ATCC 29213 and a clinical strain of *S. aureus* resistant to GEN and CIP were used. The microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) of LT on all strains of *E. coli* and *S. aureus*, while the checkerboard method was used to determine the interaction between LT and the antibiotics CIP and CMP employing combinations of these compounds at different concentrations in Mueller-Hinton broth. So, the combinations LT plus CIP and LT plus CMP for the inhibition of clinical strains of S. aureus demonstrated an increase in bacterial susceptibility to this antibiotics, being observed a decrease of 8-fold in the MIC value of the same when were combined with LT. Moreover, when LT was combined whit these antibiotics for the inhibition of *S. aureus* ATCC a synergistic effect was revealed whit CIP (FICILT+CIP = 0.5) when the MIC of CIP and LT were decreased 4 times below their individual MIC and likewise, the combination LT plus CMP demonstrated a synergistic effect (FICILT+CMP = 0.378) when the MIC of CMP decreased 4 times and the MIC of LT decreased 8 times respect their individual MIC. Regarding in both strains of *E. coli* the presence of LT did not produce changes in the susceptibility to CIP and CMP. On this basis we can conclude that LT significantly increase the antibacterial activity of CIP and CMP in strains of *S. aureus*, revealing synergistic effects in the clinical strains, while the combination of LT with these antibiotics did not alter the sensitivity of reference strains of *E. coli*. Therefore, LT proved to have a protective effect against oxidative stress induced by CIP and CMP in human leukocytes without modifying the antibacterial effect of these drugs in *E.coli* and strengthening it against *S. aureus* strains.

FM-007

ADHERENCE AND INTERNALIZATION OF *Streptococcus uberis* TO BOVINE MAMMARY EPITHELIAL CELLS

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Streptococcus uberis is one of the most prevalent environmental pathogens responsible for a significant proportion of subclinical and clinical bovine intramammary infections in lactating and nonlactating cows. Adherence of *S. uberis* to host epithelial cells has been accepted as an important initial and critical step in the colonization of bovine mammary glands, while the invasion of epithelial cells would prevent macrophages, antibodies and other antimicrobial factors from eliminating the bacteria. The aim of the present study was to investigate capability of adherence and internalization of 9 *S. uberis* isolates collected from cows with clinical and subclinical mastitis from 9 herds located in the central dairy region of Argentina. The isolates were identified by Mass spectrometer Bruker MALDI-TOF. For the assays the MAC-T bovine mammary epithelial cell line was used. Briefly, the isolates were cultivated in Todd-Hewitt broth overnight at 37°C, and a 1:50 dilution incubated for 2 h at 37°C was used as bacterial suspension. To determine internalization of *S. uberis*, bacterial suspension was co-cultured with MAC-T cells in Dulbecco's Modified Eagle's Medium at a multiplicity of infection (MOI) of 10 during 1 h at 37°C. Then, monolayers were washed three times with PBS and treated with gentamicin (100 µg/mL) for 2 h to kill extracellular bacteria. Monolayers were then washed two times with PBS and further lysed with Triton X-100 at a final concentration of 0.025% v/v in sterile distilled water. Lysates were serially 10-fold diluted and plated onto trypticase soy agar. After incubation during 18 h at 37 °C, colony forming units per mL (CFU/mL) of *S. uberis* internalized in MAC-T cells were determined by colony counting techniques. Adherence assay was performed in parallel and under the same culture conditions as described for internalization assay, but without gentamicin treatment. Data of internalization *S. uberis* were expressed as Log10 CFU/mL and the number of adherent bacteria was calculated by subtracting the number of internalized *S. uberis* from MAC-T cell-associated *S. uberis*. The isolates were able to adhere to MAC-T epithelial cells, dividing into two groups statistically different, showing that the isolates exhibit different adhesion capability to MAC-T epithelial cells. The isolates RC29 and RC37 showed the highest adhesion capability. In addition, the internalization capability of the isolates was again divided into two groups statistically different. It was evidenced that the isolates RC13, RC34, RC37 and RC38 presented the greatest capability to internalize in MAC-T cells, showing the highest arithmetic means expressed as Log10 CFU/mL and that are significantly higher than the rest of the isolates. In conclusion, the results presented in this research evidenced that adherence and internalization to MAC-T epithelial cells differ between *S. uberis* isolates and these traits were not associated with their clinical or subclinical origin.

FM-008

NAFTOKINONES AS INHIBITORS OF BIOFILM FORMATION OF A *Yersinia enterocolitica* REFERENCE STRAIN

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Yersinia enterocolitica is a Gram-negative coccobacillus belonging to *Enterobactereaceae* family. It is pathogenic to humans and warm-blooded animals. It is frequently isolated from samples of water, animals and a wide variety of foods. The main route of transmission is oral, by ingestion of water or contaminated food, where it can reach a high concentration, even if these are kept refrigerated. At 25 °C it has the ability to form biofilms, which are accumulations of bacterial cells surrounded mainly by highly hydrated polysaccharides that give the involved bacteria protection against multiple adverse environmental conditions. *Y. enterocolitica* has an intercellular communication system called *Quorum Sensing* (QS), which allows it to communicate with each other through chemical molecules called N-acyl-homserine lactones, to develop coordinated behaviors depending on cell density, such as biofilm formation, among others. The naphthoquinones are a chemical compounds group present in a great variety of plants whereby they are used as base in traditional medicine. It has been shown that some representatives of this group of compounds are able to inhibit the biofilms formation of several bacterial species. The high occurrence of strains resistant to various antimicrobials due to their indiscriminate use has led to the search for new compounds that inhibit virulence factors such as biofilm, instead of inhibiting the planktonic growth of cells, which would lead to less resistance, this is why the objective of this work was to evaluate the activity of 28 naphthoquinones, obtained by chemical semi-synthesis, in the biofilm development of the reference strain *Y. enterocolitica* WAP B1B/O:8, that carries the virulence plasmid (pYV+). For biofilm inhibitors screening, the crystal violet technique with trypticase soy broth supplemented with 0.25% glucose was used followed by the determination of the Minimum Inhibitory Concentration of Biofilm (CIMB). Finally, QS was evaluated as a possible biofilm inhibitor mechanism by measuring diameters of violacein halos produced by the biosensor strain *Chromobacterium violaceum* 026 when it is in contact with homoserine lactones fromother bacteria. Of the 28 studied compounds, six were biofilm inhibitors 1, 2, 16, 21, 22, and 23; none of them inhibited planktonic growth. The CIMB was 50 mM for compounds 1, 2 and 16; 25 mM for 21 and 22 and 3.05 mM for 23. None of these naphthoquinones showed QS inhibition, indicating that they inhibitory effect is carried out by another mechanism of action. These results demonstrate the potential of naphthoquinones to inhibit the biofilm formation of this important enteropathogenic microorganism.

FM-009

ANTIFUNGAL ACTIVITY OF STYRYLBENZENE COMPOUNDS AGAINST *Candida tropicalis*

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Styrylbenzene compounds have been extensively study because its optical properties but their potential biological properties are still undiscovered. The rigid structure of these types of compounds allows to place the therapeutic functionally in specific position, distance and angles and it helps to establish a structure-activity relationship. The synthesis of these structures is based on the Horner-Wadsworth-Emmons (HWE) reaction for the formation of the doubles bonds. This procedure shows few advantages compare with other to generate double bonds. The trans stereochemistry of the double bonds located at the core was preserved throughout the synthetic methodology. This is a critical issue because for establish a structure-activity relationship it is important that comparison among molecules are made with perfectly well known structures, included stereochemistry. On the other hand, the HWE reaction provides a methodology with an easy workup and free of catalyst that typically raise the price of the production and may interfere in the biological properties. The aim of this work was to determine the effect of styrylbezene compounds against *Candida tropicalis* NCPF 3111. The antifungal activity of four compounds obtained (AFL 8.3, AFL 10.1, AFL 5.1 and AFL 14) was tested against *C. tropicalis* NCPF 3111 by the determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The MIC of the four compounds was determined according to the M27-A3 broth microdilution reference procedure of Clinical and Laboratory Standards Institute (CLSI, 2008), at a final inoculum of 1-5 × 103 CFU/mL, RPMI media. Serial dilutions of the compounds, ranging from 0.033 to 512 mg/mL, were carried out in a microdilution plate (96 wells) containing 100 mL of RPMI. The inoculum was then added to each well. The microplates were incubated at 37 °C for 48 h. MIC was defined as the lowest concentration which resulted in inhibition of visual growth. Minimal fungicidal concentrations were determined by subculturing 100 mL of the culture from each negative well and from the positive control on sabouraud dextrose agar. All these analyses were performed in triplicate. Amphotericin B was used as control, ranging from 0.033 to 16 mg/mL. The four compounds evaluated show a greater antifungal activity against *C. tropicalis* NCPF 3111. The values obtained for MIC and MFC were 8 µg/mL for AFL 8.3, 32 and 64 µg/mL for AFL 5.1, 64 and 128 µg/mL for AFL 10.1, 16 µg/mL for AFL 14 and 0.25 for amphotericin B. The data obtained revealed a fungicidal activity against yeasts cells of the *C. tropicalis*, and open new perspectives for future research in continuation to this study, where information such as determination of the site and mechanism of action of the styrylbezene compounds could contribute to an alternative therapy against these organisms.

FM-010

IMPACT OF 2(3)-*TERT*-BUTYL-4 HYDROXYANISOLE MICROCAPSULES AT SUB-LETHAL DOSES ON BEHAVIOR OF *Aspergillus flavus* IN CULTURE MEDIUM AND PEANUT KERNELS

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Peanuts are considered to be a high-risk product for contamination with aflatoxins (AFs) since it is frequently contaminated with fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, and for the long peanut drying times and occurrence of rainy periods after uprooting. In addition, mycotoxins can be produced in grains in the field, and also during transport and storage where conditions are suitable for their production. Formulation of the food grade antioxidant of 2(3)-tert-butyl-4 hydroxyanisole (BHA) have shown antifungal effects on stored peanuts. In this work, our interest focuses on the physiological behavior of *Aspergillus flavus* affected by the exposure to sub-lethal doses of microencapsulated BHA. For this, peanut meal extract agar (PMEA), peanut kernels with modified water activity (aw) (0.96 and 0.99) and a sub-lethal dose (0.6 mM) of an BHA formulation were used. Fungal physiological aspects as growth rate, time to growth and AFB1 evolution were modified by the formulation, especially on PMEA at both aw. In general, conidial and vesicle sizes of the mold only were affected by growth substrate, being higher on sterile kernels than on PMEA, regardless of aw condition. However, positive correlation (p<0.05) observed in controls between radius and biomass responses was altered by the application of the formulation, mainly under the lowest aw in both substrate evaluated. Finally, the presence of encapsulated antioxidant showed significant change in Pearson coefficients respect to the controls for all studied parameters. As conclusion, sub-lethal doses of formulation lead in reduction of growth and toxin accumulation, but conidial and vesicle size were not affected. Results of this work indicate the need to consider both fungal primary and secondary metabolism to determine the effect of food grade antioxidant formulation in those points of the silo where not adequate concentration by an incorrect homogenization could appear.

FM-011

HARMFUL EFFECTS ON *Oryzaephilus surinamensis* (L.) AND *Tribolium castaneum* BY FOOD GRADE ANTIOXIDANTS AND THEIR FORMULATIONS IN PEANUT KERNELS

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Two important species of pest insects are *Oryzaephilus surinamensis* (L.) saw-toothed grain beetle (Coleoptera; Silvanidae) and *Tribolium castaneum*, Herbst; the red flour beetle (Coleoptera; Tenebrionidae) which attack grains, preferring stored cereal products and oleaginous seeds. Both insects could acts as vector for potential toxigenic molds as *Aspergillus* spp. and the constant migration of insect populations within granary ecosystem efficiently contributes to dispersion of viable fungal spores which are carried on the vector's body surface or are deposited with its feces. For this, the aim of this study was to investigate the effect of two food grade antioxidant as 2(3)-tert-butyl-4 hydroxyanisole (BHA) and 2,6-di(tert-butyl)-p-cresol (BHT) frees and microencapsulated on mortality, body weight and total protein content of *Oryzaephilus surinamensis* (L.) and *Tribolium castaneum*. The effect of free BHA and BHT and their formulations (F-BHA and F-BHT) at dose ranging from 10 to 45 mM, in peanut kernel was evaluated. BHA and BHT free antioxidants showed significant mortality percentages upper than 80%. Besides, microencapsulated antioxidants at 20 and 30 mM significantly affected the survival of insect populations after 45 days of exposure. Insecticidal activities of BHA and BHT formulations were estimated in 100 and 70%, respectively. Insect weight was significantly affected by all studied treatments and the major effect on *O. surinamensis* (L.) was produced by time, especially with the application of BHA follow by F-BHA and F-BHT. Body mass weight of *T. castaneum* showed the highest reduction after application of F-BHA, followed F-BHT, BHT and BHA. Total protein content of insects also was affected by both free and microencapsulated antioxidants. Formulations preserved insecticidal effectiveness of antioxidants and extended their effect for up to 45 days which could act as new strategies to control these insects pest. Besides, sub-lethal levels of these compounds results in a loss of insects' weight and change in protein content. These results showed the first step in toxicity mechanisms for free and microencapsulated BHA and BHT on *O. surinamensis* (L.) and *T. castaneum*, the two important aflatoxin fungi vectors in peanut agro-ecosystems.

FM-012

ENHANCED BIOFILM FORMATION BY *Bordetella pertussis* CLINICAL ISOLATES FROM ARGENTINA AND USA

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Bordetella pertussis is a strict human pathogen and the primary etiological agent of whooping cough or pertussis, a respiratory disease that is highly prevalent among infants. After widespread use of vaccines since the 1950s, the incidence of the disease decreased noticeably. However, in the last past decades pertussis has become re-emergent with significant mortality in infants and increase in the incidence on adolescents and adults. Physiological and pathogenic features of *B. pertussis* have been extensively studied focusing on the planktonic mode of growth of the bacterium, while only a few works so far have considered the biofilm lifestyle of this bacterial pathogen. With the hypothesis that *B. pertussis* colonizes and persists in their host through biofilm formation, in this work we examined the biofilm forming capacity of clinical isolates recovered in Argentina and USA, from 2001 to 2012, against a reference strain, Bp536 (a strain sub-cultured in vitro since 1950s). Associated with a hyper-biofilm phenotype, we found in the clinical isolates a higher autoaggregation rate, whereas no differences in cell surface hydrophobicity were observed. Autoaggregation was also evidenced by fluorescent microscopy, after initial attachment of isolates to abiotic surfaces. Confocal microscopy analysis revealed that clinical isolates produce biofilms with higher biomass, average thickness and maximum thickness; in addition, biofilms produced by Argentinean strains resulted to be more homogeneous than US isolated, which correlated with higher roughness coefficient in the laters. Expression of two virulence factors, filamentous haemaglutinin (FHA) and adenylate cyclase toxin (ACT) was affected in clinical isolates. FHA and ACT positively and negatively control biofilm formation in *B. pertussis*. We found an inverse correlation between FHA and ACT production in clinical isolates, which were characterized by the production of higher levels of FHA and lower ACT activity. We also studied the interaction of clinical isolates with epithelial cells A549, observing higher attachment in all studied strains. Moreover, higher in vivo colonization of the mouse respiratory tract by two clinical isolates was found, particularly in nasal cavity and trachea, after 4 and days post-infection. In conclusion, we have for the first time demonstrated an association between higher levels of biofilm formation in *B. pertussis* isolates with enhanced survival in an animal model of infection. Data obtained in this report also provide mechanistic explanations towards the continued circulation of *B. pertussis* and the resurgence of whooping cough. We propose that the hyper-aggregative, hyperbiofilm and hyper epithelial cell adhesive properties of the clinical strains result in the formation of robust organ-adherent biofilm communities in the nose and trachea.

FM-013

PROTECTIVE EFFECT OF PHASIN PhaP FROM *Azotobacter* sp.FA-8 AND ITS COMPARISON WITH THE CHAPERONE GroELS

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Phasin PhaP from *Azotobacter* sp.FA-8 is a polyhydroxyalkanoate (PHA) granule-associated protein that not only plays an important structural role in polymer accumulation, but has also been shown to have an unexpected protective effect in non-PHA synthesizing *Escherichia coli*. This protective effect was observed under both normal and stress conditions, resulting in increased growth and higher resistance to both heat shock and superoxide stress by paraquat. Moreover, PhaP has chaperone activity and was observed to exert a beneficial effect in *E. coli* cells producing heterologous proteins, playing an active role in protein folding and/or unfolding prevention that reduced the number and size of inclusion bodies. Further research showed that PhaP enhanced bacterial fitness in the presence of biofuels, such as ethanol and butanol, and to other chemicals, such as 1, 3-propanediol. The effect of PhaP was also studied in a *groELS* mutant strain, in which both GroELS and PhaP were observed to exert a beneficial effect on cells. These results indicated that PhaP was able to complement the phenotypic effects caused by the *groEL* mutation in a similar manner as the GroELS protein. In view of these findings, we further studied the ability of PhaP to reduce the impact of different types of stressors in recombinant *E. coli* and compared this protection with the known chaperone GroELS. To test if PhaP reduces osmotic stress, a *groELS* mutant strain, carrying plasmids pBBR1 (control), pADP2 (expressing *phaP*) and pGroELS1 (expressing *groELS*), was grown in LB medium supplemented with 0, 3 M NaCl. As it was observed previously for biofuels, both PhaP and chaperone GroELS were able to protect cells from high salt concentration. Moreover, the *phaP* expressing strain displayed faster growth than the strain overexpressing *groELS*. Taken together, these results show that PhaP protects cells from multiple stresses, expanding the applications of the multitalented phasins and opening the road for the development of more resistant strains, suitable for the synthesis of diverse biotechnologically relevant products from renewable carbon sources.

FM-014

THE LONB PROTEASE HAS A GLOBAL IMPACT ON THE PROTEOME SYNTHESIS IN THE HALOARCHAEON *Haloferax volcanii*

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Energy-dependent proteolysis is a key process in cell physiology. ATP-dependent Lon proteases are conserved among the three Domains of Life and in *Archaea* it is unusually associated to the cytoplasmic membrane (LonB subfamily). We have previously shown that LonB is an essential protease in the haloarchaeon *Haloferax volcanii,* and that suboptimal amounts of this protease affect growth rate, cell shape and produce hyperpigmentation. To better understand the biological relevance of Lon in archaeal cells, the whole proteome turnover was examined in a *H. volcanii* conditional LonB mutant (HVLON3) under reduced (- trp) and physiological (+ trp) protease levels. Liquid chromatography coupled to tandem mass spectrometry combined with stable isotope labeling was applied for the identification and quantitation of membrane and cytosol proteins affected by the LonB protease. In a previous report we showed that presence of LonB affected the degradation of many proteins and several potential LonB substrates were identified (including phytoene synthase, key enzyme in the carotenoid biosynthesis pathway). In this work we focused on the effect of LonB on the overall proteome synthesis. A total of 225 proteins displayed differential synthesis rates depending on LonB expression. Proteins related to translation, amino acids, co-enzyme, nucleotide and energy metabolism showed a decrease in synthesis when LonB was induced while those involved in transcription, environmental information processing and lipid metabolism displayed an increase in synthesis under this condition. Several proteins that showed LonB-dependent synthesis correlated accordingly with changes in protein amounts observed in our previous work that compared the proteomes of the HVLON3 mutant vs the parental H26 strain by a quantitative MS approach. For instance, a membrane protein of unknown function (HVO_A0039) whose synthesis was arrested after LonB induction, increased by up to 320 fold in HVLON3 (reduced Lon levels). This observation was also supported by an RT-PCR assay, showing that while the specific HVO_A0039 transcript was almost undetectable in H26, its level dramatically increased in HVLON3. The whole proteome turnover analysis, performed for the first time in an archaeon, shows that the membrane LonB protease not only has a global impact on protein degradation but also on the proteome synthesis in the archaeon *H. volcanii.* Supported by CONICET, UNMdP, ANPCyT and MINCyT-BMBF.

FM-015

POLYHYDROXYBUTYRATE INCREASES SURVIVAL AFTER UVA EXPOSURE IN *Pseudomonas* SPP

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Solar ultraviolet radiation (UVA; 315-400 nm) the major fraction of UV radiation reaching the Earth's surface represents one of the main environmental stress factors for bacteria. Lethal effects of UVA are due to oxidative damage of macromolecules by the action of reactive oxygen species (ROS). Antioxidative defenses and other strategies are a key factor for survival after UVA exposure. *Pseudomonas* species are ubiquitous microorganism with a wide variety of ecological niches and life styles. In this work we analyzed the survival after UVA exposure in *P. extremaustralis*, *P. aeruginosa* PAO1 and *P. protegens* Pf-5 in relation to polyhydroxyalkanoates (PHA) content. Most of *Pseudomonas* spp. produces medium chain length PHA while *P. extremaustralis* produces mainlypolyhydroxybutyrate (PHB). Cultures were grown in LB medium with or without sodium octanoate as carbon source for PHA accumulation at 30oC and 200 rpm for 24 h. Cells were washed and adjusted to OD650nm 0.4 in saline solution. Each bacterial suspension was divided in two fractions. One of the fractions was irradiated from above at a fluence rate of 20 W m-2 at the level of the free surface of the suspension, while the other fraction was covered with a black plastic sheet (dark control). Aliquots were taken through time and plate counts were performed. In absence of PHA, *P. aeruginosa* PAO1 showed a higher UVA resistance than *P. extremaustralis* and *P. protegens* Pf-5. However, in LB cultures supplemented with sodium octanoate *P. extremaustralis* presented the highest survival after UVA exposure. Moreover, *P. aeruginosa* PAO1 carrying a PHB synthase gene showed an increase in survival in cultures supplemented with sodium octanoate while a mutant strain of *P. extremaustralis* impaired of PHB accumulation presented similar survival notwithstanding the presence of sodium octanoate. To deeply study this phenotype PHB content was measure before and after UVA exposure and in dark control experiments. After 300 min of irradiation the PHB content drop in line with increased survival while in the control without irradiation the PHB content was similar to those observed at the initial time. Additionally, redox ratio was higher in cultures exposed to UVA, probably as consequence of PHB depolymerization. Our results showed that PHB accumulation increases the resistance to UVA in *Pseudomonas* species probably due to its depolymerization and the consequent NADH/NADPH availability, a key factor for stress resistance.

FM-016

ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING AQUEOUS EXTRACT OF *Bothriochloa laguroides*

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The continuing appearance of antibiotic resistance in pathogenic and opportunistic microorganisms obliges the scientific community to constantly develop new drugs and drug targets.In the current scenario, nanotechnology offers opportunities to reexplore the biological properties of already known antimicrobial materials by manipulating their size to alter the effect. Due to nanoparticles have demonstrated antimicrobial activity, the development of novel applications in this field makes them an attractive alternative to antibiotics. The aim of the present work was to determine the effect of silver nanoparticles (AgNPs) against four bacterial species and eight phytopathogenic fungi. For this, a simple and economic method of biosynthesis of AgNPs was used employing an aqueous leaf extract of *Bothriochloa laguroides* (known in Argentina as "cola de zorro") as reducing and stabilizing agent and AgNO3. *In vitro* susceptibility of the bacteria and phytopathogenic fungi to the biosynthesized AgNPs was evaluated using agar diffusion technique. The agar plate surface was inoculated by spreading a volume (100 µL) of the microbial inoculum over the entire agar surface. Then, four holes with a diameter of 6 mm were punched aseptically. Into the hole was placed: i-100 µL of water as negative control, ii- 100 µL AgNO3 solution, iii- 100 µL of the plant extract and iv- 100 µL of the AgNPs. Potato dextrose agar and trypticase soy agar were used for fungus and bacteria growth respectively. Then, agar plates were incubated at under suitable conditions depending the test microorganism (37 ºC and 28 ºC for bacteria and fungi, respectively).The results obtained showed an antimicrobial effect of AgNPs on the four tested bacterial species and on six of the eight fungi, showing greater inhibition in bacteria than in phytopatogenic fungi, for which it was decided to continue the following tests with the bacteria. The determination of minimum inhibitory concentration (MIC) was carried out through the standard tube dilution method on Mueller Hinton broth and using resazurin as indicator of cell viability. The minimum bactericidal concentration (MBC) was determined after 18 hours of incubation. The zone of inhibition varied in range of 4 to 6.5 mm for bacteria and in range of 2 to 4.25 mm for fungi with AgNPs concentration, while AgNO3 solution and aqueous extracts of *B. laguroides* did not show antimicrobial activity. MIC and MBC results showed the following order of sensitivity: *Staphylococcus aureus* ATCC 43300 <*Staphylococcus aureus* ATCC 29213 <*Escherichia coli* ATCC 25922 <*Pseudomonas aeruginosa* ATCC 27853. The synthesized AgNPs with *B. laguroides* shown good antimicrobial efficacy and may prove tobe good antimicrobial agent against wide range of microbes.

FM-017

SAFETY CHARACTERIZATION OF *Lactobacillus* STRAINS

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A wide variety of microbial species is used in food and feed production worldwide. Some have a history of safe use while others are less well understood and their use may represent a risk for consumers. Probiotics are live microorganisms which, administered in adequate amounts, exert a beneficial effect on the health of the host. Taking into account the worldwide obesity epidemic and the importance of using new probiotic strains for this disease, our working group selected strains which showed probiotic effects in animal models of diet-induced obesity. The study of beneficial properties attributed to isolated microorganisms constitutes a field of interest for the development of functional foods. A fundamental requirement that contributes to the development of these foods is to determine the degree of food safety of probiotic strains before they are marketed. Lactobacilli are generally regarded as safe (GRAS) and most of them are included in the Qualified Presumption of Safety (QPS) list of the European Union due to the long history of use in fermented dairy products and their presence in the human intestinal tract. However, certain *Lactobacillus* strains have been associated with cases of sepsis, endocarditis, or bacteremia, mostly in association with a severe underlying disease. On the other hand, the absence of acquired antimicrobial resistance is an important criterion for evaluating the safety of lactic acid bacteria used as food started or probiotics. The aim of this study was to evalue the safety of strains with probiotic potential in obesity: *Lactobacillus casei* CRL431, *Lactobacillus fermentum* CRL1446, *Lactobacillus plantarum* CRL353 y *Lactococcus lactis* 1434. Our workgroup has assessed many functional properties of this *Lactobacillus* strains, but parameters regarding safety must be studied before calling them probiotics. In this work, safety aspects of *Lactobacillus* strains were studied. None of the strains tested caused a- or b-hemolysis. All the strains were susceptible to tetracycline, clindamycin, streptomycin, ampicillin, erythromycin, kanamycin, gentamicin, vancomycin and chloramphenicol. We also did not find antibiotic resistance genes for any of the strains studied. Mice treated daily with an oral dose of 108 CFU during 21 days showed no signs of pain, lethargy, dehydration, or diarrhea, and the histological studies were consistent with those findings. No translocation of microorganisms to blood, spleen, or liver was observed. Regarding these findings, *Lactobacillus casei* CRL431, *Lactobacillus fermentum* CRL1446, *Lactobacillus plantarum* CRL353 y *Lactococcus lactis* 1434 strains are microorganism GRAS with a great potential as probiotic for obesity.

FM-018

EFFECT OF PHENOLIC COMPOUNDS ON THE GROWTH OF LACTIC ACID BACTERIA ISOLATED FROM WINE AND WINERY WASTES

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Red wines contain a high concentration of polyphenolic compounds, which are known for having beneficial effects on human health due to their potent antioxidant properties. In addition, polyphenolic compounds contribute to the bitterness, astringency and color of the wine. Phenolic composition varies among different wines depending on the type of grape used, winemaking conditions, yeast type carrying out alcoholic fermentation and weather factors. On the other hand, the major part of grape polyphenols comes from solid fruit parts, thus a high proportion of them remains in the solid residues like pomace, which is left after juice extraction during winemaking. Other winery wastes are the residues called lees that form at the bottom of recipients containing wine. However, there is a lack of information on the lactic acid bacteria metabolism isolated from winery residues and the influence of phenolic compounds. The aim of this study was to determine the effect of some phenolic acids, normally associated to wine and winemaking residues, on the growth of LAB strains isolated from Argentinean red wines, pomace, and lees. At the same time the cellular morphology and homo- and hetero-metabolism type of the tested strains were determined. Cells grown aerobically at 30 °C until late exponential phase in MRS medium enriched with L-malic acid (3 g/L), pH 4,8 (MRSM), were inoculated at a rate of 3% (v/v) in MRSM medium added with two different phenolic acids. Concentrated solutions of a hydroxybenzoic acid (gallic acid) and a hydroxycinnamic acid (caffeic acid) were prepared in ethanol (99.5% v/v) and added after sterilization to growth medium to obtain a final concentration of 250 mg/L. In the control assay ethanol (5%) was added instead of phenolic compound. Each individual assay was made in duplicate and incubated at 30ºC for 96 h. Bacterial growth was monitored by periodic measurement of optical density at 560 nm, using a microplate reader. Both phenolic acids tested had a negative effect on the growth of all *Oenococcus oeni* isolated from wine or lees, and the strongest inhibitory effect was observed with caffeic acid. In presence of caffeic acids, the growth inhibition of *O. oeni* isolated from wine (between 39-65%) was higher than the inhibition observed in *O. oeni* isolated from lees (36%). The growth inhibition detected with the addition of gallic acid was around 16-29%in *O. oeni* isolated from wine and 17% in *O. oeni* isolated from lees. The higher antibacterial effect of caffeic acid compared to gallic acid could be in relation to their propenoic chain. The growth of heterofermentative rod bacteria isolated from pomace and lees were not modified significantly, in presence of gallic or caffeic acid. Only, in one of them an increase in growth was observed in presence of gallic acid. Whereas, the growth of homofermentative rod shaped rod bacteria isolated from pomace and less was inhibited with the addition of gallic or caffeic acids.

FM-019

GLUTATHIONE ADDITION IMPROVES *Oenococcus oeni* ADAPTATION TO STRESS CONDITIONS OF WINEMAKING PROCESS

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During wine-making process after alcoholic fermentation can take place the malolactic fermentation (MLF). This secondary fermentation consists in the convertion of L-malic acid into L-lactic acid and it is an important step to obtain high quality wines. *Oenococcus oeni* is the best adapted species and is almost exclusively used as starter culture of MLF. However, viability and malolactic activity of *O. oeni* in wine depend on its resistance to several stress factors such as low pH and high concentrations of ethanol. Glutathione (GSH) is a non-proteic tripeptide that acts as an antioxidant and is considered a protective agent against adverse environments. The objective of this study was to investigate if glutathione addition can improve tolerance of *O. oeni* against stressful wine conditions. Two strains of *O. oeni*, X2L and Sb10, were cultured in MRS medium with and without GSH addition (5mM). These pre-cultures were sequentially inoculated in grape juice fermented by *Saccharomyces cerevisiae* mc2 containing L-malic acid (3 g/L). Bacterial growth was followed by viable cells counts and MLF was monitored using an enzymatic method. In addition, expression levels of *mleA* (malolactic enzyme), *hsp18* (heat shock protein) and *citE* (citrate lyase) genes were evaluated by RT-qPCR. In presence of GSH, both strains of *O. oeni* showed higher biomass after 48 h of incubation being greater the GSH effect for the strain Sb10. During the sequential inoculation in fermented wines, *O. oeni* X2L and Sb10 showed higher viability and malolactic activity when were pre-adapted with GSH completing MFL at 4 days while cells pre-cultured without GSH were not able to remove of L-malic acid after 7 days. Moreover, in presence of GSH higher expression level of *mleA* gene was found in correlation to better MLF of *O. oeni* cultures pre-adapted with GSH. Genes involved in stress response, *hsp18* and *citE*, also showed differential expression in pre-cultures grown with GSH. In conclusion the GSH addition had a positive effect on *O. oeni* growth during preparation of starter cultures and this previous adaptation step was able to improve the MLF performance of *O. oeni* when was inoculated in wines. These findings demonstrate the protective role of GSH against stressful conditions related to wine-making process.

FM-020

THE LPS CORE GLYCOSILTRANFERASE *wabH* IS ESSENTIAL FOR COLD GROWTH IN in *Pseudomonas extremaustralis*

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Temperature is a key factor for bacterial survival and growth. Although most of microorganism could suffer transient changes of temperature, the psycrophiles and psycrotolerant microorganism have developed different adaptation strategies for growth under low temperatures. In Gram-negative bacteria, the lipopolysaccharide LPS is the major component of the outer membrane. In this work we studied the impact of a mutation in the gene *wabH*, encoding for a glycosyltranferase of the LPS core, in growth and survival under cold conditions in *Pseudomonas extremaustralis*, an Antarctic bacterium. Under low temperature, the *wabH* mutant strain was impaired to grow in solid and liquid cultures and to develop visible colonies and to grow in liquid cultures. Stress resistance assays showed that the oxidative stress resistance and the sensitivity to gentamycin was similar between the wild type and the mutant strain showing that the defect of growth under low temperatures was not due a pleiotropic phenotype. Additionally, we analyzed the envelope permeability in a SDS survival colony count assay. The *wabH* strain showed lower resistance to SDS in comparison to the wild type strain (40.9±11.8 and 12.2±.0%, respectively) suggesting a more permeable state for the mutant strain. Additionally, Nanomechanical measurements using an atomic force microscopy were performed to determine the biophysical behavior of the envelope, at 30oC for the wild type, the mutant and the complemented strain and at 8oC for the wild type and the complemented strain. The nanomechanical measurements showed that the mutation of *wabH* affects cell elasticity since the *wabH* strain presented a higher Young module value (E). These results suggest a more "rigid" state in the *wabH* strain than in the wild type. Additionally, the wild type strain presented differences between temperatures, showing a lower E value at 30oC in comparison with 8oC. Our results showed a key role of core LPS in cold adaptation by affecting cell elasticity.

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MODALIDAD ORAL

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MODALIDAD POSTER

IN-001

BACTERIAL QUORUM SENSING MOLECULES MODIFY THE OXIDATIVE STRESS RESPONSE IN THE ENDOPHYTIC YEAST *Meyerozyma guilliermondii* 6N

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It has been largely described that quorum sensing (QS) systems, regulatory mechanisms whose activities are related to the cell density of the population, play key roles in the regulation of the microbial physiology. Through the production and release of signal molecules, the cell senses the density of the population and modifies its physiology accordingly, repressing or inducing genes. It is well known that QS signal molecules have also an important effect on the host. However, up to date little is known about the effect of these compounds on the physiology of fungi that can colonize the same niche than the producing bacterium. To analyze this aspect, we characterized the response to bacterial QS molecules of *Meyerozyma guilliermondii* 6N, endophitic yeast isolated from sugarcane. Noteworthy, this plant is also colonized by bacteria harbouring active QS systems. Considering what has been described in plant and mammal cells, we focused in the modification of the oxidative stress response of the yeast by the QS molecules. *M. guilliermondii* 6N was cultivated aerobically in complex medium supplemented independently with 12 different AHLs (C6-HSL, 3OC6-HSL, C8-HSL, 3OC8-HSL, 3OHC8-HSL, C10-HSL, 3OC10-HSL, 3OHC10-HSL, C12-HSL, 3OC12-HSL and 3OHC12-HSL) and the oxidative stress response to different compounds was analyzed by flow cytometry. The detection of dying cells was evaluated after staining with propidium iodide (PI), while the oxidative stress was analyzed through the detection of reactive oxygen species (ROS) with the fluorescent probes dihydrorhodamine 123 (DHR123), dihydroethidium (DHE) and dihydrofluorescein diacetate (DHFDA). Results obtained show that under the assayed conditions, the levels of ROS induced by diamide, menadione or cadmium were not modified by any of the AHLs assayed. However, measurements performed with DHE showed that a group of AHLs, in particular those with a long acyl chain (i. e., C12-HSL, 3OC12-HSL and 3OHC12-HSL), augment the levels of ROS induced by *tert*-butyl hydroperoxide, an alkyl hydroperoxide, in *M. guilliermondii* 6N, suggesting increased levels of superoxide anion. These AHLs also increased the level of dying cells of *M. guilliermondii* 6N, as determined with IP. Noteworthy, the observed responses (higher levels of ROS and dying cells) were directly related to the concentrations of the AHLs. These findings show that the response of the endophytic yeast *M. guilliermondii* to specific stressors is affected by the presence of certain AHLs. The modification of the fungal physiology by these quorum sensing molecules also suggests that the biological activity of AHLs is broader than previously known.

IN-002

INFLUENCE OF ACENOCOUMAROL ON THE INTERACTION BETWEEN *Bifidobacterium* AND THP-1 CELLS.

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Bifidobacteria are microorganisms widely used as probiotics. In previous studies we demonstrated the strain-dependent immunomodulatory effects on THP-1 cells, a model of phagocytic cells. It has been demonstrated that probiotics modify the bioavailability of certain drugs but there is no evidence of the effect of drugs on probiotic activity. Our goal is to study the effect of acenocoumarol, a widespread oral anticoagulant, on the interaction between Bifidobacteria and THP-1 cells. THP1 cells were differentiated with phorbol miristate acetate 200 nM in DMEM (10% fetal bovine serum) for 48h at 37ºC/5% CO2. *Bifidobacterium bifidum* strain CIDCA 5310 and *Bifidobacterium adolescentis* strain CIDCA 5317 were cultured (48h; 37ºC) in MRS broth in anaerobic conditions. To evaluate the effect of the drug on the phagocytosis activity, FITC-labeled bacteria were incubated with cells at multiplicity of infection=10 and acenocoumarol at different concentrations for 1h at 37ºC/5% CO2. Phagocytosis was evaluated by flow cytometry. For quenching of non-internalized bacteria, trypan blue was used. UI (Uptake Index) = Percentage of FL1 (+) cells x mean fluorescence intensity, was calculated. Intracellular localization of bacteria was evaluated by confocal laser microscopy by using Lysotracker DND-99 and Transferrin Alexa-594 as markers of acidic and recycling compartments respectively. The expression of HLA-DR and TLR2 was measured by flow cytometry after 16h of incubation with the strain CIDCA 5310 and the drug. Medium Fluorescence Intensity (MFI) was determined. A decrease ($P<0.05$) in the internalization of strain CIDCA 5310 was observed in the presence of acenocoumarol 472 µM (UI 11015.93 ± 707.46) as compared with controls without the drug (UI 14895.53 UI ± 331.61). No effects were observed on the uptake of strain CIDCA 5317. The presence of the drug did not modified trafficking of bacteria to acidic compartments and no co-localization in recycling endosomes was detected irrespectively of the presence or not of the drug. Strain CIDCA 5310 diminished the expression of HLA-DR in IFN-gamma-stimulated cells (MFI=263.89 ±10.65) as compared with controls without bacteria (1288, 02 ± 127, 28). The presence of acenocoumarol partially abrogates the effect of bacteria (481.32 ± 89.46). Interestingly, the lowest values of MFI were detected when THP-1 cells were incubated with INF-gamma in the presence of the anticoagulant alone (41.40 ± 5.64). On the other hand, acenocoumarol decreased the expression of TLR2 triggered by strain CIDCA 5310 (573.04 ± 99.36) as compared with the strain alone (1832.05 ± 433.33) $p<0.05$. Our results suggest that the immunomodulatory effects of potentially probiotic *Bifidobacterium* strains can be significantly modified by acenocoumarol.

IN-003

ISOLATION AND CHARACTERIZATION OF ENDOPHYTIC BACTERIA FROM *Handroanthus impetiginosus*.

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Handroanthus impetiginosus "pink lapacho" (Bignoniaceae) is a medicinal, ornamental and forestal tree from the northwestern of Argentina. Propagation by seeds or cuttings of the pink lapacho is difficult so *in vitro* culture methodologies and biofertilization are recommended technologies. Plants usually interact with endophytic and rhizospheric bacteria with mutual benefits. Plant growth promotion by biofertilizers is mainly attributed to improve the acquisition of essential nutrients like nitrogen and phosphorus, or modulate the level of vegetal hormones. Plant growth promoting bacteria (PGPB) isolated from pink lapacho are scarcely described. The aim of this work was to isolate and characterize native endophytic bacteria from leaves and roots of pink lapacho trees grown in National University of Luján, and to analyze their potential as PGPR. Superficial disinfected tissues were placed on nutritive agar plates and on nitrogen-free agar (Nfb) supplemented with antifungal and incubated at 30ºC until bacterial growth was observed. Morphology of colonies, Gram staining and catalase activity were determined. Indole acetic acid (IAA) production of isolated strains was colorimetrically evaluated in Luria-Bertani (LB) medium supplied with tryptophan (2.5 mg/mL) using Salkowski's reagent. Strains able to solubilize inorganic phosphate were identified using Pikovskaya agar supplemented with Ca3 (PO4)2 (5 g/L) and green-bromocresol (PVK). Phosphate solubilization index (PSI) was recorded. Strain salt tolerance was evaluated in LB medium supplemented with 5% or 10% NaCl. Bacterial identification was carried out by partial amplification and sequencing of 16S rRNA gene (V1 and V3 regions). Sequences were aligned to reference genomes retrieved from leBIBI database. Phylogenetic inference was performed by the maximum likelihood method followed by bootstrap analysis (1000 pseudoreplicates) as implemented in MEGA v6.0 software. Bacteria isolation of leaves and roots allowed obtaining 13 and 6 strains, respectively. Seventeen strains were able to grow in Nfb indicating biological nitrogen fixation. All strains were able to grow in PVK although 13 showed solubilization halo. From total isolated strains, 10 were positive for IAA production. Leaf isolates (n=8) produced IAA concentrations between 4.37 and 20.80 mg/ml whereas root isolates (n=2) produced 19.4 and 41.6 mg/ml. Of them, root isolates were able to grow in medium with 5% NaCl and only one also grew at 10% NaCl after 48 h. Among leaves endophytes, four isolates grew at both concentrations of NaCl. Molecular identification allowed determining *Bacillus*, *Paenibacillus*, *Pseudomonas* and *Methylobacterium* as endophytes genera. In conclusion, the isolated strains present plant growth promoting properties that can contribute to increase environmental sustainability in agriculture.

BIOREMEDIACIÓN Y BIOCONTROL

MODALIDAD ORAL

IN-004

BIOFILM FORMATION OF *Pseudomonas syringae* IS AFFECTED BY A GERMIN LIKE PROTEIN (GLPI)

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Biofilm formation is a necessary step for an efficient surface colonization by microorganisms. Biofilms are communities of multi-specific cells, which are organized in cumulus and adhered to each other by extracellular polymers (proteins, polysaccharids, nucleic acids) secreted by themselves. All this determines the formation of an extremely irregular film, with pores and inner channels, through which the surrounding fluids can freely flow.Inside this irregular film, the microorganisms can be specialized and they can adopt biochemical characteristics not observed in planktonic cells. This can explain phenomena like the persister cells after antibiotics treatments, pathogenicity and beneficial interactions between microorganisms and plants. In our laboratory we study a protein purified from extracellular fluid of wheat leaves. This protein was called "Germin like protease inhibitor" (GLPI) and it has multiple enzymatic activities. Besides, GLPI has a possible role in plant defense response against pathogenic microorganisms. Given the importance that biofilms formation has for the interaction between plants and microorganisms, we decided to investigate the possible effects of GLPI on this process. Biofilm formation of a phyto-pathogenic bacterium (*Pseudomonas syringae*) and a halophilic archaea (*Halobacterium salinarum*) was determinated in batch cultures. Two inert surfaces (glass and polystyrene) were tested and different GLPI dosses and times of incubation were analyzed. The results indicate that GLPI stimulates almost 100% the biofilm formation in glass by *H. salinarum*, meanwhile, it inhibits 32% the adherence of *P. syringae.* Based on this, the next objective will be study the effect of GLPI on beneficial bacteria biofilms growing upon *Solanum lycopersicum* roots and analyze the mechanism involved in this interaction.
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BIOREMEDIACIÓN Y BIOCONTROL

MODALIDAD POSTER

BB-001

MICROFLUIDIC DEVICES FOR THE ASSESSMENT OF THE PAHs REMOVAL CAPACITY BY BACTERIAL BIOFILMS

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Microfluidics is the study and manipulation of fluids in micrometer scale structures. It provides promising systems for lab-on-a-chip (LOC) applications. LOC offers faster, parallel, and high throughput (bio) chemical analysis and screening on miniaturized systems with several advantages such as reductions in sample volumes and manufacturing cost. The LOC application for the study of organisms is an emerging field, where miniaturization system benefits offer a precise spatiotemporal control over the microenvironments of soil organisms with approximation of natural conditions. The aim of our work is to propose the microfluidic device as a platform to study the capacity of PAH degradation by bacterial biofilms. The microdevices were built with glass base and PDMS cover. PDMS was mixed with curing agent in a 10:1 ratio and then the mixture was placed under vacuum to remove air bubbles, poured onto the SU8-mold and cured in an oven at 80 °C overnight. The microchip consists of an input and an output connected with four microchannels of 496 mm wide with 4 cisterns in each of 1690 mm in width and a total internal volume of 32.22 mL. The microchannels were washed with ethanol 70% and it were desinfected using NaOH 0.5 mol.L-1 for 30 minutes. For biofilm formation on the microchannels, a continuous culture of the bacterial strain *Pseudomonas monteilii* P26 was carried out using the microchip as the bioreactor. After 3 days of culture, good cell adhesion to the substrate and biofilm formation inside the microchannels were observed. After, a PAH suspension containing 50 ppm of a mix of acenaphthene, fluoranthene and pyrene was pumped through the microchip in a closed loop at room temperature for 4 days. After this time, the remaining PAH in the system was solubilized with acetone and quantified by RT-HPLC. Results showed 79.2%, 56.2% and 55.0% removal of acenaphthene, fluoranthene and pyrene, respectively. For comparison, a culture of planktonic cells of *P. monteilii* P26 was incubated in presence of the same PAHs concentration for 30 days. This culture was able to remove 76.96% acenaphthene but no significant removal of fluoranthene and pyrene was observed. Our results have shown that using the microchip as culture system improved the PAH removal capacity of *P. monteilii* P26. This microfluidic device has proved to be a valuable tool for quickly screening of PAH removal capacity by biofilms.

BB-002

EFFECT OF THE USE OF NATIVE MICROORGANISMS IN THE EFFLUENTS TREATMENT FROM TABLE OLIVE INDUSTRY.

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Mendoza is a province that produce table olives in large quantities, where the water resource is limited so it is fundamental a sustainable use of it to preserve its quality. During the production process of Spanish style fermented green olives, large amounts of effluents are generated, with physicochemical characteristics that give them high pollutant power. The wastewaters from the debittering stage is a dark brown liquid due to its content of polyphenols and high quantity of organic matter; presents high salinity and alkalinity due to the use of a 2% sodium hydroxide solution. In order to reduce these contamination parameters, different bioremediation tests were carried out, using microorganisms from evaporation ponds from the originated effluents during the elaboration of table olives. The aim of this work was to evaluate the use of microorganisms from an evaporation pond on the contamination parameters, measuring the variation of pH, total acidity, polyphenol content and organic matter content from the debittering wastewaters of Spanish style fermented green olives. Two tests were carried out using equipment with constant agitation and controlled temperature, one of them with the addition of an exogenous source of carbon (Test 1) and the other without the addition of the same one (Test 2). The volume of work was 15 liters in each one, corresponding to 25% of the debittering effluent; 10% of mud rafts (inoculum) and 65% of distilled water. It was supplemented with mineral salts and antifoaming agents. The content of organic matter, such as COD and BOD, was measured at the initial and final moment; every day was measured pH, total acidity, polyphenol content and microbial growth by surface plate counts. Isolates of cultivable microorganisms were carried out in a selective medium with the effluent to determine their nature. The duration of each trial was 14 days. The results obtained, in the Test 1, showed a great decrease of pH, arriving to 5 pH units. In contrast, in the Test 2, there was no marked pH decrease. Regarding the organic matter content, the percentage of decrease of COD and BOD5 was 91% and 94%, respectively, in the Test 1; while, in Test 2, the percent reduction of COD and BOD5 was 48% and 49%, respectively. The maximum percentage of total acidity was higher in the first test; as well as the polyphenol content declined sharply in this experiment. The presence of mixed cultures of bacteria and yeasts was detected. The results obtained are encouraging to achieve the bioremediation of the effluent and thus contribute to the reduction of the pollution generated.

BB-003

LACTOBACILLI STRAINS AND THEIR METABOLITES AS ANTAGONISTIC AGENTS OF *Escherichia coli* O157 AND NON-O157 GROWTH

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Shiga-toxin producing *Escherichia coli* (STEC) is a major foodborne pathogen causing Hemolytic Uremic Syndrome (HUS), which is a severe human disease worldwide. Several serotypes are commonly associated with human diseases but *E. coli* O157:H7 is the most frequently isolated. Nowadays, lactobacilli have gained an increasing scientific interest as a biological option for the control of this pathogenic microorganism. The objective of this study was to evaluate the inhibitory activity of lactobacilli strains on *E. coli* O157:H7 and non-O157 strains. Six strains of bacteriocin-producing lactobacilli identified as *L. fermentum* L23, *L. brevis* L52, *L. plantarum* L54 and L57, *L. cellobiosus* L56, *L. rhamnosus* L60 were tested. Thirty STEC strains, serotypes H7:O157 (n=17) and non-O157 (n=13), were selected as indicator microorganisms. The antimicrobial activity of each lactobacilli strain was evaluated by two *in vitro* techniques, the streak-diffusion and well-diffusion methods. Firstly, a central streak of lactobacilli strain was seeded on an MRS agar plate and then different STEC were perpendicularly streaked. Plates were incubated in microaerobic conditions at 37°C for 24h. Average inhibition zones of bacterial growth were measured in mm. The inhibitory activities of different metabolites released by lactobacilli were tested by the well-diffusion method. Thus, two types of cell-free supernatant of lactobacilli growth, CFS and N-CFS, were obtained. CFS contains all produced metabolites (organic acid + bacteriocin), while the neutralized CFS (N-CFS) only contains bacteriocin. Six selected lactobacilli strains inhibited 100% of STEC strains. The average values of inhibition zones by the streak-diffusion method were 18.7 mm; 30 mm; 27 mm; 29 mm; 28 mm and 20.5 mm for L23, L52, L54, L56, L57 and L60 strains, respectively. By the well-diffusion method, the SLCs showed values of inhibition halos which ranged between 22 mm and 23.7 mm, being *L. plantarum* L57 the strain with higher inhibition activity. The supernatants maintained important antimicrobial action after neutralization treatment. The mean inhibition halos obtained with N-CFS on different STEC were 16.6 mm; 15.3 mm; 17.2 mm; 16.5 mm; 15.2 mm and 16.4 mm for L23, L52, L54, L56, L57 and L60 strains, respectively. It is important to highlight that the inhibition effect found was mainly due to the bacteriocins. In relation to the total antimicrobial activity, the inhibitory action specifically due to bacteriocins reached values between 65% and 75%. Among the STEC serotypes studied, the O157 strains were the most sensitive to both, CFS and N-CFS, in comparison to non-O157 strains. In conclusion, the thorough selection of these lactobacilli strains could represent a valuable biological strategy for control of shiga toxin-producing *E. coli* strains. Furthermore, future studies employing combinations of these strains could enhance their antimicrobial power.

BB-004

BIODEGRADATION OF THIOCYANATE MEDIATED BY BACTERIA ISOLATED FROM WATERS AND SOILS FROM VELADERO INFLUENCE AREA (SAN JUAN)

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The mining company installed in Veladero, San Juan, uses cyanide compounds for gold leaching. These species react with the sulfide present in gold bearing ores, producing thiocyanate (SCN-), wich despite being less harmful still affects the development of aquatic species. Fortunately, SCN--degrading bacteria can be isolated from this environments being able to be applied to bioremediation strategies for mining wastes. The objective of this study was to isolate and select microorganisms with SCNbiodegradative capacity from areas adjacent to mining industries. Water and soil samples were taken from Veladero surroundings (M1 and M2 30 ° 03 '0.4 "S 69 ° 10' 9.8" W, M3 30 ° 10 '12 "S 69 ° 06' 14" W and M4 30 ° 12'24.6 "S 69 ° 2 '27.6 ° W). An enrichment was successfully performed in minimal medium (M9-KSCN-glucose: Na 2HPO4 6 g/L, KH2PO4 3g/L, NaCl 1g/L, CaCl2 0,01g/L, L MgSO4 0,5 g/L, FeSO4 0,04 g/L, MnSO4 0,0015g/L, KSCN 0,2g/L supplemented with glucose 10 g/L) at 20 °C or 32 °C, 120 rpm, 4 days. Then the isolation of bacterial strains capable of using the SCN - as carbon, nitrogen and sulfur sources was carried out. Colonies were separated in agar M9-KSCN-glucose, agar M9-KSCN (KSCN as carbon source) and agar M9S-KSCN-glucose (KSCN as sulfur source). Biodegradation of SCN- was studied in time in M9-KSCN-glucose medium monitoring biomass production and SCN- removal for 7 days. Bacterial growth was measured by OD at 600 nm and SCN- concentration was determined by measuring supernatant absorbances at 466 nm after the reaction with FeCl3, previously removing bacteria by centrifugation (7000 *g*, 15 min). 82 bacterial colonies were obtained and, after successive spreads, only ten colonies were able to use SCN- as carbon and sulfur source. The SCN- was totally degraded after 2 days at 20 °C by M1/S30, M1/A17 and M373 strains and SCN - biodegradation was strictly related to bacterial growth. Thus, the minimal nutrient requirements of these three strains, along with the observed rapid thiocyanate consumption will provide a potential new tool for the bioremediation of gold mining soils and waters.

BB-005

CR (VI) REMOVAL COMPARATIVE EVALUATION BY USING FUNGAL MIXED CULTURES

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The use of resistant microbial consortia in heavy metals bioremediation processes has clear advantages over the application of pure cultures. In previous studies three Cr (VI) resistant strains were isolated from Chorrillos River, San Luis, Argentina: *Candida* sp. (C), *Wickerhamomyces* sp. (W) and *Trichoderma* sp. (T) these strains removed high Cr (VI) concentrations in liquid monocultures. The objective of this work was to evaluate and compare the growth and Cr (VI) specific removal capacity of mixed cultures. Interaction tests were realized in EG agar medium (g L-1: glucose, 10; K2HPO4, 0.125; KH2PO4, 0.125; MgSO4, 0.1; yeast extract, 1 and agar, 15) to check the antagonism between selected fungi in the presence or the absence of Cr (VI). 106 yeast cells or filamentous fungus spores mL-1 (to final concentration) were inoculated in 50 mL of EG liquid medium supplemented with the mentioned above Cr(VI) concentrations during 120h, at 30°C and 200 rpm. A biotic control (BC), under the same conditions, was evaluated. Every 24 hours, growth was evaluated by dry weight, Cr (VI) removal by colorimetric method of 1, 5-Diphenylcarbazide. Total chromium concentration in supernatants was analyzed by Atomic absorption spectroscopy (Shimadzu, AA-6800) at 24, 72 and 120h. An inhibitory effect between *Candida* sp. and *Trichoderma* sp. was observed in plate interaction assay; this was only observed in the presence of 75 and 100 mgL-1 Cr (VI). This test was repeated in liquid medium, and *Trichoderma* sp. spores germination inhibition was observed under all concentrations of Cr (VI) used in presence of *Candida* sp. Based on these results two double mixed cultures were studied: C-W and T-W. The microbial growth in mixed cultures was significantly affected under all Cr (VI) concentrations, a decrease of 50-60% in C-W and 70-80% in W-T were observed respect to BC. C-W showed the higher Cr (VI) removal than T-W under all concentrations studied to shorter times. These values were 88.8% ± 0.37; 74.50%±0.88; 57.66% ±2.05 and 46.8% ± 3.88 at 25, 50, 75 and 100 mgL-1 Cr (VI) respectively. No significant differences were observed in the total chromium removal between the different times in all concentrations. Total chromium for C-W decreased by 11.3% to 26.8% with 50 and 25 mg L-1 Cr (VI) at 72 h respectively and with 75 and 100 mgL-1 remained constant. On the other hand, T-W removed 15.6 to 20.8% with 75 and 100mgL-1 respectively and with 25 and 50 mgL-1 the total chromium concentration continued constant. The C-W double mixed culture was the most efficient to remove Cr (VI). These are promising results and it is expected, in future studies, to optimizing the culture medium to achieve Cr (VI) removal process more effective, efficient and economic.

BB-006

ANTARCTIC AND SUB-ANTARCTIC MICROORGANISMS AS PROMOTERS OF VEGETABLE GROWTH AND ANTIMICROBIAL PRODUCERS FOR THE DEVELOPMENT OF NATURAL BIOPESTICIDES

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La actividad antimicrobiana de microorganismos psicrófilos y/o psicrotolerantes puede ser utilizada en la prevención/tratamiento de enfermedades que afectan a diferentes cultivos regionales o como alternativa de productos químicos, dañinos para el medioambiente. Por otro lado, las bacterias promotoras del crecimiento vegetal (BPCV) pertenecen a un grupo de microorganismos benéficos, capaces de promover el crecimiento de plantas y a su vez, protegerlas de ciertas enfermedades. Las BPCV estimulan el crecimiento vegetal a través de mecanismos directos que aseguran la biodisponibilidad de nutrientes, tales como fijación biológica de nitrógeno, solubilización de fosfatos, producción de fitohormonas, etc. Los mecanismos indirectos, generalmente suceden fuera de la planta, y están asociados a la producción de compuestos sideróforos, antimicrobianos y a la competencia por nichos ecológicos. En este trabajo, las bacterias psicrotolerantes aisladas de suelo antártico y sub-antártico identificadas como *Burkholderia gladioli, Serratia proteamaculans y Pseudomonas yamanorum* fueron estudiadas *in vitro* para evidenciar mecanismos directos e indirectos de promoción de crecimiento vegetal en plantas. Luego se seleccionaron en base a los resultados obtenidos para estudiarlas *in vivo* en cultivos de plantas de soja. *Serratia proteamaculans* fue capaz de producir sideroforos, ácido indol acético y solubilizar fosfatos. *Burkholderia gladioli* y *Pseudomonas yamanorum* sólo demostraron tener capacidad de solubilizar fosfatos. En los aislados no se observó la formación de ácido cianhídrico. Se evaluó la actividad antagonista *in vitro* de *Burkholderia gladioli, Serratia proteamaculans y Pseudomonas yamanorum* frente a diferentes microorganismos fitopatógenos, donde se observó una marcada inhibición de los tres aislamientosfrente a bacterias del género *Xanthomonas, Erwinia, Acidovorax y Pseudomonas* como así también de hongos del género *Fusarium*, *Penicillium, Macrophomina, Phomopsis, Geotrichum* y *Diplodia*. Considerando los resultados, se seleccionó a *Serratia proteamaculans*, ya que demostró tener características de una BPCV, con potencial para mejorar el crecimiento vegetal y a *Burkholderia gladioli* como agente de biocontrol, por su amplio espectro de inhibición contra hongos que afectan al cultivo de soja. Ambas fueron evaluadas *in vivo* en plantas de soja.

BB-007

BACTERICIDAL AND BACTERIOSTATIC EFFECT OF BACTERIOCINS PRODUCED BY DIFFERENT LACTIC ACID BACTERIA STRAINS ON THE *Escherichia coli* O157:H7 GROWTH

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Currently, lactic acid bacteria (LAB) and their bioactive metabolites are widely studied for the biological control of pathogenic microorganisms. Enterohemorrhagic *Escherichia coli* is responsible of outbreaks and sporadic cases of hemorrhagic colitis and hemolytic uremic syndrome. *E. coli* O157:H7 is the major serotype associated with outbreaks in humans. The aim of this work was to determine the mode of action of the bacteriocins produced by *Leuconostoc mesenteroides* B19, *Pediococcus acidilactici* B82 and *Lactococcus lactis* B87 on *E. coli* O157:H7. Cell free supernatants (CFS) were obtained from a culture of each LAB strain in MRS broth. CFS were neutralized (NCFS) with NaOH 1N to eliminate the inhibitory effect of organic acid. NCFS containing bacteriocins of each LAB were added to *E. coli* O157:H7 cultures at the beginning of the exponential growth phase and incubated at 37°C for 72 h. A culture of *E. coli* O157:H7 without bacteriocin was included as a control. At different times, bacterial growth was measured by absorbance at 600 nm, viable cell counts on TSA plates and the growth curves were performed. The control of *E. coli* O157:H7 presented an exponential growth phase between 4 h and 8 h of incubation, reaching a maximum OD value of 1.45, which was correlative to a log value of bacterial count of 9.17 log CFU-1. Culture of *E. coli* O157:H7 with added NCFS containing B19 bacteriocin, showed OD values of 0.4 at 24 h, which represents a decrease of 72.41% in this bacterial population, in comparison with the control. Bacteriocins produced by *P. acidilactici* B82 and *L. lactis* B87 maintained very low the OD values of *E. coli* O157:H7 throughout all experience. During the viable cell counts, bacteriocins B82 and B87 produced a reduction of *E. coli* counts at 24 h of 3.54 and 4.81 log CFU ml-1, respectively. Furthermore, it is noteworthy that these two last bacteriocins were able to reduce until zero the bacterial counts, which was observed for B87 at 48 h and for B82 at 72 h. In conclusion, the bacteriocins produced by *P. acidilactici* B82 and *L. lactis* B87 demonstrated a bactericidal effect on *E. coli* O157:H7 growth, while that bacteriocin of *L. mesenteroides* B19 showed a bacteriostatic mode of action.

BB-008

Cu(II) BIOSORPTION MEDIATED BY *Pseudomonas veronii* 2E CELLS AND EXTRACELLULAR PRODUCTS IN BATCH AND CONTINUOUS REACTORS.

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Industries often apply chemical treatments to remove metals from their effluents to decrease their concentration and comply with current regulations. However, these treatments are not efficient enough for the complete metal removal. When metals remain in low concentrations a complementary biological treatment is required. This work was focused on the study of the interaction between Cu (II), used in electroplating processes, and *Pseudomonas veronii* 2E free and immobilized cells, or its extracellular products, in batch and continuous systems, for the development of innovative biotreatments. Cu(II) biosorption by *P. veronii* 2E cells, grown in M9- 2% glycerol, was evaluated both free (BL) and immobilized biomass (BI). For BL, biosorption kinetics was performed at 25°C and 32°C, supplemented with 1 and 0.5 mM Cu(II) and isotherms were carried out at 32°C and 1mM Cu(II), at pH 5.5. Cu(II) concentration was monitored by the Bicinchoninic Acid method. The kinetics experiences with 0.5 mM Cu(II) revealed a maximal biosorption of 86.25% and 88.59% at 32°C and 25°C respectively. While at 1mM Cu(II) maximal sorption was 86.90% and 79.76% at 32°C and 25°C respectively. The biosorption isotherms showed a saturation at qmax=0.523 mmol Cu(II)/g biomass. For BI, immobilization was performed on sterile loofa sponges for approximately 30 days at 32°C, with minimal agitation and culture medium renewal every 2-3 days. After immobilization, the sponges were washed with ultrapure water and dried at 37°C for 48 h. The amount of attached biomass was determined by weight difference. The BI was challenged against 1mM Cu(II) and metal was quantified in supernatants. The results showed that biosorption was of 34.2% by only BI, while the BI and the matrix adsorbed a 63.1%. In addition, three desorption cycles were performed in a batch system for BL at 32°C and 120 rpm. They consisted in exposing 50 mL of a BL-Cu(II) suspension to 1, 2 and 2.5 mL of 75 mM HCl for 30 minutes. Cu(II) desorptions of 51.2% for 1 mL, 63.6% for 2 mL, and 100% for 2.5 mL of 75 mM HCl were obtained. Soluble extracellular polymeric substances (EPSs), from *P. veronii* 2E grown in M9-2% glycerol at 32°C, 120 rpm, were obtained and placed in cellulose membrane dialysis bag. 34.15 mg of EPSs were exposed to 1 mM Cu(II)in a continuous flow rate of 12 ml/h at 25 °C, registering a retention of 15.8%. This work evidenced the ability of both of *P. veronii 2E* and EPSs to be able to biosorb Cu(II). Immobilizing bacteria represents a lower cost in a bioremediation process design, since it does not require growing cells and allows loofa sponges to be recycled. The metal recovery by desorption process implies an easy separation from the sorbent. *P. veronii* 2E with its great versatility and high Cu(II) biosorption capacity proved to be applicable in industrial effluent treatments when metal concentration is not adequate to implement a chemical process.

BB-009

Pseudomonas veronii 2E: A PROMISING CANDIDATE FOR THE DEVELOPMENT OF A METAL BIOSENSOR

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Electrochemical sensors have been widely applied since the evidence of many advantages such as high sensitivity and fast response. Developments of new sensors are studied for the detection of several metals due to their environmental relevance. Particularly, the carbon paste electrode (CPE) shows wide operating potential window, modification's possibility, new reproducible surface, miniaturization, easy fabrication and low cost. The aim of this work was to develop carbon paste electrodes modified with the metal biosorbent *Pseudomonas veronii* 2E biomass (MCPE) for the determination of Cd(II) and Cu(II) by anodic stripping square wave voltammetry. MCPE were built with a homogeneous paste containing 60 % graphite power (Arcair®), 30 % mineral oil (Estrella®) and 10 % *P. veronii* 2E dried biomass. Biomass was obtained as follows: *P. veronii 2E* was grown in nutrient both for 48 h at 32 °C and 120 rpm. Then cells were harvested by centrifugation at 5432 *g* for 10 min, washed twice with ultrapure water and finally dried at 32°C for 24 h. The paste was packed into a Teflon electrode body and stored at 4°C in water. The electrode surface was smoothed on a weighing paper before use. Focusing on Cd(II) detection, the first step was Cd(II) preconcentration on the electrode surface by immersing the MCPE in stirred 1-102 µM Cd(II) solutions for 5 min. Then, the electrode was washed with ultrapure water (18,2 mW.cm) and immersed in KCl 0,1 M. Cd(II) was determined by stripping square wave voltammetry. Deposition potential and time was studied. Best results were obtained at -1200 mV and 60 seconds. Anodic peak was only observed since -1100 mV. Cadmium peak was observed at -750 mV for concentrations between 1.0 to 102.5 µM. A typical response was observed with saturation above 50 µM, suggesting an interesting linear range for analytical applications in environmental matrices. MCPE resulted a promising candidate for the development of an innovative biosensor for Cd(II).

BB-010

MICROARRAY DATA TO ELUCIDATE HYDROCARBONS DEGRADATION CAPACITIES OF *Amycolatopsis tucumanensis* DSM 45259

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The analysis of catabolic capacities of microorganisms is currently often achieved by cultivation approach and by the analysis of genomic or metagenomics datasets. Recently, a microarray system designed from curated key aromatic catabolic gene families and key alkane degradation genes was designed. The collection of genes in the microarray can be exploited to indicate whether a given microbe or microbial community is likely to be functionally connected with certain degradative phenotypes, without previous knowledge of genome data. Herein, this microarray was applied to capture new insights into the catabolic capacities of polycyclic aromatic hydrocarbon (PAH) degrading actinomycete *Amycolatopsis tucumanensis* DSM 45259. *Aromadeg* was used as data base for reconstruction of the catabolic pathway. Furthermore, to validate the predictions, removal of hydrocarbons was performed in minimal medium (MM) supplemented with 500 mgL-1 of each compound. Target analysis by Liquid Chromatography-Mass Spectrometry (LC-MS) was further used to confirm the consumption of the initial substrates. The formation of key degradation intermediates in test cultures was compared to the abiotic (culture without cells) and biotic (culture without the aromatics) control cultures. As result, a total of 23 genes related with hydrocarbon catabolism were detected. The array data supported the presence of key catabolic genes in the DSM 45259 strain genome that confer the capacity to degrade aromatic hydrocarbons (naphthalene, biphenyl, phenanthrene, anthracene, pyrene, isopropylbenzene, ethylbenzene, tetralin and benzene), heterocyclic or substituted aromatic hydrocarbons (anthranilate, aniline, quinoline, 2-chlorobenzoate, 2,4-dinitrotoluene), single alkanes (n-decane and n-tetradecane) and several intermediates of the degradation of the mentioned compounds. The detected genes allow proposing the presence of the catechol pathway, the salicylate pathway and the phthalate pathway, as well as hydrocarbon degradation lower pathways. The presumptive ability of DSM 45259 strain to use the single alkanes, benzoate, phthalate and phenol as sole carbon sources would be inferred, which was experimentally validated by cultivation and mass spectrometry. Degradation occurred in the absence of glucose as co-substrate that was previously reported to be required for the degradation of naphthalene and phenanthrene. Interestingly, while *alkB* gene encoding an alkane hydroxylase is most likely highly similar to that found in other actinomycetes, the genes encoding benzoate 1,2-dioxygenase, phthalate 4,5-dioxygenase and phenol hydroxylase were homologous to proteobacterial. This occurrence suggests that strain DSM 45259 contains catabolic genes distantly related to those found in other actinomycetes. Together, this study not only provided new insight into the catabolic abilities of strain DSM 45259, but also suggests that this strain contains genes uncommon within actinomycetes.

BB-011

PRODUCTION OF DELTA ENDOTOXINS BY *Bacillus thuringiensis* USING COMPLEX SUBSTRATES

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The *Bacillus thuringiensis* delta endotoxins are widely used as insecticidal proteins. These crystalline inclusions show a wide range of specificity for different insect orders such as Lepidoptera, Coleoptera, Diptera and also to nematodes. Moreover, productivity of crystal proteins can be regulated by optimizing the concentration of complex substrates yielding an economic medium. On the other hand, agro-industrial raw materials and waste products are constantly produced being their final disposal sometimes associated with several environmental problems. Thus, bioconversion of these cost-effective substrates that are also locally available is turning in an interesting and a useful approach. In this connection, we previously applied a sequential optimization strategy involving a Plackett-Burman and a full factorial experimental design to maximize the production of crystal proteins; as a result the following culture medium formulation was obtained (in g/l): milk serum 7.5, starch 3.0 and soybean meal 10.0. In this work, both the supplementation of 10.0 g/l of vinasse (X1) and the ratio reactor volume/working volume (X2) were evaluated using a full factorial design. The native *Bacillus thuringiensis* RT from our own culture collection was used throughout this study. The crystal protein (Cry) concentration was determined by the method of dye elution in SDS-isopropanol using bovine serum albumin as a standard. Fermentations were carried out during 72 h at 30 °C and 200 rpm. Our results indicated that X1 has a significant and a positive effect on the production of both Cry 1 Ac ($p=0.008$) and Cry 2 Ab ($p<0.001$). While, X2 ($p<0.001$) and the interaction X1-X2 ($p<0.001$) only have a significant impact on the Cry 2 Ab production. In addition, the adequacy of each model was verified by the R2 (> 88.67) and the Adj R2 (> 80.17) coefficients indicating the percentage of variability that is explained by the model. Thus, our finding revealed that a careful balance of culture conditions should be established to increase delta endotoxins production. In addition, the biological activity of the improved product against *Spodoptera frugiperda* was also discussed.

BB-012

BIOLOGICAL SYNTHESIS OF METALLIC NANOPARTICLES MEDIATED BY AUTOCHTHONOUS AND NON-PATHOGENIC BACTERIA

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The synthesis of nanoparticles (NPs), especially metallic NPs, has accrued interest due to its unique properties that make them applicable in different fields of science and technology. The most common methods for preparing NPs are physicochemical, which generally use toxic reagents and/or complex and expensive synthesis steps limiting their final application. The biological synthesis has been studied in an effort to provide a green method of NPs production. The objectives of this study were: a) To study the extracellular biosynthesis of Ag, Au and Cu NPs mediated by *Pseudomonas veronii* 2E, *Klebsiella oxytoca* P2, *Klebsiella ornithinolytica* 1P and b) To explore the biotechnological applications of Ag-NPs specifically in antimicrobial activity. The effects of pH, temperature and culture media on NPs biosynthesis were examined and monitored by UV-visible spectroscopy. *P. veronii* 2E stationary phase supernatants from PYG medium (g/L: peptone casein 2.5, yeast extract 1.25, glucose 0.5), pH 7, 4 h darkness, 38 °C, 120 rpm showed maximal production of Ag-NPs. Cu-NPs were obtained by *P. veronii* 2E, *K. oxytoca* P2 and *K. ornithinolytica* 1P growing cells, in double concentrated PYG (25°C, 5 days, 120 rpm). Au-NPs were formed -after 24 h exposure to AuCl4 – 0,01% (m/v) - by both growing cells and culture supernatants of *P. veronii* 2E, *K. oxytoca* P2, *K. ornithinolytica* 1P in 1:2 diluted PYG and M9 (supplemented with glucose 5%(w/v) or glycerol 2%(v/v)). The characterization of the NPs biogenesis such as shape, monodispersity and size were studied using Scanning Electron Microscopy, SEM. The antimicrobial activity of purified Ag-NPs was tested towards reference species by diffusion in Muller-Hinton agar. The results showed an antibacterial effect for *Pseudomonas aeruginosa*, *Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Mycobacterium smegmatis* and *Escherichia coli.* Thus, Ag-NPs produced by a non-pathogenic bacteria result functional as antimicrobial agent. These results suggested the possibility of using a green, simple and eco-friendly biosynthesis as an approach in an innovative method to produce Cu, Ag and Au NPs. Future steps involve the detection of intracellular biosynthesis of Au and Cu NPs by transmission electronic microscopy, TEM.

BB-013

ARSENITE REMOVAL BY HETEROTROPHIC CONSORTIUM DOMINATED BY *Paenibacillus profundus*

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Arsenic (As) toxicity for living species depends on their chemical form; As(V) is a molecular analog to phosphate thus it can enter the cell using phosphate transporters and it might inhibit the production of energy by oxidative phosphorylation, meanwhile As(III) is generally considered more toxic because it binds to sulfhydryl groups which interferes with protein structure and function and it is also more mobile in the environment. Thus, the oxidation of As(III) to As(V) would be an important step to decrease toxicity and mobility of As in the environment. Natural environments exposed to arsenic, even those with low content, have been the source of microorganisms able to tolerate and metabolize arsenic compounds. The Copahue geothermal area, an extreme environment of volcanic origin located in Neuquen province (Argentina) with low As soluble concentration, has been the source of As tolerant species found in different enrichment cultures. Interestingly, *Paenibacillus profundus* was the most abundant microorganism identified in an heterotrophic consortium able to tolerate 20 mM of As(III). This culture also showed positive amplification for the *aioA* gene which encode for a periplasmic enzyme implicated in one of the main arsenic detoxification mechanisms in heterotrophs due to its ability to oxidize As(III) in to the less toxic As(V). The aim of this study was to evaluated the capacity of As(III) removal by this heterotrophic consortium called Het(As+3 20 mM). The consortium was inoculated in an Erlenmeyer flask with LB medium and supplemented with 100 ppm of As(III). Non-inoculated Erlenmeyer flask was set as sterile control. Both, culture and control, were made in triplicate and incubated in agitation at 30ºC. Samples were collected at initial time (T0) and at the exponential phase of microbial growth (T1). As(III) concentration was measured by Electrochemical Anodic Stripping using a gold disc electrode with a mercury film. The culture inoculated with the consortium Het(As+3 20 mM) decreased 41% the As(III) content, while in the sterile control the decrease was only 14%. This work evidence the ability of this consortium to remove As(III) from the medium making it a functional alternative to bioremediation processes in As contaminated sites. On the other hand, this is the first report of *Paenibacillus profundus* capacity to remove the As(III).

BB-014

CRY PROTEIN ANALYSIS WITH MOSQUITOCIDAL ACTIVITY AND ANTIMICROBIAL PEPTIDE SEARCH FOR THE CONTROL OF PATHOGENS VECTORIZED BY MOSQUITOES

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Bacillus thuringiensis is an entomopathogenic bacteria that produces a parasporal inclusion composed by Cry proteins, toxic against mosquitoes and other insects. When this crystal is ingested by insect larvae, is solubilized in alkaline gut environment and proteolytically cleaved by gut proteases. After binding to the specific receptors on the brush border membrane of the midgut epithelium, the activated toxin would lead to insect death. Recently a novel polycation peptide, BTM-P1 with antimicrobial (AM) activity, was described based on the amino acid sequence of domain I of some Cry protoxins. *Aedes aegypti* is the principal vector for Zika, chikungunya, yellow fever, and dengue worldwide and itis the main target in vectorial control program. Some *Culex* species are vector of some encephalitis viruses with relevance in public health. In this context, cry toxin studies result interesting as a putative alternative for biological control of *Aedes* and *Culex* mosquitoes. On the other hand, AM peptides can be used for the control of human pathogens vectorized by these insects. In this work we analyzed several Cry proteins isolated from a native strain with mosquitocidal activity against *Ae. aegypti*, *Aedes* (*Ochlerotatus*) *albifasciatus*, *Culex pipiens* and *Culex apicinus*. The isolated toxins were identified as Cry4-like1, Cry4-like 2, Cry19-like1, Cry19-like2 and Cry24Ca by phylogenetic analysis. We performed the alignment by ClustalW in MEGA Software between all known Cry proteins with mosquitocidal activity and the ones isolated from the native strain and we constructed a tree using the statistical method UPGMA. Then, we analyzed structural differences among native Cry proteins plus other toxins near the native ones according to the previous phylogenetic tree. We focused on: i) number of a-helix, ii) large of b-sheets, iii) loops similarity and iv) conserved motifs of functional importance. Cry native toxins show the typical domains (I, II and III) present in Cry mosquitocidal proteins. Structural analysis revealed that a motif located in a5 is conserved in all native Cry; this motif is involved in oligomerization which is necessary for pore formation. Moreover, specific residue located in a4-a5 loop is highly conserved among all native Cry and it is involved in lipid membrane interaction. In this context, we hypothesize that native Cry have mosquitocidal activity and Cry4-like1 and Cry4-like2 have at least the same efficiency that Cry4Aa and Cry4Ba. Furthermore, Cry proteins will be cloned and expressed in an heterologous system and the toxic activity will be measured by bioassays against mosquito larvae. *In silico* analysis shows that domain I of all native Cry toxins have hydrophobic regions, which could be used as templates for the generation of putative AM peptides. In conclusion, native Cry toxins are a promising option as biological agents for mosquito control or for the control of pathogens vectorized by them.

BB-015

NATIVE BACTERIOPHAGES INFECTING *Bacillus thuringiensis:* MORPHOLOGY AND THERMOSTABILITY

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The production of bioinsecticides based on *Bacillus thuringiensis* (*Bt*) is sensitive to bacteriophage infection. As lytic bacteriophages, they could act directly on the bacteria cells and/or bacteria could have prophages integrated to the genome or as a plasmid. Consequently, the isolation, characterization and identification of the viruses infecting entomopathogenic strains are crucial for improvement the industrial process. *Bt* RT (EF638795.1) is a native strain isolated from an indigenous *Spodoptera frugiperda* (*Sf*) larva, and its efficacy against different pests including *Sf* has been proven. In this work, we proposed the characterization of two environmental bacteriophages (M3 and M4) active against *Bt* RT. Those viruses were previously isolated from soil samples from Tucumán. Thermostability of M3 and M4 was evaluated as follow: Single lysis plaques of M3 and M4 phages were propagated in LB cultures of *Bt* RT. Suspensions were filtered (0,22 µm) and titrated by the Double Agar Overlay Plaque Assay. Thermal assays were carried out at 45, 50, 55, 60, 65, 70 and 75°C during 30 min. Samples were taken every 10 min. Later, 3 µl of each suspension were dropped onto a Bt RT lawn and incubated at 30°C ON. As a result, after 30 min of treatment at 60°C both bacteriophage suspensions were completely inactivated. For Transmission Electron Microscopy, a drop of purified bacteriophage was negatively stained with 1% uranyl acetate on formvar-coated copper grids and photographed on a Zaiss EM109 microscope (Oberkochen, Germany). This study showed tailed bacteriophages. So, according to the morphological analysis, both bacteriophages belonging to the order Caudovirales. In this order, M3 belong to the *Siphoviridae*family based on the observed non-contractile and flexible tail (length= 293 nm; width= 9nm) with a capsid width= 58.6 nm and a capsid height= 55 nm). M4 showed a morphology similar to the *Myoviridae* family with the peculiar contractile tail (length= 66 nm; width= 18 nm) and with an isometric head with a diameter of ~66 nm. In general, both phages exhibited similar behavior regarding temperature. The results obtained will allow us to design more effective control procedures to avoid contamination in fermentations with loss of the product which may be partial or total depending on the type of bacteriophage. In additionthe isolated bacteriophages could be also used for the phagotyping of this bacterial species. This work was supported by FONCyT (PICT 2011-2158 and PICT 2015- 2596), CONICET (PIP 339) and UNT (PIUNT E548/3).

MICROBIOLOGÍA AMBIENTAL Y DEL SUELO

MODALIDAD ORAL

MS-001

IDENTIFICATION OF 29 COMPLETE BACTERIAL GENOMES FROM TWO SMALL DAIRY INDUSTRY WASTEWATER STABILIZATION PONDS

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Stabilization ponds are the most common treatment technology in developing countries due to their low operation and maintenance costs. Normally, they are organized in several serial ponds that combine anaerobic and facultative processes. The microbial community composition in two stabilization ponds systems of dairy industries was analyzed using whole genome shotgun sequencing. Samples were collected from six full-scale stabilization ponds belonging to two small dairy industries (CYC and AUR) located in the center of Santa Fe in Argentina. CYC treatment consisted in four serial ponds, while AUR have two serial stabilization ponds. For this work, we carried out a contig binning strategy aiming to reconstruct complete genomes from the metagenomic reads. First, reads were assembled using IDBA_UD and bins were constructed using MaxBin2. The quality of the binning was evaluated using CheckM, which uses collocated sets of genes that are ubiquitous and single-copy within a phylogenetic lineage. Later, these bins were manually corrected, removing contigs with marker genes corresponding to distant lineages and all reads mapping to each bin were re-assembled in order to produce a better assembly with less contamination. The bins were taxonomical classified using marker genes from CheckM and annotated using the RAST annotation server. Last, to give extra support to the classification, the bins were placed in a phylogenetic tree and the predicted gene composition was compared to its nearest neighbor. In total, we found 29 complete bins (>90% of the markers present) with low contamination (<5% of duplicated markers), 20 from CYC samples and nine from AUR. Based on the markers found and the placement in the phylogenetic tree, 11 of CYC bins classified as Firmicutes, mainly Clostridiales. The rest corresponded to Proteobacteria (four), Bacteroidetes (four) and Spirochaetes. Among the AUR bins, four Proteobacterias, two Verrucomicrobias, two Actinobacteria and one Synergistetes were identified. All bins shared a high percentage of the genes with their closest neighbor in the same lineage, which supports the assignation proposed. The bins AUR008, AUR009, CYC015 and CYC067 had a larger proportion of unique genes, which suggest that these bins could represent more distant organisms. The reconstruction of complete genomes from metagenomic data is not only key to obtain information about uncultured microorganisms, but also to understand the process of substrate processing in systems like stabilization ponds, and represents a new way to identify genes of biotechnological interest.

MS-002

DETECTION OF CO-OCURRENCE PATTERNS IN SOIL MICROBIOTA USING GRAPH THEORY ON AMPLICON BASED METAGENOMIC DATA

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Soil microbial communities have a central role in the sustainability of agricultural systems, such as organic matter formation and losses, agrochemicals degradation and nutrient cycling. Also, there are microorganisms that have direct interactions with plants acting as phytopathogens, PGPR or symbionts. A common approach to study environmental microbiomes is through metagenomics. This is the study of genetic material of a whole microbial community involving DNA extraction from soil samples and next generation sequencing. A typical step in the analyses of metagenomic data is the clustering of DNA sequences in operating taxonomical units (OTUs) followed by, if possible, taxonomic designation of the OTUs. Soil is one of the environments with the largest diversity and quantity of microbial taxa; however, the structure and interaction of its microbial communities remain largely unknown. The aim of this study was to analyze the taxonomic profiles of OTUs and their interactions across different types of soils through a meta-analysis of six studies and use of co-ocurrence network methods. The dataset comprised 202 samples from six different environments: grasslands, crop soils, shrublands, and coniferous, tropical and mixed forests. First, the short-sequence DNA reads were aligned with a reference alignment. Then we determined the taxonomic designation of the reads at the family level using the 16S rRNA SILVA database with the mothur software. Then we filtered out contaminants, chimeras and non-target sequences. We further filtered our dataset by removing reads from families that could not be potentially amplified by the two different PCR primers pairs used in the different studies. After that we applied a subsampling method to equalize the sequencing depth of the different studies. Through the use of hierarchical clustering and NMDS we detected a tendency of the samples to group by study rather than by type of environment. To remove this bias we built co-occurence graphs for each study and then fused them using the Similarity Network Fusion method, obtaining a consensus network. This application of network analysis allowed the identification of the most important and hidden co-ocurrence patters within the microbiomes. We topologically characterized the network by calculating global and nodal parameters and found five significant clusters or communities of co-occurring families (graph-communities). These patterns of meta-communities were consistent across a wide number of environments. The simultaneous analysis of hundred of samples allowed us to caracterize how the taxons were related, we also discovered hidden connection patterns and found five graph-communities validated with a permutation test. Finally, we detected significative associations amonggraph-communitiesand environments.

MS-003

ANALYSIS OF SOIL BACTERIAL COMMUNITIES ASSOCIATED TO GENETICALLY MODIFIED DROUGHT TOLERANT CORN PLANTS

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The use of genetically modified (GM) plants have been developed worldwide since the last 30 years, these crops dominated the agricultural production systems in Argentina in a few years. It is known that different plant species influence the composition and activity of the rhizosphere microbiota through the modification of physicochemical factors in soil. Since microorganisms play a fundamental role in soil processes and plant growth, it is of the utmost importance to understand plant-microorganism interactions, including how GM plants can affect the soil microbial communities. The aim of this work was to analyze the bacterial community diversity associated to GM maize plants resistant to drought stress. In order to analyze whether GM plants impact on the soil bacterial community, experiments under controlled conditions were carried out. Plants were grown in pots with soil from Río Cuarto (RC) and Inés Indart (II) in a growth chamber for 60 days. Two lines of maize were used, one GM that over express the *Hahb4* gene and the wild-type line (B104). Soil moisture was maintained at 100 or 30% of soil field capacity. Bacterial diversity of soil samples was analyzed by amplicon sequencing of the16S ribosomal RNA gene V1-V3 region using Illumina MiSeq. The two analyzed soils were different in terms of a diversity but not differences in a diversity were found by comparing treatments within soils. In II there were no differences in b diversity between treatments, whereas in RC we found differences when comparing the samples with different corn line. The composition of the major groups was similar between soils, with *Proteobacteria* being the most represented with around 30% of the total reads, followed by *Acidobacteria* (17%) and *Planctomycetes* and *Verrucomicrobia* (10%) each one. In all samples the predominant genus was *Acidobacterium* with 14 to 20% of the reads and most genera had a relative abundance of 0.01-0.1%. Analysis were performed to determine bacterial genera affected by each irrigation condition and corn line. We found 23 genera significantly affected ($p<0.05$) by moisture level in II soil samples and 59 in RC samples. We also observed 29 genera that showed significant differences in relation to the corn line in II and 61 genera in RC soil samples. Treatments had less effect on II as compared to RC. In II, no significant differences were observed either with the irrigation regime or the type of plant and there were fewer affected genera. In RC soil samples a significant effect of the plant type was observed but not of the moisture. In conclusion, overexpression of the Hahb-4 gene in maize plants did not affect the bacterial communities of rhizosphere under the experimental conditions of the study. However, communities of soil bacteria were affected to some extent. In some cases the effects between treatments were not significant, but allowed us to detect differences in bacterial communities between the soils analyzed.

MS-004

SEASONAL CHANGES IN MICROBIOTA DIVERSITY OF SOILS FROM MORENO DISTRICTBUENOS AIRES EXPOSED TO INTENSIVE PERIURBAN AGRICULTURE.

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In Buenos Aires Metropolitan Area, small farm clusters are mostly located in the western districts as Cuartel V, Moreno. In this area, horticulture has been developed with high crop rotation and intensive use of pesticides and chemical fertilizers, evidencing soil deterioration. The aim of this work was to seasonally monitor bacterial diversity of a horticultural soil (S) and a reference soil without any declared practice in the last 20 years (R) to collect information for the design of future restoration strategies. Soil samples were taken in 2014 and 2016 springs, and 2015 and 2016 falls. The environmental conditions differenced each sampling time: spring 2014 was a rainy season with continuous flooding events, while fall 2015 and 2016 and spring 2016 were characterized by an increasing dry weather. The crop changed according the farmer´s needs, so the sample collection was before harvest for strawberries (spring 2014) and cabbages (fall 2015), post-harvest for red peppers (fall 2016) and of a resting soil in treatment with poultry litter as fertilizing amendment (spring 2016). Applications of agrochemicals depended on the crop. In S samples, chlorpyrifos, atrazine, trifluralin, deltamethrin, l-cihalotrin, iprodione, endosulfan, traces of procymidone and Cu-based products (Cotacuatro) were detected. Bacterial diversity was analyzed by the use of high throughput sequencing of the V1-V2 region of the 16S rRNA gene by Illumina Miseq and the obtained sequences were evaluated *in silico* using Qiime pipeline. All R soils showed less richness and diversity than S soils (measured by CHAO1 and Shannon index respectively). Phylum analysis of R soils seemed relatively constant in time, enriched in *Proteobacteria* (Class *Alphaproteobacteria*,Order *Rhizobiales*) and *Acidobacteria*. The effect of the intensive use of S soils was specially proved by differences in *Chloroflexi*, *Bacteriodetes*, *Actinobacteria* and *Proteobacteria* abundances. The evidence of the seasonal change in S soils was the increase in *Bacteroidetes* and Class *Betaproteobacteria* (Order *Burkholderiales*) in the flooded sample of spring 2014. The poultry litter addition to S soils during spring 2016 clearly affected the composition of *Firmicutes* and *Actinobacteria* fractions. Before spring 2016, *Firmicutes* main Class was *Clostridia*, while after poultry litter incorporation, *Firmicutes* main Class became *Bacilli* as in the amendment. Similar changes occurred within *Actinobacteria* Phylum from the Class *Thermoleophilia*, Order *Solirubrobacterales* to the Class *Actinobacteria*, Order *Actinomycetales*, dominant in the amendment. A weak recuperation trend of S soil microbiota was registered in fall 2015 samples as consequence of their post-harvest collection during an inactive period. These results clearly show the perturbation of microbiota, consequence of the climate and intense use of periurban agriculture soils and will contribute for further actions to improve environment quality.

MS-005

ADVANCES IN THE SEARCH FOR ELECTROGENIC HALOPHILIC MICROORGANISMS

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Electrogenic microorganisms are typically anaerobic and are able to channel their respiratory electrons to a polarized electrode. This capacity is of interest for bio-electrochemical technologies such as microbial fuel cells (MFC). If able to grow at NaCl concentrations ranging from 100 to 390g.l-1, microorganisms are recognised as hyperhalophiles, but so far no microorganisms have been described that meet both characteristics. Hypersaline environments typically present low oxygen solubility and are inhabited by a variety of microaerophilic and anaerobic microorganisms, but life at high salt concentrations is energetically expensive, due to the energy investment in sustaining internal homeostasis. This fact reduces the range of useful combinations between oxidants and reducers that can support cell growth, remaining unknown if polarized electrode can serve to these aims. In order to determine the presence of electrogenic hyperhalophilic microorganisms in the microbial community of Salitral Negro, enrichments were performed on liquid culture medium using fumarate as the only electron acceptor. This community was replicated to minimal media with acetate or lactate as an electron donor, in combination with different oxidizing compounds that would act as acceptors: NaNO3, Na2SO4, Thiosulfate, DMSO and a polarized graphite electrode. DNA extraction was performed on cultures that were positive for growth the gene encoding the 16S subunit of the rRNA was amplified. Amplification products were separated by electrophoresis on denaturing gradient gels (DGGE). DNA bands were extracted and re-amplified for further identification by sequencing. It was observed that the community enriched in fumarate reducers, was composed of both archaea and bacteria indeed capable of respiring NO3 -, SO4 -2, Thiosulfate and also a graphite electrode polarized to a positive potential. As the electrode was polarized to an oxidation potential a positive current could be recovered, presumably due to the growth of a biofilm using lactate as the electron donor. Work is still in progress to isolate anaerobic microorganisms capable to reduce the different compounds in order to learn more about these communities.

MICROBIOLOGÍA AMBIENTAL Y DEL SUELO

MODALIDAD POSTER

MS-006

SELECTION, CHARACTERIZATION AND EVALUATION OF PLANT GROWTH PROMOTING BACTERIA ISOLATED FROM ANDEAN VEGETATION IN SOYBEAN CROPS

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In a global scale, Argentina is the leading exporter of soybean oil and flour and the third largest producer of soybeans. Therefore, the cultivation of soybean is of great importance for the national economy. The production of soybean depends upon the soil as their main natural resource to ensure a good productive capacity. Therefore, the physical, chemical and biological properties of the soil need to be preserved. These properties are altered by the indiscriminate use of chemical fertilizers. Their negative environmental impact, and the continuous increase in their price as an agricultural resource, leads us to look for new alternatives that reduce the negative impact on the soil and, in turn, allow us to continue achieving optimum crop yields. This has led to a trend towards "clean production", aimed at reducing the use of chemical in fertilization and controlling phytopathogens. One of these cleaner alternatives is the one that involves biological means that do not harm the ecology, through the use of plant growth promoting bacteria. These bacteria can be free-living, and when they are associated with roots, near or inside the tissues, they increase the absorption of nutrients, fix atmospheric nitrogen, solubilize phosphates, produce growth regulators and siderophores, as well as reduce the attack of pathogenic microorganisms and insects. In this way, co-inoculation techniques in soybean with plant growth promoting rhizobacteria (PGPR) are of great interest since they would allow a reduction in the use of fertilizers, increase crop yields, decrease production costs and reduce environmental impact. The general objective of this work was to evaluate the growth promoting effect of previously isolated bacteria from the Andean vegetation rhizosphere, on soybean seeds grown in saline soil. For this purpose, the characterization and identification of 55 bacterial strains were carried out. This allowed selecting those with the higher potential for growth promotion, for later germination tests in the laboratory. The characterization consisted of the following biochemical tests: siderophores production, catalase activity, fixed nitrogen, protease production, solubilized phosphorus, and indoleacetic acid production. The results of these tests determined that from the 55 bacterial strains, 51 of them produce siderofores, 43 possess the catalase enzyme, 22 fixate nitrogen, 35 produce the protease enzyme, 41 solubilize phosphorus, and 48 produce indoleacetic acid. These results allowed the selection of 17 bacterial strains that were considered to have the potential of promoting plant growth, to be inoculated in soybean seeds and then, cultivated in soil. The differentstrains were identified by sequencing of 16sDNA genes. In addition, the following plant growth parameters were evaluated on the germinated plants: root length, stem diameter, fresh and dry weight, and nodule production. The most favorable results allow establishing those bacterial strains with better potential for plant growth promoting.

MS-007

EFFECT OF DIFFERENT NUTRIENT SOURCES IN THE BIOREMEDIATION OF SOILS CHRONICALLY CONTAMINATED WITH HYDROCARBONS AT CARLINI STATION, ISLA 25 DE MAYO, SOUTH SHETLAND ISLANDS, ANTARCTICA

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The logistics involved in the operation of Antarctic stations and the development of scientific activities entail the risk of contamination by fuels. Previous studies have shown that biostimulation of soils chronically contaminated with Antarctic Gasoil (AGO) produces significant increases in the removal of hydrocarbons (HC). In this work, we report on the effect of amendment with N and P in different formulations: inorganic salts, NH4NO3 y Na2HPO4, with (IN) and without (ANA) oxygenation by mixing; Nitrofoska® granular fertilizer (NPK) and OSEII® commercial product (CP), on the efficiency of HC removal from soils with chronic contamination by gasoil, compared to a system without nutrient addition (CC). The soil was collected from an area affected by the dripping of a connection between two diesel fuel pipes. Treatments were done in triplicate, simulating biopiles containing 15 kg of soil, which were sampled over 50 days, analyzing the changes in the bacterial communities involved and the quality and quantity of the remaining HC. Total heterotrophic aerobic (THAB) and hydrocarbon degrading bacteria (THDB) counts were performed on CPS agar and Gasoil agar. The quantification of hydrocarbons was performed by infrared spectrophotometry (modified ASTM D066 method) and by GC-FID. The V3-V4 region (GC-341F and 518R primers) of the 16S rRNA gene was partially amplified in all soil samples to obtain a fingerprinting of the bacterial communities by DGGE (45% -60% denaturing urea/formamide gradient, gels run at 60V and 65 °C for 16h). Both the presence of numerous bacterial populations and changes in their relative proportions in the community throughout the trial as well as the decrease in HC concentration in CC systems indicate that, even without nutrient aggregation, the native microbiota can metabolize HC with just aerating the soil and at the expense of the few available nutrients. However, biostimulated systems resulted in markedly higher HC removal with respect to CC, with CP as the most efficient. Bacterial counts supported these results, showing a greater proportion of degrading populations in biostimulated systems. With respect to the communities involved, although it is expected that any intervention in the microenvironment of microorganisms will influence its structure, particularly the aggregate of inorganic salts (IN, ANA) and granular fertilizer (NPK) selected communities less diverse than in CC, while the addition of OSEII® (CP) product led to the development of more diverse communities. Although natural attenuation contributes to the removal of HC in Antarctica, the activity of microorganisms plays a key role in accelerating this process, which is important in Antarctica given the short periods in which the soils are thawed and accessible to carry out a treatment. The addition of nutrients to balance metabolism favors the biodegradation of HC and increases their removal in shorter times.

MS-008

CHARACTERIZATION OF MINERAL-ASSOCIATED MICROBIAL ECOSYSTEMS (EMAM) IN LAGOONS OF THE ANDEAN PUNA

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The Andean Microbial Ecosystems Associated with Minerals (EMAM) are associations of bacteria, cyanobacteria and Haloarchaea that influence or induce the precipitation of minerals in lagoons, hydrothermal vents, fumaroles of volcanoes and Puna salares. They include microbial mats, microbialites, biofilms and endoevaporites. Those in the Puna are the highest described so far and due to the extreme conditions that give rise to altitude (high UV radiation, low O2 pressure, abrupt temperature changes, oligotrophy, etc.) these ecosystems are the most similar to the primitive Earth that is known in the planet. The general objective of this work is to characterize microbial ecosystems of different high altitude lagoons (Ojos de campo, Pozo Bravo, and a fumarola near the Diamond Lagoon) from the province of Catamarca, Argentina, including microbial mats and microbialites of sites not previously studied. DNA isolation of the samples were performed using different protocols, including CTAB, methods used for saline sediments, and kits for processing microbial mats (MoBIO). Subsequently a DNA quality analysis was performed by electrophoresis and UV absorption spectroscopy. The success of the methods employed was confirmed by PCR reactions with oligonucleotides to amplify the 16S rDNA gene (F27 and R1492). Amplicons libraries from the V4 region of the 16S rDNA gene, using the Bakt-341F and Bakt_805R oligonucleotides were constructed. These libraries were sequenced on the Illumina platform. Diversity data by 16S rDNA were analyzed using the QIIME software. This analysis allowed to define the biodiversity of the microbial ecosystems under study. The results indicate the presence of archaea and bacteria in these samples. A comparison of the study environments with other Puna environments previously analyzed shows that they are grouped with other microbial mats, differentiating them from other microbial communities with structures such as evaporites, but a clear difference is not seen with the microbialites. Some specific groups are more abundant in evaporite mats, such as the genus *Salinivibrio*. Microbial mats are divided into two classes. In the first group, there are abundant *Alphaproteobacteria* of the family *Rhodobacteraceae* and *Gammaproteobacteria* of the family *Marinicellaceae*, whereas in the second group there are important *Deltaproteobacteria* of the family *Desulfobacteraceae*. This work represents a new contribution to the bioprospecting of Andean Puna environments, and will help to select sites for further exploration.

MS-009

SELECTION OF VINASSE-DEGRADING MICROORGANISMS

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One of the main problems of the sugar-alcohol industries is the generation of large volumes of vinasse. The release of this acid effluent generated during the alcoholic distillation causes an undesirable environmental impact as consequence of the high content of organic matter and other toxins. Many technologies based on vinasse conditioning by microbial pathways are continuously evaluated in order to mitigate their environmental impact. In the current study, a preliminary selection of microorganisms from the soil with the potential to grow and degrade effectively the sugarcane vinasse was performed. To perform the isolation, two vinasse concentrations in distilled water (10 and 30%, v/v) added with 2% agar (w/v) were used as selective solid media (VS10 and VS30). An aqueous suspension obtained from a sugarcane vinasse-contaminated soil was inoculated in the VS media. A suspension obtained from same soil but without vinasse was also inoculated in these media to be used as controls. After 48 h of incubation at 30°C, spores of the microorganisms developed in the VS media were harvested and quantified to be inoculated in a liquid medium consisting of 30% vinasse in distilled water (VL30). At the 72 h of incubation in VL30 at 30°C, 150 rpm, the biomass concentration was measured by estimation of the dry weight at 80°C, while the biological oxygen demand was quantified by BOD5 standard method. Under the current assay conditions, microbial growth was not detected in the VS media from the soil samples without vinasse. However, two types of colonies from soil exposed to raw vinasse were isolated using VS30 as a selective medium. Microscopical observations of the two isolates denoted as V1 (spores pink) and V2 (green spores), revealed sparsely branched and septate hyphae as well as the presence of aspergillary heads. Both isolates were able to grow in VL30 and remove over 50% of the biodegradable organic matter, suggesting a proportional reduction in the effluent toxicity. This finding could be promising in terms of the future application of both strains for recovery of effluents with high load organic like vinasse. Supported by PICT 2015 Nº 0297 and CONICET.

MS-010

CHARACTERIZATION AND PROPERTIES OF A MICROBIAL EMULSIFIER PRODUCED FROM CRUDE GLYCEROL

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Bioemulsifiers (BE) are amphipathic molecules used in the bioremediation field due to the role in emulsification, solubilization, and removal of hydrophobic compounds from the environment. The ability of a spore-forming bacterium to produce BE using crude glycerol as a cheap feedstock was previously detected. In the current study, partially purified BE was characterized. The ability of the microbial product and of two commercial synthetic agents such as sodium dodecyl sulfate (SDS) and Triton X-100 (TX-100) to emulsify hydrophobic substrates was also comparatively evaluated. Culture supernatant containing BE was filtered through a dialysis tubing cellulose membrane (Typical molecular weight cut-off = 14,000 Da). The concentrate obtained was then used as BE source, and subjected to hydrolytic treatments with proteinase K (30 U mg-1 at 37 °C for 4 h), commercial lipase from *Candida rugosa* (100 U mg-1 at 37 °C for 1 h), and acid hydrolysis (10% HCl at 100 °C for 10 min) in order to estimate the role of peptides, lipids and sugars on the BE nature. The biodegradability of BE, SDS and TX-100 was assayed using the BOD/COD ratio, with BOD and COD as the biological and chemical oxygen demand, respectively. BOD and COD parameters were determined according to the Standard Methods for the Examination of Water and Wastewater. Finally, it was evaluated the emulsifying ability of the three agents on hydrophobic substrates (kerosene, toluene, chloroform, chlordane pesticide and vegetable oils), determining the emulsification index for each substrate after being left to settle for 24 h (E24). All hydrolytic treatments significantly reduced the BE activity on the kerosene, suggesting that the microbial product could have a protein fraction as well as sugar and lipid fractions. On the other hand, a virtually negligible BOD/COD ratio was detected for SDS and TX-100 (0.070-0.172), confirming the extremely low biodegradability of these synthetic products. However, BOD/COD ratio was significantly increased for BE (0.386), so confirming its biodegradable character. Finally, a differential performance of the BE, SDS, and TX-100 to emulsify the hydrophobic compounds was detected: a similar performance of the three agents to emulsify substrates as kerosene and toluene were detected, with E24-values of 61% and 62%, respectively. However, chloroform was only effectively emulsified by the BE, with an E24-value increased 4-fold compared to those detected for the synthetic agents. While SDS had poor ability to emulsify a pesticide such as chlordane (E24 = 18%), the BE and TX-100 were optimal emulsifying agents for this substrate, with similar E24-values between them (61%). Finally, only the BE was able to emulsify vegetable oils as sunflower, canola, and grape, with E24-values that ranged from 38% to 51%. These results could encourage the application of a biodegradable microbial product to achieve the effective removal of hydrophobic pollutants, without detriment to the environment. Supported by PICT 2015 Nº 0297 and PIP 0372.

MS-011

SYNTHESIS OF BIOFILM, POLYHYDROXYALKANOATES ACCUMULATION AND PROTEOLYTIC ACTIVITIES BY *Bacillus subtilis* subp. *spizizenii* USING GLYCEROL AS CARBON SOURCE

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Bacillus subtilis subsp *spizizenii,* a PGPR organism, synthesis biofilm at the liquid-air interface. Biofilm is a survival strategy, giving protection against environmental fluctuations in humidity, temperature, pH and nutrient availability. These characteristics are important for its use as bioinoculant. Glycerol is an industry by-product suitable as carbon source. Yeast extract is a possible source of amino acids. Bacterium survival is benefited by the reserve material polyhydroxyalkanoates (PHAs). The objectives of this work were the production of a biofilm with sessile PGPR bacteria suitable as a biofetilizer using glycerol in a medium supplemented with yeast extract; the study of biofilm characteristics, PHAs accumulation and proteolytic activities. *Bacillus subtilis* subsp. *spizizenii* free of plasmids was used. The culture media were basal salt medium supplemented with amino acids or with yeast extract (YE) (0,2; 0,5 and 1%); with or without 1% glycerol. The biofilm formation was determined in static conditions at 96 h of incubation at30 °C. Bacterial growth was measured as optic density at 600nm (OD). Protease activity was determined with azocasein. The PHAs were determined by crotonic acid formation. Data were analyzed by ANOVA test. *B. subtilis* needed specific amino acids (glutamic acid, aspartic acid or lysine but not tryptophan) to synthesize biofilm (BF) using glycerol. For glutamic, the minimum needed was 0,07%. The bacterium formed biofilm using YE (without glycerol) at concentrations higher than 0,5% (0,062 mg BF/ml). With 1% glycerol and 1% YE, biofilm formation increased (423%) and planktonic cells decreased (810%). Biofilm robustness was greater with glycerol. At 0,2% YE, with or without glycerol, there was not biofilm synthesis, glycerol increased 220% plancktonic growth. High proteolytic activity (around 90 U/ml) was observed in the medium with only YE. With glycerol there was proteolytic activity only when YE was higher than 0,5%, being maximum for 1% YE. PHAs accumulation in biofilm sessile cells was lower for YE 1% than for 0.5% (29.3 and 65.1 mg PHAs/mg BF respectively). With glycerol, PHAs concentrations in biofilm were similar for both yeast concentrations (around 8 mg PHAs/mg BF). The maximum PHAs accumulation (756 mg PHAs/OD) in the planktonic cells was with 1% YE and 1% glycerol, for the others assay conditions the accumulation were similar (around 185 mg PHAs/OD). Amino acids were needed for the synthesis of a biofilm by *B. subtilis* using glycerol as a carbon source. Due to the bacterium proteolytic capacity, yeast extract was a suitable source of the amino acids. *B. subtilis* accumulated PHAs in the sessile cells of the biofilm. This results indicate that a basal salt medium with 1% Glycerol (as a carbon source) and 1% Yeas extract (as a source of amino acids) was suitable for the production of a robust biofilm by *B. subtilis*, that accumulated PHAs, characteristics suitable for its use as biofertilizer.

MS-012

SEARCH FOR BIOLOGICAL SURFACTANTS IN HALOPHILIC BACTERIA

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Biological surfactants are secondary metabolites of amphipathic nature that lower the surface tension of solutions allowing the formation and stabilization of heterogeneous systems such as emulsions. Halophilic and/or halotolerant microorganisms have the ability to grow in extreme environments using adaptations that allow them to withstand osmotic stress, being the production of biosurfactants one of those strategies. Physicochemical characteristics of these biomolecules allow them to be used in a variety of applications such as in bioremediation, industrial processes, medicine, and biological control. The aim of this work was to search for halophilic and/or halotolerant bacteria that produce biosurfactant molecules for future applications in different bioremediation strategies.
Water and soil samples were taken from El Salar de Hombre Muerto, located in the Puna of Atacama in the limit between Catamarca and Salta provinces. A total of 106 bacterial strains were isolated and their ability to grow in defined medium (DM: L-asparagine, 0.5 g/L; K2HPO4 , 0.5 g/L; 0,28 ml/L micronutrient solution (FeSO4.7H2O, 0.01 g/L; MgSO4.7H2O, 0.2 g/L) and glucose, 10.0 g/L) without or with the addition of 2 M (DM2) or 4 M (DM4) NaCl, were tested. Hemolytic capacity of strains was studied using blood agar medium as a preliminary evaluation of biosurfactant activity. After 48 h bacteria's ability to lyse partially (alpha hydrolysis) or totally (beta hydrolysis) red blood cells was evaluated. A quantitative evaluation of biosurfactant activity was then performed. For that, 700 µl of culture of each strain grown in DM, DM2, and DM4 for 24 h (30 °C and 250 rpm) were mixed with 700 ml of kerosene. The mixture was mixed-vortex for 1 minute and 1 hour and 24 h after shaking the height of the emulsion formed was measured in order to evaluate its stability. The emulsion index was calculated as the quotient between the height of emulsion and the total height of liquid in the tube. As a result of this work, we observed that 67% of the strains studied had capacity to perform hemolysis of the red blood cells, indicating the ability of isolated bacteria to produce bioemulsifiers. Twenty nine strains presented alpha hemolysis and 42 strains beta hemolysis. These results were in agreement with the quantitative test, where 75 strains presented emulsifying capacity after 1 h, whereas only 42 of those showed stability after 24 h. The emulsion index varied from 2 to 52%. Based on the results obtained in the present work it can be concluded that the microorganisms isolated from El Salar del Hombre Muerto have the capacity to produce biosurfactants, which should be characterized in the following investigations.

MS-013

IDENTIFICATION AND ANALYSIS OF BACTERIA ISOLATED FROM EL SALAR DEL HOMBRE MUERTO

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Saline environments harbor microorganisms adapted to extreme conditions. These organisms possess some properties and share some features that allow them to live in such hostile environment. In previous assays, several microorganisms were isolated from El Salar del Hombre Muerto, a salt flat area in the northwest of Argentina, where mainly lithium is extracted. Their characterization allowed us to establish the presence of a wide diversity according to their macro and micromorphology. They were able to grow using different carbon and nitrogen sources. Quantitative and qualitative assays to establish the tolerance against lithium were carried out. Five bacterial strains were selected as the most tolerant for further studies; among them there were cocci and bacilli, Gram positive and negative, non-pigmented and pigmented cells and some of them were biofilm forming organisms. In this work, the microbial identities of five strains (SA211, FAMB1, SX139, HA120A and SFS) isolated from El Salar del Hombre Muerto were studied and the phylogenetic closeness within themselves and with strains from the NCBI database was determined. In order to know the proteins involved in lithium tolerance a proteomic study was conducted. Selected microorganisms were grown in a defined culture media with two different concentrations of lithium chloride. Analyzing the crude cell extracts by mono-dimensional polyacrylamide gel electrophoresis, a differential protein profile was observed among cells grown at 10 and 30 g/L of LiCl. Those proteins would be involved in the ability of these bacteria to survive and grow in adverse conditions. Microorganisms were also identified by sequencing the 16S rRNA gene for which first we extracted the nucleic acids and then amplified through PCR with universal primers. Identity estimation of these five microorganisms was performed by comparison with sequences from the NCBI (National Center for Biotechnology Information) database and the phylogenetic tree was constructed using MEGA software. Three of these strains were classified within the gender *Bacillus* (HA120A, SX139 and FAMB1), SA211 was closely related to *Micrococcus* genus and SFS belonged to the genus *Halomonas*. The analysis of the sequences let us conclude that although sharing the ability to tolerate high concentrations of lithium chloride, the selected strains possess a wide variety of different properties and identities, even though most of them belong to the *Bacillus* genus. In a bacterial community, diversity is one of the most important features, because it allows adaptation to environmental shifts, for example, anthropogenic contamination. Interestingly, it is known that the *Halomonas* genus harbor alkalitolerant microorganisms, some strains from the *genus Bacillus* have the ability to produce exopolymers and some strains from the *Kocuria* genus degrade hydrocarbons, just an example. Further studies are needed to elucidate the capabilities of these strains.

MS-014

THE SOCIAL LIFE OF MEMBERS OF THE SUGARCANE MICROBIOME

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Manipulation of soil and plant associated microbiomes holds great promise for contributing to a more environmentally benign agriculture. Even though numerous microbial isolates have been proposed for their use in agriculture, the typical approach designed to fine-tune the plant physiology involves the use of single microbial species. This approach clearly under-exploits the potential of microbiomes and highlights the need of more information regarding the ability of members of such microbiomes to interact with each other and with the plant. Distinctive microbial communities can be found at different plant structures (i.e. roots, stems and leaves) and it is expected that a functional basis underlays those specific associations. In order to gain some insight into the functional and metabolic characteristics of the sugarcane-associated microbiome, we isolated bacteria from this niche and evaluated bacterial interactions between members of the community. For that, plant stems were sampled with a cork borer, plant tissue was ground and subsequently plated in a culture medium containing salts, aminoacids and sucrose. After 5 days of incubation at 30° C, sugarcane endophytes were selected based on differential colony morphologies and growth rates. A subset of phylogenetically different isolates was selected after sequencing each isolate 16S rDNA. With this subset, which included species belonging to the *Acinetobacter, Agrobacterium, Beijerinckia, Kocuria, Microbacterium, Pantoea, Pseudomonas Rhizobium, Rothia* and *Sphingomonas* genera, we performed pairwise co-cultures on the same solid medium used for isolation. Microbial interactions were followed over time and the type of interaction was documented. Even though the majority of pairwise co-cultures showed no apparent interaction, several combinations revealed different types of interactions including growth inhibition, commensal and mutualistic growth promotion, colony morphology changes, pigment production and an unanticipated interaction consisting in growth inhibition of one of the isolates in the immediate proximity to the other isolate but growth promotion at a distal position. Initial attempts to reveal the chemical nature behind some of the observed interactions indicate that siderophores may play a key role on them.

MS-015

ARSENIC TOLERANCE AND PRESENCE OF SPECIFIC RESISTANCE GENES IN HETEROTROPHIC CONSORTIA OBTAINED FROM COPAHUE GEOTHERMAL SYSTEM

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The major sources of arsenic (As) pollution are related to anthropogenic intervention. This carcinogenic metalloid can be found mainly in two oxidizing states: As(III) and As(V), being the first the most toxic form. Fortunately some microorganisms are able to metabolize arsenic contaminants through several specific enzymes and proteins contributing to remediate polluted sites. The present work describes the influence of As on microbial growth and the presence of a variety of genes related to As metabolism and As resistance in two heterotrophic consortia obtained from a sample collected from Caviahue-Copahue geothermal system (Salto del Agrio) and enriched in LB medium at 30°C supplemented with increasing concentrations of NaAsO 2 or Na2HAsO4.7 H2O. The consortia proved to be able to adapt to concentrations up to 20 mM of As(III) (culture called Het(As+3 20 mM)) and 450 mM of As(V) (culture called Het(As+5 450 mM)). The phylogenetic analysis indicated the prevalence of *Paenibacillus profundus* in both cultures. Control cultures, without As added, were carried out to compare growth profiles ((As+3 0 mM) and (As+5 0 mM)). Growth was measured by OD600 every 24 hours. After 4 days of incubation, Het(As+5 450 mM) reached their maximum OD600 meanwhile Het(As+5 0 mM) did that after 3 days; Het(As+3 20 mM) reached their maximum OD600 after 8 days instead of 2 days for Het(As+3 0 mM. Due to the lower toxicity of As(V), its influence on growth of Het(As+5 450 mM) was less than As(III) on Het(As+3 20 mM). The growth profiles also showed that the cultures supplemented with As had longer lag phases than the controls without As, although final concentration of cells was similar in both. The screening of As resistance genes was done using 12 primer sets. Interestingly, Het(As+3 20 mM) showed positive amplifications with 6 primers set corresponding to two types of *arrA* genes that coded for a respiratory arsenate reductase, the *acr3* gen that coded for an arsenite transporter, two types of *aioA* genes that coded for an arsenite oxidase and the *arsC* gen that coded for a cytoplasmic arsenate reductase. Curiously, the culture Het(As+5 450mM) did not amplify with any of the primers assayed in spite of a great optimization effort. Our study opens the door for the study of many diverse aspects of As tolerant microorganisms, from the genetic resistant characteristics to the potential use in the remediation of contaminated sites.

MS-016

METAGENOMIC ANALYSIS OF MICROBIAL MATS FROM BRAVA AND TEBENQUICHE LAKES

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In this work, nucleic acid-based molecular methods, geochemical measurements and physico-chemical characteristics were combined to investigate microbial sedimentary ecosystems of Laguna Tebenquiche and Laguna Brava. Molecular diversity of two hypersaline microbial mats was compared by WGS sequencing of environmental DNA from the mats. Brava and Tebenquiche are lakes in the Salar de Atacama, Chile, where microbial communities are growing in extreme conditions, including high salinity, high solar insolation, and high levels of metals such as lithium, arsenic, magnesium, and calcium. Evaporation creates hypersaline conditions in these lakes and mineral precipitation is a characteristic geomicrobiological feature of these benthic ecosystems. Microsensor measurements on the mats allowed determination of depth profiles of O2 and sulfide, showing active production and respiration. The mat from Brava was more rich and diverse, with a higher number of different taxa and with species more evenly distributed. At the phylum level, Proteobacteria, Cyanobacteria, Chloroflexi, Bacteroidetes and Firmicutes were the most abundant, including ~75% of total sequences. At the genus level, the most abundant sequences were affilitated to anoxygenic phototropic bacteria from the genera *Roseoflexus, Chloroflexus* and *Oscillochloris*, followed by cyanobacterial genera such as *Microcoleus, Cyanothece* and *Nostoc.* In Tebenquiche mats, Proteobacteria and Bacteroidetes covered ~70% of the sequences, with Cyanobacteria and Firmicutes including 5% each. Over 13% of the sequences were affiliated to *Salinibacter* genus, thus addressing the lower diversity. Other Bacteroidetes genera with more than 1% abundance included *Rhodothermus* and *Bacteroides.* Finally, the Alphaproteobacteria *Rhodospirillum* was also important. In spite of the differences at the taxonomic level, the two mats were functionally similar. Thus, similar roles could be fulfilled by different organisms. For example, primary production, performed mainly by photosynthetic organisms from the phyla Cyanobacteria and Chloroflexi in Brava, is performed by members of Alphaproteobacteria in Tebenquiche. Further comparison with other microbial mats will allow identifying unique genes from these environments related to their extreme characteristics.

MS-017

Rhodopseudomonas palustris AZUL: A NEW MEMBER OF THE ELECTRO-ACTIVE BACTERIA CLUB?

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Among current alternatives for non-polluting electricity generation, some depend on the activity of biological catalysts. The use of electrogenic bacteria in bio-electrochemical systems is a good example that calls particular attention due to its low cost, innocuousness and potential applications. In the last years, the search for microorganisms capable of exchanging electrons with a polarized electrode has become significant when aiming at improving the production of electrical energy in such systems. In this work, we explore the capacity of an autochthonous strain of *Rhodopseudomonas palustris*, (AZUL strain) of growing in continuous culture electrochemical bioreactors interacting with an electrode. *R. palustris* belongs to the group of purple non-sulphur photosynthetic bacteria and has an extraordinary metabolic versatility which allows it to grow under any of the four modes of metabolism: photoautotrophic, photoheterotrophic, chemoautotrophic and chemo-heterotrophic. A phylogenetic analysis based on 23S rRNA gene indicated that strain AZUL has a closest match to CGA009, TIE-1 and DX-1. The latter two are strains which interaction with an electrode has been previously reported. *R. palustris* AZUL was capable of growing in bioreactors, forming biofilms on graphite electrodes under photoheterotrophic conditions. Electrochemical assays showed that biofilms formed on electrodes under various applied potentials (polarized) had specific redox signals which varied with light, polarization time and potential value. Despite these signals, current obtained from biofilms in cronoamperometric assays did not overcome 9.5μA/cm2; moreover, we could not see any relationship between current magnitude and biomass accumulation on the electrode. At least two well defined redox pairs centered at 0,4 and 0,6V (Ag/AgCl- 3M NaCl) were detected whose amplitude depended on imposed conditions. On the other hand, SEM images revealed differences in the structure of biofilms grown on polarized and non-polarized control electrodes. These results indicate that *R. palustris* AZUL possesses extracellular electron transfer mechanisms which do not seem to be directly related to cellular respiration, but probably to a mechanism of protection against oxidative stress. Due to its ease of culture in laboratory and its extraordinary metabolic versatility, the study of optimal conditions in which this bacterium exchanges electrons to a polarized electrode in a bioelectrochemical system, deserves to be extended aiming at developing technological applications.

BIOTECNOLOGÍA Y FERMENTACIONES

MODALIDAD ORAL

BF-001

EFFECT OF NANO-MICROMETRIC TOPOGRAPHIES ON EARLY STEPS OF BIOFILM FORMATION

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Biofilms are defined as communities of microorganisms that live attached to a surface. They can include a single bacterial specie or multiple species and are formed on both abiotic and biotic surfaces. This well-known phenomenon has undesirable effects for industrial or medical surfaces. Surface properties impact on the first steps of biofilm formation. Nature offers multiple solutions to biofilm formation. An important number of biological surfaces prevent microbial colonization due to their surface topographies, e.g.: the shells of mollusks and crabs and the skin of marine mammals and sharks. These facts have encouraged research of bioinspired surface designs. The main objectives of this work were to produce micro-nanometric hierarchical topographies and to analyze the influence of the topography on the bacterial adhesion. The hierarchical surface was designed using surface plasma oxidation of uniaxial stretch of polydimethylsiloxane (PDMS) films. This method has the advantage to allow designing sub-micrometric wrinkle topographic surfaces changing the plasma time exposition and the uniaxial stretch. Different topography surfaces were obtained, surface has wrinkles with different wavelength (from 500 to 3000 nm) and amplitude (from 80 to 700 nm) parameters. The bacterial adhesion on these novel hierarchical surfaces was evaluated through exposing them to a culture of *Pseudomonas protegens* Pf-5 for different times. The bacterial attachment was evaluated taking images of the wrinkled and smooth surfaces using an Atomic Force Microscopy (AFM). The initial results of this study suggests that wrinkled surface with a wavelength of 1000 nm (aprox. bacteria size) delay the bacterial adhesion and, on the other hand, wrinkled surface with a wavelength of 3000 nm enhance and encourage bacterial adhesion. These results demonstrate the importance of the topographic surface to inhibit or stimulate the biofilm development.

BF-002

METABOLIC ENGINEERING OF A DIAZOTROPHIC BACTERIUM IMPROVES AMMONIUM RELEASE AND BIOFERTILIZATION OF PLANTS AND MICROALGAE

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The biological nitrogen fixation carried out by some Bacteria and Archaea is one of the most attractive alternatives to synthetic nitrogen fertilizers. However, with the exception of the symbiotic rhizobia-legumes system, progress towards a more extensive realization of this goal has been slow. In this study we manipulated the endogenous regulation of both nitrogen fixation and assimilation in the aerobic bacterium *Azotobacter vinelandii*. Substituting an exogenously inducible promoter for the native promoter of glutamine synthetase (GS) produced conditional lethal mutant strains unable to grow diazotrophically in the absence of the inducer. This mutant phenotype could be reverted in a double mutant strain bearing a deletion in the *nifL* gene that resulted in constitutive expression of *nif* genes and increased production of ammonium. Under GS non-inducing conditions both the single and the double mutant strains consistently released very high levels of ammonium (> 20 mM) into the growth medium. The double mutant strain grew and excreted high levels of ammonium under a wider range of concentrations of the inducer than the single mutant strain. Induced mutant cells could be loaded with GS at different levels, which resulted in different patterns of extracelular ammonium accumulation afterwards. Inoculation of the engineered bacteria into a microalgal culture in the absence of sources of C and N other than N2 and CO2 from the air, resulted in a strong proliferation of microalgae that was suppressed upon addition of the inducer. Both single and double mutant strains also promoted growth of cucumber plants in the absence of added N-fertilizer, while this property was only marginal in the parental strain. This study provides a simple synthetic genetic circuit that might inspire engineering of optimized inoculants that efficiently channel N2 from the air into crops.

BIOTECNOLOGÍA Y FERMENTACIONES

MODALIDAD POSTER

BF-003

THERMOTOLERANCE OF THE IMMUNOBIOTIC *Lactobacillus rhamnosus* CRL 1505: EFFECT OF INTRACELLULAR POLYPHOSPHATE INCLUSIONS

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The immunobiotic *Lactobacillus rhamnosus* CRL 1505 (CRL-1505) is produced as dehydrated powder by spray drying for subsequent addition into different food matrix to improve transportation and storage. The success for having a high survival rate depends on the thermotolerance of the strain during the drying process.Previous studies put in evidence the accumulation of inorganic polyphosphate (polyP) as cytoplasmic inclusions by the cells when CRL-1505 was grown in a culture medium with high inorganic phosphate. The accumulation of these inclusions, reported in other lactic acid bacteria, depends on the concentration of inorganic phosphate in the culture medium and its presence could be related to osmotic, oxidative and acid stress response mechanisms. The aim of this work was to evaluate the effect of cytoplasmic polyP inclusions on the thermotolerance of the strain CRL-1505. Fermentations were performed at 37°C under free pH in MCM medium with and without addition of inorganic phosphate. A DAPI-based fluorescence technique was used as a measuring of intracellular polyp accumulation. Cells from both culture media were harvested at the stationary phase, suspended in phosphate buffer, and exposed to heat shock (60°C, 5 min). The cell viability before and after heat shock was determined by plate count and flow citometry using the BD™ Cell Viability Kit. No significant differences (p ³ 0.05) in cell growth were observed but the polyP level was lower (*ca.* 40%) in cells grown without inorganic phosphate addition. The subsequent exposure of these cells to heat shock resulted in a greater loss of cell viability (3.0 ± 0.5 Dlog CFU/mL) compared to those of high polyP content (2.0 ± 0.2 Dlog CFU/mL). These results were confirmed by flow cytometry. The strain CRL-1505 with low polyP content displayed 85.9 % dead cells after heat shock while it was 50.4% in cells with high level of polyP. From these results, we conclude that the thermotolerance of CRL-1505 strain was dependent on the phosphate concentration in the culture media and, consequently, on the levels of polyP in the cells.

BF-004

INDIGO BIOSYNTHESIS BY *Pseudomonas monteilii* P26 PLANKTONIC AND IMMOBILIZED CELLS

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Indigo is used as dye in the textile industry. Its production by conventional methods involves the use of toxic precursors, the consumption of high amounts of energy and the production of toxic waste. Indigo biosynthesis mediated by microorganisms could be an environmentally friendly and more efficient alternative for indigo production. The aim of this work is to study different conditions for indigo production using *Pseudomonas monteilii* P26 resting cells suspensions and to use the best condition obtained for the production of indigo by immobilized cells. Immobilization was carried out by biofilm formation using two methods: a continuous culture and a sequential batch reactor process. The carrier used was polyurethane foam (PUF). Results showed that the best condition for indigo production using cell suspensions was obtained when indole was added as aqueous solution at low cell concentration. Previous indigo biosynthesis induction by the addition of naphthalene resulted to be unnecessary. Indigo production by immobilized cells was also evidenced. This work is a first approach towards the optimization of indigo biosynthesis for the development of cleaner production processes.

BF-005

OPTIMAL CONDITIONS FOR THE PRESERVATION OF *Pseudomonas tolaasii* IEXb, A MICROORGANISM USED AS GRASS BIOFERTILIZER

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Phosphorus (P) is one of the most required inorganic nutrient for microorganisms and plants. Phosphate Solubilizing Bacteria (PSB) exert a relevant role in the P cycle on the soil, mobilizing deposits of insoluble phosphates. PSB belong to the group of Plant Growth Promotion Rhizobacteria (PGPR). In previous studies, PSB were isolated from Argentinian Puna (Salta and Jujuy) and characterized for their ability to solubilize tricalcium phosphate and hydroxyapatite. Other properties of PGPR were also evaluated, such as indole acetic acid and siderophore production, and ability to biocontrol against phytopathogen fungi. *Pseudomonas tolaasii* IEXb was selected for its ability to promote the plant growth and to increase performance of maize culture. The aim of this work was to evaluate different preservation treatments of *P. tolaasii* IEXb biomass, to formulate a product with shelf life according to its application on field. Biomass production was performed in a 10 L fermenter (New Brunswick Scientific Microferm Fermentor MF-214), using 5 L of a low-cost culture medium (1% whey permeate and 1% soybean meal), at 30°C, initial pH = 6.0, with agitation (200 rpm). Growth kinetic parameters were determined up to 8 h of culturing, through viable cell counts on agar plate. The produced biomass was harvested, washed and subjected to different preservation processes. A factorial experimental design 3 x 2 was applied to determine the effects of three formulations (cell suspensions in LB broth-20% glycerol, cell suspensions in LB broth-10% lactose, and lyophilized bacterial cells in 10% whey permeate-5% sodium glutamate) and two storage temperatures (4°C and 25°C) on the *P. tolaasii* IEXb viability during 150 days. Samples were taken during storage and the numbers of colony forming units (CFU) per mL (CFU/mL) were determined. Data were evaluated applying the general linear model of analysis of variance (MINITAB 17 statistical software). Maximal *P. tolaasii* IEXb biomass (6.2 x 108CFU/mL) was reached at 8 h of culture, with a growth rate of 0.87 h-1. All the factors evaluated significantly affected the *P. tolaasii* IEXb viability during storage. The increase of storage temperature exerted a marked negative effect on the bacterial survival in liquid formulations, mainly in the presence of lactose (absence of viable, cultivable cells at day 28, when storing at 25°C). However, high numbers of viable cells were recovered from lyophilized powders stored at 25°C (3.88 x 10 8 CFU/mL and 1 x 107 CFU/mL at days 42 and 96, respectively). After 150 storage days at 4°C, similar numbers of viable cells were recovered from cell suspensions in the presence of glycerol or lactose (around 5 x 108 CFU/mL), viability being lower than in lyophilized powders in whey permeate-sodium glutamate (1 x 109 CFU/mL). In conclusion, these results allowed the determination of the most suitable conditions for the preservation of *P. tolaasii* IEXb for its use as biofertilizer.

BF-006

KIWI CV HAYWARD: CHARACTERIZATION OF ASSOCIATED MICROBIOTE AND EVALUATION OF TECHNOLOGICAL ALTERNATIVES TO OBTAIN A HIGH QUALITY PROCESSED PRODUCT

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Kiwifruit production in Argentina is in continuous growing, becoming more important mainly in Southeast of Buenos Aires province. Mar del Plata and the surrounding areas are placed in the most suitable latitude for cultivation. The production of kiwifruit in our country is oriented to fresh market. Consumers demand safe, healthy and easy-to-use food. It is necessary to investigate alternatives of industrialization of these foods. Minimally processed products could allow adding value to fruits that do not meet quality standards. An important factor that guarantees shelf-life is to reduce the initial microbial load of the selected raw material. Disinfection is essential to ensure hygienic-sanitary quality of the product. The objective of this work was to characterize the microbiota of kiwifruit and to evaluate different disinfection alternatives to achieve a minimally-processed, safer and longer-lasting product. The fruit was harvested at 6.5% total soluble solids, subjected to the curing process and stored for 3 months in a refrigerator at 0°C. After this time, the kiwifruits were immersed in a solution of sodium hypochlorite (NaClO) (300 ppm, pH:6.5 at 6°C), or in sterile water (control). Microbial load and composition were determined by quantifying, using conventional methodologies, total mesophilic aerobic bacteria (TMAB), filamentous fungi (FF) and yeasts (Y). Subsequently, the fruits were peeled, washed and cut into slices. Disinfection treatments applied were: 150 ppm NaClO, 1% (w/v) lactic acid (chitosan solvent), 1.5% (w/v) chitosan (Sigma Chemical), and sterilewater (control). The slices were air-dried and placed in bags of low oxygen barrier at 4°C. TMAB, FF and Y were quantified at 0, 7 and 9 days. TMAB were characterized using conventional microbiology protocols (Gram staining technique, microscopic observation of cell morphology). Coliforms, *Salmonella spp*. and *Listeria monocytogenes* were characterized using a specific protocol (ISO 4831, 6579, 11290 rules) and informed as presence/ absence. Disinfection of the whole fruit with 300 ppm of NaClO was effective in the reduction of BAMT, FF and Y. There were also significant differences in the presence of coliforms between the disinfection treatment and the control. In the minimally processed product, all treatments reduced the burden of FF compared to the control treatment. Disinfection with chitosan or NaClO also controlled Y and TMAB significantly reducing its load. Of the total bacterial isolates characterized, 71% corresponded to Gram (+) and 29% to Gram (-). Regarding Gram (+) 30% presented coccus and 70% bacillary morphology, of which 10% showed resistance structures (endospore). None of the treatments showed coliforms, *Salmonella spp*or *L. monocytogenes*. Regardless of the disinfection treatment, the loading of Y, FF and TMAB increased significantly during storage at 4°C.It can be concluded that natural coatings such as chitosan could be a sustainable alternative to replace NaClO.

BF-007

GLOBAL REGULATORS AS NON-TRADITIONAL TARGETS TO ENHANCE THE PRODUCTION OF BIOTECHNOLOGICALLY RELEVANT COMPOUNDS IN *Escherichia coli*

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Transcriptional regulation in *Escherichia coli* comprises a network of specific and global regulators. Some of the latter control operons related to central metabolism affecting carbon flow and reducing power. Mutations in these genes may be advantageous for the production of compounds of biotechnological interest. Among different global regulators that affect central metabolism we studied ArcA and CreC, Cra and Rob. ArcA is known to be one of the main regulators that affects C metabolism in response to O 2 availability, while CreC is known to respond to both C source and aeration. The majority of Cra targets are genes coding for the enzymes involved in central carbon metabolism. Rob is involved in antibiotic resistance, solvent tolerance and affects some genes of glucose metabolism and TCA cycle. To investigate the effect of deleting these regulators on the production of industrial compounds, cultures of mutants in each regulator carrying the genes for synthesis of 1,3-propanediol from *Klebsiella pneumoniae* were carried out in M9 or LB medium supplemented with glycerol. Different conditions of oxygen availability were assayed: (I) low aeration (125 rpm and 1:2 flask-volumen:medium-volume ratio) and (ii) aerobiosis (200 rpm and 1:10 ratio). Glycerol 10 g·liter-1 and 30 g·liter-1 were used, respectively. All regulators affected the synthesis of this diol in both aeration conditions and culture media. In M9 medium most relevant effects where seen forboth D*arcA* and D*creC* mutants which had higher yields (p/s) of 1,3-PDO whengrown in low aeration. Furthermore, a slight increase in titers of this compound were observed for D*arcA* mutants. As for LB, microaerobic cultures showed a significant decrease of 1,3-PDO titer for DarcA mutants in contrast to those in minimal medium. Additionally, a slight increase of 1,3-PDO titer for D*cra* mutants was achieved in aerobic conditions. Remarkably, a 2-fold higher titer of this solvent was observed for D*arcA* strain compared to wild type. Mutants carrying the genes for polyhydroxybutyrate synthesis from *Ralstonia eutropha* were also evaluated for the production of this polymer in M9 glucose 30 g·liter-1 in low aerobic conditions described above. Slight increases in PHB accumulation for D*rob* and D*creC* mutants were observed. In conclusion, all global regulators seemed to affect carbon distribution and to behave differently in the aeration and culture medium conditions tested. Results obtained indicates that D*cra* and D*arcA* mutant could be potentially interesting for 1,3-PDO production. As for PHB, although slight effects were seen in the microaerobic conditions, more experiments must be done to evaluate the roles of these regulators on polymer production. Furthermore, results were strongly dependent on culture conditions that need to be optimized to design more efficient and sustainable processes for biocompounds production.

BF-008

PRODUCTION OF A PROTEASE ACTIVITY FROM *Bacillus thuringiensis* RT IN A COMPLEX MEDIA

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Bacillus thuringiensis is a Gram-positive, facultative anaerobic bacterium that produces crystal proteins with insecticidal properties. In addition, it is an excellent producer of proteases and other lytic enzymes that can increase the insecticidal effect by acting in synergy with the crystal protein. In this work, the production of proteases was carried out by submerged fermentation using a complex media for possible use in different industries such as food, chemical, textile and bioinsecticides. The strain *Bacillus thuringiensis* RT (Genebank: EF638795, 16S partial sequence) was used. The production was carried out in a Labfors 3 fermenter (Infors HT, Bottminguen, Switzerland) in 3000 ml at 30°C and free pH. A complex culture media called M5 developed from economical substrates was used; it contains (g/L): cerelosa 2, whey 10, skim milk powder 5, vinasse 5, sucrose 2, starch 2 and soybean meal 7. Protease activity was determined in triplicate in culture supernatant every 24 h during 5 days. One hundred and twenty ml of supernatant was mixed with 480 ml of azocasein (1% w/v) in phosphate buffered saline. One unit of activity is defined as the amount of enzyme that produces an increase in 0.01 OD420 in 30 min at 30°C. The values of protease activity during fermentation were (U/L): 0.13 ± 0.02 x 106 (24 h), 0.26 ± 0.02 x106 (48 h), 0.35 x106 ± 0.01 x106 (72 h), 0.98 ± 0.07 x106 (96 h) and 1.36 ± 0.09 x106 (120 h). Thus, the maximum value of protease activity was obtained at 120 h (a = 0.05) displaying a volumetric productivity of 11421.97 U/Lxh. In addition, the final pH value of the culture was 9.51. In conclusion, this lab-scale study gives useful information to continue with the scaling-up of the process. This work was supported by FONCyT (PICT 2011-2158 and PICT 2015- 2596), CONICET (PIP 339) and UNT (PIUNT E548/3).

BF-009

IDENTIFICATION AND HETEROLOGOUS EXPRESSION OF *Enterococcus faecalis* ESTERASES FOR THE PRODUCTION OF SHORT CHAIN FATTY ACIDS COMPOUNDS THAT CONTRIBUTE TO FLAVOR GENERATION IN CHEESES

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Enterococcus strains usually dominate the non-starter flora of traditional cheeses such as Mozzarella and Fontina. They contribute positively on the development of flavor compounds during ripening. The most important enzymatic activities of non-starter lactic acid bacteria involved in flavor production are proteolysis and lipolysis. As the applications of enzymes in the food industry are expanding in this project we have performed a screening of *E. faecalis* esterases in order to produce flavor-enhancing esterases in a GRAS host. First, twenty-three hydrolases with possible esterase activity were identified in the available genome of *E. faecalis* JH2-2 using *in silico* tools. In an attempt to identify wall-anchored or secreted enzymes the program SignalP 4.0 was subsequently used. Hence, nine out of twenty-three hydrolases showed to have a signal peptide indicating possible secretion. Four of these hypothetical esterases coding genes, named *estA*, *estB*, *estC* and *estD,* were cloned in pET 28b and expressed in *Escherichia coli* DH5a. Then, cell fractions of IPTG-induced recombinant strains were obtained and analyzed. The presence of EstB and EstC was only observed in the cytoplasmic fractions which suggests that neither of the putative enzymes could be recognized or transported by *E. coli* secretion system. On the other hand, EstD was not detected in soluble form under tested conditions. Interestingly, EstA putative esterase could be recovered in the periplasmic fraction which indicates that the hypothetical signal peptide is being recognized and the protein secreted by the *E. coli* Sec system. In an attempt to corroborate the hydrolytic capability of the putative enzymes, the esterolysis of p-nitrophenyl (pNP) monoesters of fatty acids were evaluated. EstA showed to hydrolyze only short chain acyl pNP derivatives (C4), while EstC over C4, C16 and C18 acyl pNP derivative. Noteworthy, short-chain free fatty acids are indicators of quality and source of flavor in cheese. In order to broaden the knowledge of EstA and EstC regarding its origin, a phylogenetic analysis was performed. As a result, EstA and EstC were identified in all *E. faecalis* strains analyzed and in lesser extent within the genus. Finally, in order to study the contribution of EstA in the production of cheese sensorial relevant compounds, constructions of GRAS EstA–producing strains were conducted. In order to do so, a codon optimized version of *estA* was synthetized and cloned in pNZ8048, a NICE (nisin-controlled expression) system vector, which derives from the *nis* operon (*nisABTCIPRKEFG*). As hosts, *L. lactis* NZ9000 and a derivate strain deficient in ClpP and HtrA major proteases were employed. As an alternative expression tool, the promotor of NICE system was also exchanged by the promotor of P170 expression system which is up-regulated as lactate accumulates in the growth medium. Currently, the best combination of host, promotor type, and expression conditions are under evaluation.

BF-010

LIGNINOLYTIC ENZYME ACTIVITIES DURING PHENOLIC CONTENT DEPLETION BY *Aspergillus* IN SUBMERGED FERMENTATION OF ALPERUJO EXTRACT

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During olive oil extraction process, it is obtained a semi-solid by product called "Alpeorujo" (AL). AL is composed by mashed olive pulp, olive stone and vegetation water. AL contains highly contaminant phenolic compounds (PCs), sometimes reported as responsible for the toxicity attributed to the AL. Biological treatments such as Solid-State Fermentation and co-composting have been proposed for the AL stabilization and detoxification, but during these processes a lixiviate containing PCs is produced. Submerged Fermentations would be more suitable for the treatment of this liquid waste, not only for detoxification but also for antioxidant recovery. The aim of this work was to state the enzyme activities Laccase and Lignin-peroxidase during the submerged fermentation (SF) of AL-extract by *Aspergillus LR*. Extract of AL was made mixing 1Kg of AL with 2 Kg of distilled water, and them pressed and filtered. The pH of the extract was set to 4.5 using HCl 0.1N. SF was carried out in Erlenmeyer flasks containing 25 ml of AL extract, inoculated with 108 conidia/ml of *Aspergillus LR* (isolated from AL, at the IBT-UNSJ), and incubated at 28 °C in orbital shaker (130 rpm) for 30 days. Samples were taken daily. PCs were measured as Total Phenolic Compound using Folin-Ciocalteu reagent. Laccase (Lac) and Lignin-Peroxidase (LiP) activities were quantified using ABTS and Veratryl alcohol as substrate, respectively. One Unit of enzyme activity was defined as 1 µ-mol of oxidized substrate/minute and reported as U/ml. Initial PC content of AL-extract was 167 mg/flask. During the first 6 days, PCs decreased 30%, followed by additional 20% decrease until day 30. Color removal was in accordance with PCs decrease. Final PC content was 78 mg/flask. Both enzymatic activities Lac and LiP were detected during the SF. There was a strong correlation between both enzyme activities and PCs until day 15, showing coincident peaks for both enzymes (0.5 U/ml for Lac, and 10 U/ml for LiP when PC content was 162 mg/flask). After that, Lac activity decreased up to 0.1 U/ml, but LiP showed activity near 8-9 U/ml. These results are aligned with research reports about these enzymes, capable of transforming complex phenolic compounds into simpler ones. Main conclusions are: i) *Aspergillus* LR grew in AL extract containing highly concentrate PCs; ii) Laccase and Lignine-Peroxidase activities are related to PCs depletion; iii) there was color removal. Future essays would be focused in finding parameters to optimize SF for enzyme production and also in studying antioxidant capacity of the remaining phenolic compounds after enzyme degradation.

BF-011

SELECTION AND CHARACTERIZATION OF NATIVE BIOSUPPRESSIVE/BIOCONTROLLERS YEASTS OVER WINE SPOILAGE YEASTS

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In last decades were incremented the researches about selection of autochthonous non-*Saccharomyces* yeasts as beginners of enological fermentations. Better adapted to climate zone conditions and the must to ferment, conferring advantages during the process. Several descripted like positive to wines flavor. Big economic losses are produced due to contaminated fermentations with *Dekkera*, these confer fenolic/medicinal, barn and horse sweat flavor. Also by *Zygosaccharomyces* due to re-fermentations, turbidity and dissolved CO2. Generally wineries use SO2 as antiseptic. Nevertheless, strains of both species are SO2 tolerant. Moreover this component could generate health problems, so it is seek to reduce it use. Yeasts conveniently inoculated are able to prevent/reduce the activity of other microorganisms. Killer phenomenon is an example, where yeasts holders secrete proteins inducing lethal changes to sensible. Objective: Characterize enologically non-Saccharomyces native biocontrollers yeasts due to its capacity to generate positive attributes to wine flavor. 12 non-*Saccharomyces* with biocontrol over 4 strains of *D.bruxellensis* and 4 *Z.rouxii* were evaluated about it capacity to ferment in growing reducing sugars (30°Bx), ethanol (8°,10°,12°,14°), SO 2 (50ppm,100ppm,200ppm,300ppm) and low temperature (15°C). Moreover was evaluated SH 2 production and enzymes: b-glucosidase, pectinase and protease. BHu4, BMp29 y BMp49 were able to ferment under growing conditions of SO2, ethanol, high reducing sugars and low temperature; liberate protease and to produce low SH2. Nevertheless, these did not liberate b-glucosidase and pectinase. Conclusion: Selected yeasts are object of study to apply in co-inoculums with *Saccharomyces* to obtain healthy wines and with certain positive flavor characteristics.

BF-012

TAILORING A MYCELIUM-BOUND LIPASE ACTIVITY BY SUBMERGED FERMENTATION USING *Aspergillus niger* MYA 135: CULTURE MEDIUM OPTIMIZATION

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Lipases (EC 3.1.1.3) are important industrial enzymes due to their versatile applications. They catalyze a variety of reactions such as hydrolysis, esterification, transesterification and interesterification. In addition, the biocatalyst design by using the medium engineering strategy is an alternative way to obtain catalytic efficiency and specific characteristics like substrate specificity, stability, etc. In this sense, the application of statistically-based assays such as the central composite design (CCD) becomes a powerful tool. Previously, among eleven variables, the carbon/nitrogen ratio and the concentration of both olive oil and FeCl3 were identified as significant parameters for the production a mycelium-bound lipase activity from *Aspergillus niger* MYA 135. The aim of this work was to tune this whole-cell biocatalyst by submerged fermentation using a CCD. Thus, the level of those important input factors was optimized to maximize the lipase production. All experiments were performed in duplicate and analyzed with the Minitab software for Windows. The hydrolytic activity was measured with *p*-nitrophenyl palmitate (C16) as substrate. The molar extinction coefficient of *p*-nitrophenol (*p*-NP) under the given assay conditions was 0.00979 µM-1 cm-1. One unit of enzyme activity (U) was defined as the amount of biocatalyst that released 1 µmol of *p*-NP per min. Specific activity was expressed as U per gram of mycelium dry weight. As a result, the optimal culture conditions were the following: 1.63 % olive oil (X1), 0.08 g/l FeCl3 (X2) and a carbon/nitrogen ratio (X3) of 0.24. Under these conditions the mycelium-bound lipase activity was 2.04 ± 0.13 U per gram of dry weight. The effect of each variable was also analyzed; the linear coefficient of X3, all interaction terms coefficients and the quadratic coefficients of X12, X22 and X32 were significant, as their P values were below 0.05. In addition, the P-value for the lack-of-fit test (0.118) showed the adequacy of the obtained model. This is also verified by the R2 (98.25 %) and the Adj R2 (97.56 %) coefficients indicating the percentage of variability that is explained by the model. In conclusion, the response surface methodology was successfully used to increase the production of a mycelium-bound lipase six times. Finally, the performance of these biocatalysts in the biodiesel synthesis was also discussed. This work was supported by FONCyT (PICT 2011-2158 and PICT 2015- 2596), CONICET (PIP 339) and UNT (PIUNT E548/3).

BF-013

ALCOHOLS PRODUCTION FROM COMPLEX CARBON SOURCES IN TWO STRAINS OF *Thermoanaerobacterium thermosaccharolyticum*

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Fossil fuels generate large amounts CO2, emissions and toxic byproducts, and are obtained from non-renewable resources. In this context, biofuels represent an eco-friendly and sustainable alternative, and those obtained from lignocellulosic biomass are of special interest because they can be obtained from otherwise wasted agricultural crop residues. Degradation of lignocellulosic biomass and xylan was described in many organisms including bacteria belonging to the *Clostridium* genus. In this work we analyzed degradation of xylan and lignocellulosic biomass (sugarcane agricultural residue) by two strains of the related genus *Thermoanaerobacterium Thermoanaerobacterium thermosaccharolyticum* GCU5 isolated in our laboratory and the collection strain *T. thermosaccharolyticum* DSM 531. These strains are anaerobic thermophilic butanol and ethanol producers. The sugarcane agricultural residue used consists mainly of three polymers: cellulose (34,2%), hemicellulose (24,1%) and lignin (18,5%) along with smaller amounts of, extractives (8,8%) and ash (13,6%). Also, the heteropolymer xylan represents the most abundant hemicellulosic polysaccharide. To identify genes encoding the key enzymes in xylan degradation we performed a genomic analysis using RAST annotation Server, Blast algorithm and Biocyc database collection. Five enzymes were identified in both strains: Endo-1,4-b Xylanase (EC 3.2.1.8), b-Xylosidase (EC 3.2.1.37), a-N-Arabinofuranosidase 2 (EC 3.2.1.55) and Acetyl Xylan esterase (EC 3.1.1.72). We also searched the genomes of both strains for genes coding for enzymes involved in the degradation of cellulose. We identified genes encoding for b-glucosidase (EC 3.2.1.21), 1,3 b-Cellobiosidae (3.2.1.91) and putative Endo-1,4-b-D-Glucanase. Given that these strains carry the genes responsible for xylan and cellulose degradation, we decided to evaluate their ability to grow on these substrates. The strains were grown at 60°C in TSD medium supplemented with xylan or sugarcane agricultural residue as only carbon sources, using the Hungate method. After 48 hours we observed that GCU5 and DSZ 531 were able to grow in both carbon sources. Finally, we measured ethanol and butanol production by Head Space GC-FID. For this, the culture supernatants were diluted ½ in K2CO3 1g/ml, heated at 60°C for 1h and analyzed in a GC-FID. Although both strains were able to produce ethanol on TSD xylan and TSD sugarcane biomass, the production was higher by the strain *T. thermosaccharolyticum* DSM 531. In conclusion, we could demonstrate that two strains of *T. thermosaccharolyticum* are able to grow and produce solvents from complex substrates such as xylan and lignocellulosic biomass. These results are interesting for the development of biofuels, and especially relevant for our country, because sugarcane biomass is an economic substrate that is generated as a contaminating residue during sugarcane harvest.

BF-014

ARCHAEAL CAROTENOIDS: OPTIMIZATION OF A PROTOCOL FOR THE PURIFICATION OF BACTERIORUBERINS FROM THE HALOARCHAEON *Haloferax volcanii*

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Carotenoids are isoprenoid pigments produced by plants and microorganisms. Due to their antioxidant capacity they are beneficial to human health and have numerous applications. Haloarchaea are prokaryotic organisms that thrive in high salt environments (> 2 M NaCl) and produce orange/red carotenoids with C50 (bacterioruberins, Bctr). Haloarchaea represent a novel source of bioactive compounds, including carotenoids, which have been characterized to a limited extent, and thus, still remain unexploited. The aim of this work was to optimize a protocol for the purification of carotenoids from the haloarchaeon *Haloferax volcanii* and characterize their antioxidant properties. This procedure may facilitate obtaining samples enriched in haloarchaeal carotenoids to explore their bioactive properties and evaluate their potential applications. Two protocols were assayed for the extraction of carotenoids from cells of a genetically modified overpigmented *H. volcanii* strain (HVLON3), previously constructed in our laboratory: PI (acetone: methanol, 1:1, v/v) and PII (100% acetone). The carotenoid-enriched extracts were then fractionated by thin-layer chromatography (TLC) obtaining similar yields (84-91 %) of the carotenoid fraction, denoted as BR1, with an estimated Rf = 0.25. Both protocols coupled to TLC removed several contaminants, particularly PII, which allowed elimination of the spot BR2 (Rf = 0.29). Identification of carotenoids, (mainly Bctr) in BR1 as well as in the whole extracts was performed by HPLC analysis. Two main peaks with retention times (Rt) of 3.00min and 4.00-5.14 min were evidenced. Based on their spectral analysis, that with Rt = 4.00-5,14 corresponded to Bctr showing 3 peaks with maximal absorbance in the range of 467-526 nm. The former with Rt = 3.00 min (maximal absorbance at 270-290 nm) so far could not be identified but may be a precursor or degradation product of Bctr. The antioxidant capacity of the *H. volcanii* Bctr was evaluated based on their radical reactivity using the DPPH method coupled to EPR detection and compared to that of b-carotene (BC) as a reference, using various carotenoid concentrations. The antioxidant capacity was 3 fold higher for Bctr compared to BC based on estimated EC50 of 4.46 x 10-5 M and 1.39 x 10-4 M, respectively. Supported by EXA-731/15-UNMdP, PIP1106-CONICET and PICT1477 (RDC); PIP 0804/14 and 15/E710 UNMDP (MSC).

BF-015

TWO-COMPONENT SYSTEMS FOR KETONE OXIDATION: TYPE II BAEYER-VILLIGER MONOOXYGENASES

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The Baeyer-Villiger biooxidation of ketones is a valuable reaction for the preparation of esters or lactones in both academic and industrial fields. Baeyer-Villiger monooxygenases (BVMOs) are enzymes that catalyze the insertion of one atom from molecular oxygen into the substrate, whereas the other atom is reduced to water at the expense of NAD(P)H. In particular, type II BVMOs are multicomponent enzymes formed by a flavin reductase that reduces FMN in a NADH-dependent manner and a monooxygenase subunit that oxidizes the substrate at the expense of reduced FMN. These enzymes are interesting systems for biocatalytic applications due to their ability to accept bicyclic ketones as substrates and to use NADH instead of the more expensive NADPH. In this work, we identified one gene coding for a putative monooxygenase component in the genomes of *Bradyrhizobium diazoefficiens* and *Mycobacterium tuberculosis* by bioinformatic analysis. The retrieved proteins shared 31-35 % sequence identity with other monooxygenase components of type II BVMOs from *Pseudomonas putida* previously reported [1]. A similar analysis was performed for the putative flavin reductase genes using the sequences of known reductases as queries for blast searches. Several genes were retrieved and we selected those that located near to the identified monooxygenase genes in these genomes and encoded proteins with sequence homology to the flavin reductase Fred from *P. putida* [1]. We cloned each pair of selected genes coding for the monooxygenase and reductase subunits and expressed them in *Escherichia coli*. The flavin reductase components were soluble whereas both monooxygenase components were obtained in the insoluble fractions. In order to overcome this drawback, different expression conditions were assayed. We tested diverse induction temperatures, inducer concentrations, culture media, host *E. coli* strains and the co-expression of molecular chaperones. Then, we evaluated the biocatalytic activity of these BVMOs by biotransformations in recombinant whole-cell systems (growing and/or resting cells) with the ketone (±)-*cis*-bicyclo[3.2.0]hept-2-en-6-one as a model substrate. Our results indicated that the rhizobial type II BVMO was not active under the assayed conditions and the type II BVMO from *M. tuberculosis* was able to oxidize this fused ketone. Funded by ANPCyT, CONICET, UNR. [1] Iwaki, H, Grosse, S, Bergeron, H, Leisch, H, Morley, K, Hasegawa, Y, Lau, PCK (2013). Appl Environ Microbiol, 79, 3282-3293.

BF-016

SCREENING OF AUTOCTHONOUS LACTIC ACID BACTERIA WITH THE ABILITY TO INCREASE THE BIOACTIVE POTENTIAL OF PHYTOCHEMICALS FROM ANDEAN GRAINS: ANTIOXIDANT ACTIVITY

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Currently there is a global revaluation of pseudocereals (quinoa and amaranth), ancestral Andean grains, due to their high protein content and nutritional value, health benefits, and they do not contain gluten. Pseudocereals are rich in phytochemical compounds: phenolic compounds (PC), alkylresorcinol, lignans, phytosterols and tocopherols, which possess potential antioxidant properties with beneficial effects on consumers´ health. PC in plant substrates are found as dimers, glycosylated linked or derived esters that do not possess biological activities. It has been shown that these compounds can be transformed into bioactive compounds when subjected to a fermentation process. Lactic acid bacteria (LAB), GRAS (Generally Recognized As Safe) microorganisms, form part of the autochthonous microbiota of diverse ecosystems. During fermentation, LAB modify the physicochemical and functional composition of plant substrates by changing the ratio of anti-nutritive / nutritive components. Although fermentation by LAB on different substrates has been well studied, little is known about its effect on gluten-free substrates such as quinoa. At the same time, information regarding the fermentation of pseudocereals by autochthonous LAB and the influence of PC on their growth and antioxidant activity (AOA) is scarce. Based on this, the overall objective of this study was to select autochthonous LAB that can increase the antioxidant activity of bioactive compounds present in quinoa. Growth, acidification capacity, AOA (DPPH and ABTS) and PC (Folin-Ciocolteau) concentrations of 43 LAB strains isolated from sourdoughs and grains of quinoa and amaranth were evaluated. Two controls were carried out without inoculum, C1 and C2, in which the volume of inoculum was replaced with tap water or an acidic solution, respectively. The results allowed the selection of three LAB strains: *Leuconostoc* (*Lc.*) *mesenteroides* subsp. *mesenteroides* CRL 2131, *Lactobacillus* (*L.*) *plantarum* CRL 1973 and CRL 1964. The selected strains increased the AOA from 25 to 31% with respect to C1 and from 6 to 12% with respect to C2 (DPPH method) and from 30 to 36 % with respect to C1 and 19 to 26%, with respect to C2 (ABTS method). The PC concentration increased from 13 to 25 mg GAE / 100 g dough respect to controls. The three strains were used as starter cultures for the production of biscuits with quinoa flour. The results showed that the ability of the three LAB strains to increase the AOA and PC content in the food was in line with those obtained in *in vitro* tests. In conclusion, the selected LAB (*Lc. mesenteroides* subsp. *mesenteroides* CRL 2131, *L. plantarum* CRL 1973 and CRL 1964) possess important characteristics that give them the potential to be used as starter cultures for the design of quinoa-based functional foods.

BF-017

SUBSTRATE SCOPE OF A RHIZOBIAL BAEYER-VILLIGER MONOOXYGENASE EXPRESSED IN *Escherichia coli*

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Type I Baeyer-Villiger monooxygenases (BVMOs) are flavoenzymes that depend on FAD and require NADPH as electron donor to catalyze the monooxygenation of a carbonyl substrate in the presence of molecular oxygen. These enzymes oxidize cyclic or linear ketones to produce lactones or esters which are valuable precursors for the synthesis of biologically active compounds and natural products. Due to their strict dependence on NADPH for activity, biotransformations in whole-cell systems represent a valuable experimental strategy for synthetic applications. Our group is interested in expanding the number of biocatalysts for Baeyer-Villiger oxidations. We have identified several putative genes coding for typical type I BVMOs in the genome of *Bradyrhizobium diazoefficiens* by sequence homology analysis using representative BVMOs as queries. In this study, we selected one of these genes, named *bj02* that encodes a protein with the characteristic consensus sequences and dinucleotide-binding motifs of type I BVMOs. We performed a phylogenetic analysis with different recombinant BVMOs to predict the location of the new enzyme in the inferred phylogenetic tree. The gene of interest was cloned and expressed in *Escherichia coli*. Then, we assessed the substrate preferences of this novel BVMO by whole-cell biotransformations under growing conditions. Several ketones with different structures were tested as substrates. Most of them were converted to their corresponding esters or lactones suggesting potential applications in the future. Funded by ANPCyT, CONICET, UNR.

BF-018

PRODUCTION OF A LIPASE ACTIVITY BY SOLID STATE FERMENTATION USING *Aspergillus niger* MYA 135: IMPACT OF DIFFERENT HUMECTANT MIXTURES

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The solid state fermentation is an economical alternative for production of industrial enzymes such as lipases mainly because this technology proposes the reuse of agro-industrial waste as well as adding value to those residues. Lipases (EC 3.1.1.3) are triacylglycerol hydrolases that catalyze the hydrolysis of triglycerides to free fatty acids and glycerol. In non-conventional systems these enzymes can also catalyze esterification, transesterification and interesterification reactions. The aim of this work was to evaluate the production of a lipase activity from *Aspergillus niger* MYA 135 by solid state fermentation using different humectant mixtures. As solid substrate, washed sugarcane bagasse was used. Humidity was adjusted to 90 % with the following mixtures containing (in g/l): 1) *M1*: Vinasse 5.0, molasses 5.0, milk serum 10.0, peptone 5.0, cerelose 5.0, waste frying oil 10.0, FeCl3 1.0, CaCl2 0.5. 2) *M2*: Sucrose 10.0, KH2PO4 1.0, NH4NO3 2.0, MgSO4 0.2, CuSO4 0.06, FeCl3 1, olive oil 20. 3) *M3*: Sucrose 10.0, KH2PO4 1.0, NH4NO3 2.0, MgSO4 0.2, CuSO4 0.06, yeast extract 1.0, peptone 5.0, olive oil 20. Humectant mixtures M1 and M2 were previously used as culture media for *A. niger* MYA 135 lipase production by submerged fermentation. Humectant mixture M3 was formulated from literature data. Reactors were inoculated with 105 conidia per gram of solid substrate and incubated at 30°C during 48 h. Then, the fermented substrate was dried at 45 °C and used as enzyme source. The hydrolytic activity was measured with *p*-nitrophenyl palmitate (C16) as substrate. The molar extinction coefficient of *p*-nitrophenol (*p*-NP) under the given assay conditions was 0.0073 µM-1 cm-1. One unit of enzyme activity (U) was defined as the amount of biocatalyst that released 1 µmol of *p*-NP per minute. Specific activity was expressed as Unit per gram of dry fermented substrate (U/gdfs). All experiments were performed in triplicate and analyzed with the Minitab software for Windows. Under our assays conditions, the specific lipase activity obtained from sugarcane bagasse supplemented with M1, M2 and M3 were 626.7, 978.1 and 252.8 U/gdfs, respectively. In addition, the performance of the biocatalysts produced by submerged fermentation using the same liquid mixtures was also discussed. This work was supported by FONCyT (PICT 2011-2158 and PICT 2015- 2596), CONICET (PIP 339) and UNT (PIUNT E548/3).

BF-019

METAGENOMIC DIVERSITY DURING START UP STAGE OF ANAEROBIC DIGESTERS

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Anaerobic digestion constitutes a sustainable process widely used for organic waste management that has the advantage of generating biogas in addition to the stabilization of organic matter. The process of anaerobic digestion depends on the assembly of a complex microbial community, in which methanogenic archaea and syntrophic bacteria are the leading actors. It has been observed that for certain substrates, a suitable inoculum source may be critical for efficient biogas production. However, the availability of adequate inocula is severely limited at the local level, due to the very low degree of adoption of technologies based on anaerobic digestion. This work aims at understanding the adaptation of inocula arising from different sources to food waste. Four lab- scale anaerobic reactors (5 L) were operated during 91 days and fed daily with increasing concentrations of food waste. Reactor operational parameters, including biogas production, volatile solids (VS), alkalinity and volatile fatty acids concentration (VFA) were measured on regular basis. Metagenomic DNA was obtained from sludge samples taken weekly. Methanogenic archaea abundance was estimated using qPCR assays, whereas total microbial community analysis was conducted using amplicon sequencing with primers for the V3-V4 rRNA16S region. Specific biogas production (Biogas/VS) and volatile fatty acids (VFA) varied depending on the inoculum´s source and feeding rate. Using USEARCH, OTUs were defined at 97%, obtaining 2690 OTUs that then were classified with rdp database. Under our experimental set up, adaptation to food waste was associated to the presence of acetoclastic methanogenic archaea from *Methanosaeta* genus, which were mapped with high abundance when biogas production was higher and identified as key taxa associated to process stability. This results underlies the critical role of the inoculum source for reactor start up. We are currently analyzing methanogenic archaea applying amplicon sequencing of the *mcrA* gene, which encodes methyl coenzyme-M reductase.

BF-020

SELECTION OF LACTIC ACID BACTERIA AS STARTER CULTURES FOR THE MANUFACTURE OF FERMENTED VEGETABLES

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Canned vegetables like pickles are well-accepted products consumed as appetizer or side dish with a main meal. Most fermented vegetable products marketed in the northwest of Argentina are traditionally obtained through a process of spontaneous fermentation carried out by the epiphytic microbes present on the raw materials that may include lactic acid bacteria (LAB). LAB contribute significantly to the flavor, texture, nutritional value and safety of fermented food products. In consequence, their addition as starter cultures for fermentation represents an important progress in the manufacture of high quality and reproducible products. At present, there are no industrial starters of autochthonous LAB for pickles fermentation in our region, which could provide the local market with products of appreciable organoleptic properties and longer shelf-life. Therefore, the objective of this work was to technologically characterize strains of LAB previously isolated from vegetables in order to select the most suitable as starters for the manufacture of fermented vegetables. For this purpose, six LAB strains isolated from pickles, olives and chesses made with unpasteurized milk and selected previously by their acidification rates, were identified by 16S rRNA gene sequencing and technological characterized regarding relevant properties for pickles manufacture such as: growth in the presence of increasing concentrations of NaCl (2, 4, 7 y 10%); at different pH (5.5, 5.0, 4.5 and 4) and production of antimicrobial substances against pathogens (*Escherichia coli* and *Listeria sp.).* The compatibility of the six LAB strains was tested by the production of inhibitory substances and set agar growth. Strains used in the present study were identified as *Lactobacillus rhamnosus* GS21 and GS43, *L. plantarum* GS31 and GS34, *Weissella viridicens* GS25 and *W. paramesenteroides* GS35. At initial pH 4.5 and 7% NaCl, the strains that showed the highest growth were *L. plantarum* GS34 and *L. rhamnosus* GS43. These microorganisms were also able to inhibit by acidity both pathogens tested and showed no incompatibility between each other that could prevent their use as mixed cultures. Properties exhibited by the selected LAB strains make them eligible as new starter cultures for obtaining fermented vegetable products with high hygienic and sensory quality.

BF-021

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM LENTILS (*Lens culinaris*) AND PEA (*Pisum sativum*) FLOURS

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Lentils (*Lens culinaris*) and peas (*Pisum sativum*) are the most consumed varieties of legumes in Argentina. These pulses and their derived products are considered of great nutritional value due to their high protein and dietary fiber contents. However, flours obtained by mill of these seeds have to be subjected to additional industrial processing in order to improve their technological properties that allow obtaining products with higher rheological and organoleptic quality. In this regard, fermentation with lactic acid bacteria (LAB) represents an attractive option for food industry since these microorganisms are able to remove antinutritional factors and release several metabolites that positively impact in the product. Therefore, the aim of this work was to isolate LAB strains from flours of lentils and peas and characterize them technologically in order to select the most appropriate for fermentation of these food matrices. Four samples of lentils and one sample of peas obtained from local markets were ground individually, mixed 1:1 with sterile distilled water and incubated aerobically at 37°C for up to 5 days with daily backslopping for the enrichment of the endogenous microbiota. Samples taken at 0, 1, 3 and 5 days were spread on selective agar for LAB (MRS with cycloheximide 0.1 % and LBS), and also plated in PCA and HyL media to estimate total aerobic mesophiles and fungi/yeasts respectively. Evolution of pH was followed during fermentation. Colonies grown in MRS and LBS that were Gram positive and catalase negative were differentiated by REP-PCR. The isolates with different profiles were identified by sequencing the gene encoding for 16S ribosomal RNA. Initial average counts of 2.07x104 and 7.8x103 CFU/g for lentils and peas, respectively, reached values near to 108-109 CFU/g after 24 hours of fermentation at the fifth backslopping cycle, whereas counts of fungi and yeasts started at 8.25x102 and 5x101 CFU/g for each type of flour, remained at 104 CFU/g during successive days of backslopping and decreased by 1-2 logarithmic orders at the end of the fermentation cycles. Lactic microbiota began to dominate after the first backslopping cycle with values of 1.05x107 CFU/g in lentils flour and 4.4x105 CFU/g in peas, reaching final values of 4.11x108 and 6.4x109 CFU/g respectively. Regarding pH, it decreased in all doughs from 6.4-6.0 to 4.5-3.8 at the end of fermentations. LAB isolates were identified as *Enterococcus durans (1 strain), E. faecium* (2), *Lactobacillus plantarum* (1), *Lactococcus garvieae* (2), *Weissella cibaria* (5), and *W. paramesenteroides* (7). Further studies on technological properties of these strains for selection of the most appropriate to be applied to development of a legume based functional food are at present ongoing.

BF-022

INFLUENCE OF METAL IONS ON b-D-GLUCOSIDASE AND CELL MORPHOLOGY OF *Rhodotorula glutinis* AND *Rhodotorula mucilaginosa*

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The cell wall is a complex and dynamic structure. It is also a site of diverse enzyme activities. The balance between its synthesis and lysis is critical, since wall synthesis in the absence of lysis can cause excessive wall thickening and possible growth arrest; while, lysis in the absence of synthesis would produce bursting of the cell. Therefore, within permissible limits, the net balance between wall synthesis and wall lysis will exert a marked influence on the cell growth and morphology. In this work, the b-D-glucosidase activity was firstly selected as a marker of the wall lytic potential of both *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* using p-nitrophenyl-b-D-glucopyranoside as substrate. Secondly, a doses-response study involving several metal ions was conducted; as a result, the FeCl3 and MnCl2 significant increased the biomass-bound b-D-glucosidase activity from *R. glutinis* and *R. mucilaginosa*, respectively. Thirdly, to test the influence of each metal ion, 6 mM of either FeCl3 (for *R. mucilaginosa*) or MnCl2 (for *R. glutinis)* was supplemented to a medium with lower nitrogen content (MLN). The MLN without any addition was taken as the control. Cultures were incubated at 30°C and 180 rpm during six days. Thus, the major and minor diameter of *R. mucilaginosa* increased from 3.40 ± 0.23 µm to 5.28 ± 0.40 µm and from 2.60 ± 0.23 µm to 5.20 ± 0.55 µm, respectively, in the presence of 6 mM FeCl3. And, the major and minor diameter of *R. glutinis* increased from 4,90 ± 0.47 µm to 5.48 ± 0.42 µm and from 2.23 ± 0.53 µm to 4.95 ± 0.49 µm, respectively, in the presence of 6 mM MnCl2 . So, from these results it might be concluded that the supplementation of either FeCl3 or MnCl2 to the culture medium induced morphological changes in both oleaginous yeasts. In addition, the biomass and lipid concentration obtained after fermentations were also discussed. This work was supported by FONCyT (PICT 2011-2158 and PICT 2015- 2596), CONICET (PIP 339), CIUNSa Proyect "A" Nº 20/C237 and UNT (PIUNT E548/3).

BF-023

AMPLIFICATION AND CLONING OF CARBOHYDRATE ACTIVE ENZYMES FROM *Pycnoporus sanguineus*

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Lignocellulosic biomass, which is composed of cellulose, hemicellulose and lignin, is an attractive substrate for the feed, pulp and paper industries and in the production of second generation bioethanol. Cellulose is a large linear homopolymer of beta-1,4 linked D-glucose while hemicelluloses are ramified heteropolymers with a xylan backbone. Complete breakdown of cellulose and xylan generate glucose and xylose that can be fermented to ethanol and hence their importance in the obtention of biofuels. The present work focuses on one of the critical steps for the utilization of biomass that is the complexity to breakdown of the lignocellulosic wall. The general aim is the development of enzymatic complexes of fungal recombinant cellulases, hemicellulases and accessory enzymes and their potential industrial applications. Using the transcriptomic information of expressed genes from the xylophagous fungus *Pycnoporus sanguineus* BAFC2126, we identified the coding sequences for several putative carbohydrate active enzymes selecting three of them for further analysis. We have amplified and cloned the coding sequences of a GH3 b-xilosidase (2270bp), a key enzyme for obtaining the fermentable pentose xylose from xylobiose, a GH43 arabinofuranosidase (940bp), which helps in the debranching of xylan, and an AA9 (former GH61) lytic polysaccharide mono-oxigenase (1040bp) which has been reported to enhace glucanase activity. *In silico* analysis of proteins encoded by cloned sequences showed identities of 85%, 88% and 80% respectively with other fungal enzymes of the corresponding families. All the three enzymes were cloned in vectors for expression in Pichia pastoris for recombinant production as secreted proteins, purification and further characterization.

BF-024

CHARACTERIZATION OF THE BIOACTIVE PROPERTIES OF CAROTENOIDS ISOLATED FROM AN OVERPRODUCER STRAIN OF HALOPHILIC ARCHAEA

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Archaea have evolved with distinct ecophysiological adaptations consistent with the extreme environments they inhabit, thus, their molecules may present novel characteristics. An example of these are bacterioruberins (Bctr), orange/red carotenoid pigments that exert a protective effect against the harmful effects of UV light. Due to their antioxidant capacity, carotenoids have numerous applications; however, the bioactive features of haloarchaeal Bctr have been scarcely explored. A target field for antioxidants is the protection of sperm from cryodamage. Although semen cryopreservation has contributed to the expansion of artificial insemination, this technique did not prove to be completely efficient in species such as sheep. Cryopreservation induces oxidative stress, altering the motility and viability, increasing the influence of calcium. The aim of this investigation was to explore the bioactive properties of Bctr focusing on their protective effect on ram sperm after freezing/thawing. Bctr was extracted from a Bctr-overproducer mutant strain of the haloarchaeon *Haloferax volcanii* using acetone-methanol solution (1:1, v/v). The organic extract enriched in Bctr was evaporated and preserved at-20 °C protected from light and before use it was solubilized in ethanol. The antioxidant capacity of Bctr extracts with different times of storage was evaluated using the 2,2diphenyl-1-picrylhydrazyl (DPPH) radical spectrophotometrically and was also compared to that of b-carotene. It was confirmed that the antioxidant capacity of Bctr was 2.5-3.0 fold higher than that of b-carotene and it was also observed that the extracts did not decrease their antioxidant activity after several months of storage. The effect of the Bctr extracts was evaluated on different ram sperm parameters and compared to b-carotene. Once thawed and selected, sperm was incubated with different Bctr or b-carotene concentrations or the respective controls. At different incubation times, the pattern of tyrosine phosphorylated proteins was evaluated by Western Blotting and various sperm parameters were analysed by flow cytometry including viability, mitochondrial functional status, lipid peroxidation, ROS content and DNA damage. Treatment of sperm cells with 2.5, 7, 15 and 20 µM Bctr for 120 min. significantly increased the percentage of viable cells and decreased the proportion of cells with intracellular ROS, compared to their respective controls, and the effect was similar to that observed for b-carotene. In conclusion, although fertility trials are needed, Bctr is a potential antioxidant that can be applied to ram semen to improve insemination yields. Supported by EXA-731/15-UNMdP, PIP1106-CONICET and PICT1477 (RDC); PIP 2014 0273 (AC).

BF-025

OCCURRENCE OF LACTIC ACID BACTERIA WITH POTENTIALITY TO PRODUCE BIOGENIC AMINES IN TUCUMAN WINES

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During vinification process, under uncontrolled conditions certain lactic bacteria (LAB) could produce biogenic amines (BAs) in wine. The main BAs present in wine are putrescine, cadaverine, histamine and tyramine. The prevention of the production of BAs in wines is an important topic because they can produce toxic effects and sometimes affect the quality and organoleptic properties of the final product. A selective isolation for wine lactic acid bacteria was carried out from samples obtained at the end of vinification process from different wine cellars located at "El bañado de Quilmes" and "Chañar solo" in the district of Colalao del Valle, Tucuman. A total of 30 randomly selected lactic acid bacteria were first submitted to a phenotypic characterization according to the carbohydrate fermentation profile procedures adapted for microplate. The positive fermentation test was evidenced when the indicator turned from violet to yellow after 72 h incubation at 28°C. The homo and heterofermentative metabolism of isolates were also assayed utilizing the HDD medium. All isolates were then submitted to a screening for biogenic amine production according to Maijala procedures, utilizing a decaboxilating medium supplemented with 0,1% of the precursor amino acid (tyrosine, histidine, lysine, arginine and ornithine) in agar medium. The positive results were evidenced as the turning of the indicator color from yellow to purple. A total of 20 isolates resulted positive for tyrosine decarboxylase activity, 17 for histidine decarboxylase activity, 19 for both lysine and arginine decarboxylase activity, and 21 were positive for ornithine decarboxylase activity. These amino acids are precursors of the several biogenic amines as tyramine, histamine, cadaverine and putrecine. These results put in evidence the presence of several LABs with capability for biogenic amines production isolated from wines produced in the region of the Calchaquí Valleys at Tucumán province.

BIODIVERSIDAD

MODALIDAD ORAL

MICROBIOLOGÍA MOLECULAR

MODALIDAD ORAL

BD-001

METAGENOMIC APPLIED TO MICROBIAL DIVERSITY STUDY IN A ZONE AFFECTED BY AN ACID MINE DRAINAGE: RELATION BETWEEN PHYSICOCHEMICAL PARAMETERS AND TAXONOMIC GROUPS

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The most documented pollution associated with abandoned mining areas is the acidic water produced by the oxidation of sulfides ores exposed to surface. This acidic water is known as Acid Mine Drainage (AMD) and enhances the mobility of heavy metals from soil and sediments, what poses a threat to the environment. Likewise, AMD affected environments are low-complexity natural systems regarding to biodiversity. In San Luis-Argentina, the drainage that originates in a gold mine abandoned since 1894 is released to La Carolina stream and possesses AMD characteristics. In previous works, we reported the influence of the mine drainage on physicochemical parameters of La Carolina stream sediments. The aim of this study is to determinate the influence of these mine drainage on microbial diversity and to stablish specific relations between microbial taxonomic groups and physicochemical parameters of the affected zone. Twelve sediment samples from inside the mine (7) and from La Carolina stream (5), before and after receiving the mine drainage, were selected to total DNA isolation, which was performed using DNA soil kit, MOBIO. Metagenomic sequencing of PCR amplified products of 16S rRNA and 18S rRNA genes was carried out by MR DNA (www.mrdnalab.com, TX, USA) on MiSeq (Illumina) platform. Microbial diversity results were analyzed trough *Silva NGS* (https://www.arb-silva.de/ngs). In order to stablish specific relation between microbial taxonomic groups and physicochemical parameters of the zone, a Canonical Correspondence Analysis (CCA) was performed. CCA showed that prokaryotic diversity is affected majorly by pH values, favoring the presence of phyla *Actinobacteria* and *Gamma-proteobacteria* in samples characterized by low pH values (inside the mine). Phyla *Nitrospirae, Chloroflexi, Delta-proteobacteria, Thaumarchaeota* and *Euryarchaeota* are majorly abundant in samples that presented high concentrations of heavy metals. Likewise, *Alpha-proteobacteria* is abundant in samples that were taken in presence of sunlight (stream samples). Regarding to eukaryotic diversity, the greatest incidence is given by the sunlight presence. All samples taken inside the mine, in the absence of light, showed to fungi and protists members as the most abundant microorganisms. Those samples taken in the presence of light, presented to algae (green algae and diatoms) as the most abundant microorganisms. After receiving the AMD, the stream presents a decrease in diatoms abundance and green algae predominate, probably due to the acidification of the watercourse because of the incidence of the AMD in down-stream samples. It can be clearly observed that the AMD released to La Carolina stream influences on eukaryotic and prokaryotic diversity, favoring the presence of some microorganisms over others, depending of the physicochemical characteristics of the zone. This study shows the incidence of mining activities on natural sources, even long time after their closure.

MM-001

IDENTIFICATION OF SECRETED CELLULASES AND HEMICELLULASES FROM A NATIVE ISOLATE OF *Cellulomonas sp*. AND RECOMBINANT EXPRESSION OF A GH10 ENDOXYLANASE

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We have isolated a novel bacterial strain from the genus *Cellulomonas*, named B6, from a forest soil consortium. *Cellulomonas* sp. B6 has the ability to degrade both cellulose and xylan and to grow in a minimal saline medium supplemented with either carboxymethylcellulose (CMC), or lignocellulosic biomass, such as sugar cane residue (SCR), extruded barley straw (BSE) and extruded wheat straw (WSE). In order to identify the secreted enzymes responsible for the activity, we undertook a shotgun proteomics approach. Extracellular proteins from a culture grown on WSE were precipitated and both the whole secretome as well as 1D-SDS-PAGE fractions were analyzed by mass spectrometry (CEQUIBIEM, FCEN, UBA). Identified proteins were compared to the previously obtained annotated genome of *Cellulomonas* sp. B6 and analyzed manually using the dbCAN algorithm from the CAZy database. Potential enzymatic activity was assigned using the BLAST algorithm from NCBI. We identified two potential exoglucanases of GH6 and GH48 families, eight GH10 and one GH11 xylanases, four potential GH9 and one GH6 endoglucanases and a GH74 xyloglucanase. Only some of these enzymes had been previously identified under other growth conditions, such as CMC or SCR, suggesting that growth on different biomasses can result in differential expression and secretion of hydrolytic enzymes. In order to evaluate the activity of some of these enzymes, a GH10 with a carbohydrate binding module CBM2 was selected. We amplified and cloned the coding gene and the protein was heterologously expressed without its signal peptide as a His-tag N-terminal fusion and purified by affinity chromatography. The activity of the purified enzyme, named rGH10XynC, was evaluated on xylan and lignocellulosic biomass. We confirmed the EC 3.2.1.8 xylanase activity by hydrolysis of xylan to xylobiose (X2) and xylose (X1). Optimal activity was observed at 50°C in a pH ranging from 5 to 7,5. Activity on extruded barley straw (BSE) also resulted in conversion to xylo-oligosacharides, X2 and X1, demonstrating that rGH10XynC is active on the xylan contained in biomass. These results suggest that *Cellulomonas* sp. B6 is a good source of enzymes with potential industrial applications- such as the production of paper pulp, animal feed and second generation bioethanol.

MM-002

Stenotrophomonas maltophilia ISOLATES FROM CYSTIC FIBROSIS PATIENTS IN ARGENTINA: GENOTYPIC AND PHENOTYPIC CHARACTERIZATION

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Stenotrophomonas maltophilia (Sm) is a nosocomial multidrug-resistant pathogen. Respiratory tract colonization by Sm is commonly seen in cystic fibrosis (CF) patients, but its pathogenic role has not been fully established. However, chronic Sm colonization should be considered as a chronic infection because of the associated specific immune response to Sm. The aim of this study was to characterize 8 Sm isolates obtained between 2014-2016 from sputum of 3 CF patients attending hospitals in Argentina: 5 isolates from a 22-year-old female (1318, 1321, 1326, 1336 and 1340) and B8 from a 5-year-old female (Buenos Aires City), and SF2 and SF3 from a 14-year-old male (Santa Fe City). The reference strain K279a isolated from the blood of an oncologic patient was included. ERIC-PCR revealed a great diversity among the isolates, only 2 isolates (1318-1326) belong to the same cluster. Biofilm formation and phenotypic traits associated to biofilm development (swimming, twitching and EPS production) were studied. Biofilm formation and EPS (extracellular matrix) production were evaluated in microtiter plates. The biomass, quantified by measuring the OD540 of crystal violet, ranged from 0.49 to 1.51. Among the studied isolates, only 1336, SF2 and SF3 formed strong biofilms as well as K279a. The other Sm CF isolates produced low amounts of biomass and EPS. Only SF2, SF3 and K279a showed high levels of EPS production. Regarding swimming and twitching motilities, SF2 and SF3 produced diameters similar to those of K279a, while the other isolates do not posses these types of motility. Phenotypic and genotypic characteristics related to proteolytic and nitrate reduction abilities were also investigated. Detection of *narG* (coding for membrane-bound nitrate reductase) and *stmPr-1* (coding for extracellular alkaline serine protease) genes by PCR was done with primers designed in this study. All the isolates except 1336 amplified for *stmPr-1*. However, only B8, SF2, SF3 and K279a produced zones of hydrolysis on casein agar plates, suggesting that the other isolates could have mutations in *stmPr-1*. On the other hand, the nitrate reductase activity was only detected in K279a. However, *narG* was detected in K279a and SF2, suggesting that SF2 could have mutations in this gene. In conclusion, the 5 isolates collected sequentially from a female patient do not posses motility (associated to flagellum and type IV pili) nor proteolytic activity, and only 2 isolates belong to the same cluster. Four of them formed biofilms with lower biomass and EPS production than the non-CF K279a strain. Thus, they expressed lower virulence factors than K279a. In contrast, the 2 isolates collected from a male patient showed a profile similar to K279a. None of the CF isolates showed nitrate reductase activity that supports growth in the absence of oxygen. This is the first report on characterization of Sm recovered from CF in Argentina. More studies on a possible Sm adaptation to CF airways are needed.

MM-003

THE *Acinetobacter baumannii* XERC/D SITE-SPECIFIC RECOMBINATION SYSTEM MODULATES PLASTICITY OF PLASMIDS CARRYING GENETIC ELEMENTS CONFERRING *bla*OXA-58 -MEDIATED CARBAPENEM RESISTANCE

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Plasmids from *Acinetobacter* are considered to be key genetic factors in the spread of multidrug resistance (MDR). It has been reported the presence of XerC/D-like sites flanking discrete DNA modules carrying the *bla*OXAplasmid-borne genes, which suggests that their dissemination in the *Acinetobacter* is mediated by site-specific recombination. We characterized here the plasmids carried by two clonally-related MDR *A. baumannii* clinical strains Ab242 and Ab825 belonging to the CC104 clonal complex in order to follow the evolution of mobile elements harboring resistance genes. Sequencing of Ab242 and Ab825 plasmids was done by 454 pyrosequencing method. They were classified on the basis of *A. baumannii* PCR-based replicon typing method. Plasmids bearing resistance determinants isolated from these bacteria were thoroughly characterized by cloning, sequencing and database searching. Their dissemination was evaluated by their ability to confer resistance to sensitive *A. baylyi* strains. We detected novel plasmids in both strains by sequencing, for the case of Ab242: pAb242_9 (9 kbp), pAb242_12 (12 kbp) and pAb242_25 (25 kbp); and for Ab825: pAb825_12 (12 kbp) and pAb825_34 (34 kbp). pAb242_9 contains a replicase (Rep) belonging to GR4 group; the pAb242_12 Rep shares 50% of identity with GR12 Rep; and pAb242_25 is a multi-replicon which harbors a RepB corresponding to GR10, and its additional Rep shows 100% of amino acidic identity with Rep of *A. baumannii* p11921. The pAb825_12 is identical to that described for Ab242, and pAb825_34 is a multimer formed by the junction of pAb242_25 and pAb242_12. The pAb242_25 carries a genetic element (9.6 Kbp) including IS*Aba825-bla*OXA-58 and *aphA6* flanked by XerC/D-like sites. This whole structure was found in opposite orientations within both pAb242_25 and pAb825_34 plasmids revealing thus its inversion mediated by XerC/D-sites. Deeper analyses uncovered the presence of XerC/D-like sites distributed around all mentioned plasmids. Seventeen different XerC/D-like sites were found and a consensus site was obtained. In addition, a concatemer formation between pAb242_25 and pAb242_12 through XerC/D-like sites was observed upon *A. baylyi* bacterial transformation employing Ab242 plasmid content. Thus, the pAb242_37 cointegrate uncovers that these sites are involved in recombination events. Further analysis of Ab242 plasmidic regions delimited by XerC/D-like sites exposed a high degree of identity with others from *Acinetobacter* plasmidome, suggesting that these structures could be mobilized by recombination processes. Overall evidences suggest that these sites are involved in recombination events (platforms inversion and cointegrates formation) leading to the mobilization of the resistance genes. These observations add up to the diversity of genetic plasticity mechanisms that modulate plasmid evolution and shed light on the mechanisms involved in the antimicrobial resistance dissemination.

MM-004

WHERE, WHEN, HOW AND WHY? : STUDY OF THE INTERACTION BETWEEN THE CYTOPLASMIC MEMBRANE AND PLSX, A KEY PHOSPHOLIPID SYNTHESIS ENZYME FROM *Bacillus su*btilis

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PlsX is a pivotal enzyme for phospholipid synthesis in Bacteria, catalyzing the interconversion of acyl-ACP to acyl-phosphate, which is subsequently utilized by the membrane-bound PlsY acyltrasferase on the pathway to membrane phospholipid biosynthesis. A thorough knowledge of PlsX is relevant due to its role in coordinating fatty acid and phospholipid synthesis in Gram-positive bacteria. Recently, we employed green fluorescent protein fusions to investigate the subcellular localization of this protein and analyzed its possible interaction with the cell division machinery of *Bacillus subtilis.* PlsX was shown to be homogeneously distributed as a peripheral membrane protein, as expected of a protein that participates in the synthesis of new membrane. Although PlsX was essential for viability, its depletion did not affect formation of the cell division Z-ring, and PlsX did not colocalize with the divisome, the multiprotein complex that executes division. These data suggest that coordination between division and membrane synthesis may not require physical or functional interactions between the divisome and phospholipid synthesis enzymes. In the present study, we applied genetic, biochemical and biophysical assays to demonstrate that PlsX´s membrane localization is due to a direct interaction between the protein and the phospholipid bilayer. An unstructured loop of PlsX was identified as the lipid-binding moiety and a stretch of hydrophobic residues was critical for this binding. Interestingly, the interaction between PlsX and membrane phospholipids was necessary for efficient phospholipid synthesis and viability *in vivo,* but was not required for the acyl-ACP:phosphate transacylase enzymatic activity *in vitro.* We speculate that membrane attachment *in vivo* is required for the proper delivery of PlsX´s product, acyl-phosphate, to its PlsY partner protein. Furthermore, we also demonstrated that a highly conserved amphipathic helix in PlsX is involved in the dimerization of this protein, which is indispensable for both its membrane localization and for acyltransferase activity. These results highlight the importance of spatial organization for the proper functioning of certain biochemical pathways. In addition, they contribute to the field of antibacterial drug discovery, considering the importance of bacterial lipid synthesis as a target for new antimicrobial compounds.

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MM-005

EMPLOY OF ATOMIC FORCE MICROSCOPY FOR THE ANALYSIS OF THE METABOLIC RESPONSE OF BACTERIA TO STRESS CONDITIONS IMPOSED BY ANTIBIOTICS

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To control the rapid spread of bacterial infections, fast, sensitive, and reliable methods for quantitative assessment of antimicrobial activities are needed. Current growth-based methods are time-consuming, not capable of distinguishing between bactericidal and bacteriostatic effects and fail to detect "non growing but metabolically active" bacteria. Most antibiotics inhibit processes that are major consumers of cellular energy output, affecting metabolic activities. Fluctuations of highly sensitive atomic force microscope (AFM) cantilevers can be used to detect low concentrations of living bacteria and to characterize their metabolic activity within minutes. In the case that the microbial cells, adhered to the cantilever, are exposed to a particular stress condition such as antimicrobial agents, this interaction could produce changes in the cantilever oscillation which would let assessing the efficiency of the drug on target cells metabolism. In the present study, we optimized an assay for determine the minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) through the analysis of changes in metabolic activity of bacteria incubated under the presence of different antibiotics using ultrasensitive AFM cantilevers. *Bordetella pertussis* and *Escherichia coli* were incubated in presence of different antibiotics (macrolides and ampicilin) concentrations. To attach bacteria to the cantilever´s surface it was previously incubated with glutaraldehyde and then with bacterial suspension. Finally the vibrational response of the cantilever was registered through the incidence of a laser, upon exposure of antibiotics mentioned. The results of AFM device were validated with the ones coming from traditional triphenyl tetrazolium chloride (TTC) method, and the traditional MIC and MBC analysis by the broth dilution method (CLSI). We first prove that the new device is sensitive enough to show by an analysis of variance of cantilever oscillations, the metabolic activity of cells attached to the surface of the cantilever as well as to reflect the increase of adhered cells. It was then determined that in the presence of bactericidal concentrations of antibiotics (data taken from planktonic cell assays), after only 40 min of incubation there is a significant metabolic response. Finally, MIC and MBC were determined in the presence of increasing concentrations of antibiotics. The results obtained from AFM device were tested against the traditional MIC and MBC methods according to CLSI and the derived method from the TTC. This work identifies a link between cellular respiration and cantilevers oscillations demonstrating that the metabolic state of bacteria could be evaluated by the variance of cantilevers movements. Our data show that antibiotics disturb the metabolic state of bacteria and that the answer of this device is enough sensitive and fast to characterize the metabolic state of bacteria in different environments.

MM-006

CATALASES IN *Acinetobacter*: KATG SIGNAL PEPTIDE LEADS FUNCTIONAL FOLDING AND PERIPLASMIC LOCALIZATION

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The genus *Acinetobacter* includes a broad group of physiologically versatile bacteria occupying different natural ecosystems. Some species of the genus are becoming emerging model organisms because of their genome plasticity and the resulting adaptability to environment. *Acinetobacter sp.* Ver3 is a gamma proteobacterium isolated from high altitude Andean wetlands. This polyextremophile was able to grow under hostile environmental conditions such as intense UV-B radiation, high salt concentration or the presence of arsenite up to 10mM. Interestingly, total catalase activity in *Acinetobacter sp*. Ver3 free cell extracts is about 15 times higher than those found in control collection strains. After a genome pyrosequencing strategy and annotation (http://rast.nmpdr.org), two genes were identified, corresponding to a monofunctional catalase *kat*E and a bifunctional catalase-peroxidase *kat*G. Although displaying a significant degree of structural conservation among all monofunctional catalases, Ver3 KatE exhibits one of the highest catalytic efficiencies reported up to date (*k*cat = 1,29.107 ± 0,25.107 s-1). Hydrogen peroxide sensitivity assays in the presence of the KatE inhibitor 3-amino-1,2,4-triazole revealed a significant decrease of tolerance in Ver3 when compared to the response of several collection control strains. These results indicate a critical role of the cytosolic KatE in peroxide detoxification. The katG gene codifying a bifunctional catalase-peroxidase was cloned and overexpressed as recombinant protein employing chaperone helpers in the bacterial host *E. coli* BL21 [pKJE7]. Protein sequence analysis using the algorithms JGI (img.jgi.doe.gov), PSORT (psort.hgc.jp) and SignalP (www.cbs.dtu.dk), suggests the presence of a targeting signal peptide of 19 residues. Posterior subcellular fractionation of transformant E. coli cells expressing Ver3 KatG shows its periplasmic localization. When katG was overexpressed after removing the N-terminal leader sequence, the enzyme was unable to reach the periplasm. Moreover, the protein product accumulated in cytosol as an haem-bound inactive enzyme. These results reveal that polyextremophile *Acinetobacter sp*. Ver3 takes advantage of both, a cytosolic highly efficient monofunctional catalase and a bifunctional catalase-peroxidase as periplasmic antioxidant barrier.

MM-007

CHARACTERIZATION OF A TYPE I-F CRISPR-CAS SYSTEM FROM THE CLINICAL ISOLATE *Shewanella* sp. Sh95

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Clustered regularly interspaced short palindromic repeats (CRISPRs) and their associated genes (*cas*) are essential components of the adaptive immune system of bacteria, which provides protection against bacteriophages and plasmids. CRISPRs are composed of short nucleotide palindromic repeats interspaced by short segments of foreign DNA, called spacers. *Shewanella* sp. Sh95 is a gram-negative rod isolated from an ocular infection. The *Shewanella* genus thrives in several aquatic environments and it is known for its potential in bioremediation and biocell fuels. More recently, they have been considered an emergent opportunistic pathogen. We previously sequenced the complete genome of strain Sh95, where a CRISPR array was found. The aim of this study was to characterize and understand the evolution of CRISPR-cas system from *Shewanella* sp. Sh95. The CRISPR array comprised 152 repeats (28 bp-long) and 152 spacers (32 bp-long). Upstream of the array we identified the cascade genes: *cas1*, *cas2/3*, *csy1*, *csy2*, *csy3* and *csy4*. Analysis of these genes confirmed that the CRISPR-cas system of *Shewanella* sp. Sh95 belonged to the subtype I-F. Comparative analysis with other *Shewanella* genomes revealed that this system is poorly conserved in the genus. Furthermore, the insertion site of this system occurred at the gene *radC* and downstream of the phage transcriptional regulator *alpA*. Strain Sh95 system had two types of repeats with highly similar sequences. Analysis using the Mfold software showed that both types of repeats conserved the secondary structure necessary for processing the CRISPRs RNA. On the other hand, spacer analysis showed that some of these elements might interfere with the expression of essential genes of phages from the families Lambda and Mu, providing infection immunity. Noteworthy, we found several spacers along the CRISPR array interfering with the same genes, which revealed a chronology of the infections caused by these phages. Experimental analysis confirmed that *cas* genes were expressed under different nutrient and stress conditions by qRT-PCR assays which suggests that Sh95 CRISPR-cas system is still active. Last, we evaluated plasmid loss upon transformation of pCR2.1, pACYC184 and pCR-SmaI2 into strain Sh95 to evaluate the ability of the CRISPR-cas system to eliminate exogenous material, which resulted in their complete loss after 5 days except for plasmid pCR-SmaI2 which was lost after 4 days. Taking together our results suggest that *Shewanella* sp. Sh95 adapted to clinical setting by acquiring beneficial traits, such as a type I-F CRISPR-cas immune system. This feature will provide a clear advantage in order to survive in a nosocomial environment to avoid infection by different bacteriophages.

MICROBIOLOGÍA MOLECULAR

MODALIDAD POSTER

MM-008

METABOLIC ADJUSTMENTS AT THE CENTRAL CARBON PATHWAYS ASSOCIATED WITH MISREGULATED PHB STORAGE IN A *Sinorhizobium meliloti* MUTANT LACKING THE SMALL REGULATORY RNA MmgR

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S. meliloti is a soil-dwelling alpha-proteobacterium capable of engaging into a symbiotic interaction with the roots of host legumes, after which it becomes able to reduce the atmospheric nitrogen (N) in exchange for organic carbon (C) sources. Rhizobial metabolism is known to be highly versatile, and must be tightly regulated given the sharp contrast that exists between the free- and the plant-associated- lifestyles of the bacterium. Regulation of metabolism is executed at multiple levels, from gene expression to enzymatic activities. In this regard, several non-coding small RNA regulators have been so far described to be involved in the regulation of the expression of membrane metabolite transporters in *S. meliloti.* We have previously reported that upon N exhaustion and in the presence of an available C source –a condition that resembles the soil environment-, the widely conserved non-coding small RNA MmgR is strongly induced in *S. meliloti* and negatively regulates the accumulation of the major C and reducing power storage polymer polyhydroxybutyrate (PHB) by setting a limit to the amount of synthesized polymer. Moreover, we have shown that MmgR modulates the number and the morphology of the intracellular PHB granules, by regulating the expression of the granule associated phasin proteins PhaP1/2. Despite the reported positive role of PHB in multiple metabolic aspects related to the cellular needs for C and energy, it seems reasonable that the accumulation of the polymer and its associated granule proteins has to be limited. We wondered whether the impairment to properly define the architecture and size of PHB granules in *mmgR* mutant rhizobia was associated with an underlying metabolic deregulation that could negatively impact on bacterial fitness and which would impose a natural form of selective pressure to preserve this regulatory mechanism. For this purpose, we have characterized the impact of an *mmgR* mutation on the global metabolic status of rhizobia. A quantitative metabolomic profiling of a set of 40 metabolites that are involved in the major rhizobial metabolic pathways was performed by gas chromatography coupled to mass spectrometry. A mild repression of C source assimilation (sucrose), as well as slight changes in the size of the pools of various metabolites involved in the Krebs-cycle (e.g. alpha- etoglutarate and malate) and in secondary metabolism (e.g. b-alanine) were detected in the *mmgR* mutant cells bearing higher amounts of intracellular PHB than those in the wild-type. The negative effects on the rhizobial fitness of such a mutation should be investigated under conditions that simulate the natural environment that rhizobia inhabit.

MM-009

EXTRACELLULAR FACTORS THAT ALTER THE STRUCTURE OF THE *Rhizobium leguminosarum* BIOFILM MATRIX

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The ability to form biofilms confers bacteria several advantages to survive in unfavourable environments. One of the key steps in the formation of a biofilm is the production of an extracellular matrix. Gram negative bacteria called rhizobia are able to form symbiotic atmospheric nitrogen fixing nodules with legumes thus contributing to sustainable farming practices. Rhizobia rely on biofilms to inhabit either the soil or the competitive nutrient - rich rhizosphere. Understanding of the process of a proper biofilm formation is crucial to further expand the knowledge of this symbont and its interaction with plants. Formation of the capsular (CPS) and extracellular polysaccharide (EPS) is a key process in the formation of a mature biofilm in *Rhizobium leguminosarum*. Besides, mutants lacking the PrsDE secretion system were found to be defective in the formation of a mature biofilm. This system secretes an unusually high number of substrates, including the Ply glycanases, which cleave the polysaccharide chains shortening their length, and the Rap proteins such as RapA. Rap proteins share at least one EPS/CPS-binding domain (Ra: *Rhizobium* adhering) and, in general, harbour another specific domain. In particular, RapA is only made of two Ra domains and has been shown to be involved in adhesion to the legume's roots, infection competitivity alteration of the EPS/CPS balance and biofilm formation. Interestingly, RapA was found strongly associated with the bacterial cell surface. In the current study we aimed to further understand the role of PrsDE-dependent extracellular proteins in biofilm formation. Firstly, to identify all PrsDE substrates, we performed a proteomic analysis by comparing the "secretome" of the reference strain *R.l.v.* 3841 with the *prsD*::Tn*5* isogenic mutant. We confirmed all substrates that were previously described; besides, we found a new set of PrsDE-dependent extracellular proteins, including a new Rap protein, which we called RapD. This protein harbours a N-terminal Ra domain and another C-terminal domain of unknown function. Using polyclonal antibodies generated against RapD, we found that, unlike RapA, RapD was solely detected in the extracellular medium. This observation suggests that RapA and RapD might play different roles in the modulation of the extracellular matrix structure. Ongoing studies are focused on determining the effect of the absence or the overproduction of RapD on biofilm formation and the interaction with the legume roots.

MM-010

BIOFILM-FORMING CAPACITY AND MUPIROCIN RESISTANCE IN METICILLIN-RESISTANT *Staphylococcus aureus* CLINICAL ISOLATES OBTAINED FROM HOSPITALIZED CHILDREN

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Methicillin-resistant *S. aureus* (MRSA) carriage raises the risk of adverse health outcomes in hospitalized children producing sepsis and death. Nowadays, management of infections caused by this human pathogen is currently a challenge in all health institutions. To control MRSA carriage, topical mupirocin ointments is worldwide used for skin and soft-tissue infections. However, an increased usage of this antibiotic has promoted the presence of clinical resistant isolates, which often cause treatment failures. Another serious challenge to the clinicians is the treatment of biofilm-associated infections producing an increase in antibiotic tolerance to most compounds. In fact, biofilm production is considered as another mechanism of antibiotic resistance in MRSA having a high impact on the selection of therapeutic regimens. Besides, biofilm-producing bacteria are usually a source of recurrence of local, systemic or chronic infections in children, which are very difficult to treat with antibiotics. Here, the aim of this work was to explore the level of mupirocin resistance and the biofilm-forming capacity in MRSA isolated from bloodstream of pediatric inpatients in a tertiary referral hospital located in Buenos Aires, Argentina. We performed a cohort retrospective study over the period between 2011– 2015. Samples of blood from inpatients were cultured and *S. aureus* isolates were identified by usual biochemical test followed by MALDI-TOF MS. Standard microtiter plate assays were used to assess the level of mupirocin resistance. The content of biofilm was carried out by the violet crystal test. Mupirocin-resistant MRSA clinical isolates were observed in 2.5% (5/217) of pediatric inpatients. In consequence, a low prevalence of mupirocin-resistant MRSA isolates among hospitalized children was found. However, all of them exhibited minimum inhibitory concentrations of mupirocin > 512 µg/ml, demonstrating the existence of a high degree of resistance to this antibiotic. To make the situation most problematic, our results showed that all mupirocin-resistant MRSA isolates were capable to produce biofilm in a moderate to strong level. We observed that biofilm was detected in all MRSA clinical isolates regardless of their pattern of antibiotic resistances. Mupirocin-susceptible MRSA isolates were also biofilm-producers. In summary, we observed a low incidence of mupirocin resistance in MRSA and all of them are capable of producing biofilm. This knowledge will be useful for development of effective strategies to management of infections caused by this human pathogen in hospitalized pediatric patients.

MM-011

Streptococcus uberis: SUSCEPTIBILITY TO PENICILLIN AND PRESENCE OF GENES OF PENICILLIN-BINDING PROTEINS

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Streptococcus uberis is one of the most prevalent environmental pathogens responsible for a significant proportion of subclinical and clinical bovine intramammary infections in lactating and non-lactating cows. This agent is not controlled effectively by current mastitis control practices. For a mastitis treatment to be successful, it must include the selection of appropriate antibiotic agent. Wide application of antimicrobials in veterinary practice results in selective pressure and selects resistant variants. The molecular mechanism of resistance of Streptococcus sp. is based on acquisition of mutations in penicillin-binding proteins. Although it is true that no high-level penicillin resistance (MIC 16 pg/ml) has yet been reported for these bacteria throughout the world, isolates with intermediate resistance (MICs ranging from 0.5 to 16 pg/ml) have been described. As yet, nothing has been reported about the susceptibility to penicillin among S. uberis isolates from cattle with mastitis in Argentina. The aim of the present study was determined the susceptibility to penicillin and the presence of genes coding for penicillin binding proteins (pbp) in 34 S. uberis isolated of bovine subclinical and clinical mastitis from 19 herds located in the central dairy region of Argentina. The minimum inhibitory concentration (MIC) was determined according to CLSI (2012). Isolates were categorized as susceptible, intermediate and resistant based on interpretive criteria developed by the NCCLS (2008). In addition, the presence of the pbp2A (SUB1733), pbp1B (SUB0114), pbp1A (SUB1407) and pbpx (SUB 1419) genes that encoding penicillin binding proteins were detected by pCr. The association between MIC and the presence of pbp genes was determined by contingency tables. In our study, MIC for penicillin against S. uberis isolates ranged from 0.09 pg/ml to 0.83 pg/ml. All isolates were susceptible to penicillin (MIC < 1 pg / ml). The presence of four PBP genes was confirmed in all S. uberis isolates by PCR. No correlation between the MIC and the presence of the pbp genes in 34 S. uberis was observed by chi-square test (x2). In conclusion, the present work demonstrates that S. uberis is susceptible to penicillin, which would demonstrate the absence of mutations in the detected genes. So the use of penicillin as a first-line therapeutic choice can still be recommended in veterinary medicine in Argentina.

MM-012

DETECTION OF *Yersinia enterocolitica* IN PURE CULTURE BY PCR USING INTERNAL (IAC) AND EXTERNAL (EAC) AMPLIFICATION CONTROLS

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Yersinia enterocolitica, an enterobacterium present in certain foods, can cause infections in humans. Its detection in foods is possible by PCR targeted to the *16S rDNA* gene using the primer pair 16SYerF-16SYerR. However, food samples are complex matrices and they might contain PCR inhibitors. To avoid false negative results, it is advisable to introduce a competitive internal amplification control (IAC) that is co-amplified with the target DNA sequence by the same primer pair in PCR. The IAC and target DNA products (*16S rDNA* amplicon) can be identified by their different molecular (710 and 300 bp, respectively). Thus, the IAC is incorporated into the PCR reaction mixture where will compete for primers with the target gene. In contrast with positive samples where two bands (*16S rDNA* and IAC) will be observed, negative samples will show only one band corresponding to IAC. In the present study, we included an IAC previously designed by this research team, in a well-known PCR protocol to amplify the *16S rDNA* gene from *Y. enterocolitica*. The IAC was introduced in the PCR reaction and amplified when *Y. enterocolitica* cultures was assayed. When Y. enterocolitica concentration was 4.5 x 103 CFU ml-1 in pure culture, the IAC at concentration of 2.94 ag µl-1 in the PCR reaction mixture was co-amplified with the *16S rDNA* sequence, producing bands of 710 and 300 bp, respectively. These *Y. enterocolitica* values were considered the detection limits of the duplex PCR. Furthermore, an external amplification control (EAC) which has the same size as *16S rDNA* amplicon (300 bp) was amplified in parallel as a positive control regarding future studies where unknown samples will be analyzed. The specific detection of *Y. enterocolitica* by PCR including IAC and EAC might be achieved directly on food samples when the pathogen load reaches concentrations of at least 4.5 x 103 CFU ml-1.

MM-013

RESPONSE OF *Acinetobacter Baumanni* AGAINST AGENTS THAT CAUSE DNA DAMAGE

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Acinetobacter baumannii (Ab) is a multidrug-resistant nosocomial pathogen that possesses ability to form biofilms and survives desiccation. Desiccation and desiccation-rehydration cause DNA lesions, which result in elevated mutagenesis on planktonic cells. The rise of resistant pathogens and chronic infections tolerant to antibiotics presents an unmet need for novel antimicrobial compounds or treatments. Nitrofurans are a family of antibiotics that acts primarily through the formation of N2 deoxyguanosine adducts in DNA. These adducts may be mutagenic or block replication, and therefore, their presence can be lethal to the cell. Nitrofurazone (NTZ) is used topically in the treatment or prophylaxis of infections of wounds and burns. Several groups are developing hydrogels and catheters impregnated with NTZ. The aims of this work were to study the mutagenic activity of NTZ on biofilm and planktonic cultures, and the mutagenic effects of desiccation on biofilms of Ab. Studies were done with Ab82, strain isolated from a surgical wound. The MICs of NTZ and rifampicin (Rif) were determined by the standard microdilution method. The minimal biofilm inhibitory concentration (MBIC) of NTZ was determinated in a microplate assay. Mutation frequencies for resistance to Rif were determined for planktonic and biofilm cultures treated with different concentrations of NTZ or dried biofilms formed onto coverslips. Bacterial cells were recovered from dispersed biofilms and planktonic cultures. Next, the cells were grown in LB prior to plating in LB with 50 µg/ml of Rif. Mutation frequencies were expressed as the number of Rif resistant mutants as a fraction of the viable count. The mutation frequencies are the mean values from two independent assays The MIC values for NTZ and Rif were 16 µg/ml and 4 µg/ml, respectively; and the BMIC for NTZ was 128 µg/ml. The frequency of selection of Ab82 Rif resistant mutants increased 6.7 fold when the bacteria were grown as biofilms (planktonic 4.7 x 10-9, biofilm 3.2 x 10-8, $P < 0.05$). Under planktonic conditions the mutation frequency upon treatment with the DNA damaging agent NTZ at 4 x MIC, and 1024 µg/ml were 1.2 x10-7 ($P < 0.01$) and 3.1 x 10-9, respectively. On the other hand, the frequency of mutation increased in biofilms exposed to the MBIC of NTZ (1.7 x 10-7, $P < 0.01$). Interestingly, in biofilms exposed to 1024 µg/ml the mutation frequency did not show a dose-response relationship (1.2 x 10-7). The frequency was also elevated in cells from biofilms exposed to a desiccation-rehydration process (3.8 x 10-7, $P < 0.01$). In the course of the assays with NTZ we observed several larger and mucous colonies than those of the wild type strain. One of them showed more ability to form biofilms and larger and denser twitching areas with multilayered rafts and larger microcolonies. In this report we presented evidence that Ab82 increased the mutation frequency and generate phenotypic diversity under treatments which cause DNA lesions.

MM-014

HALOARCHAEA FROM THE ANDEAN PUNA: BIOLOGICAL ROLE IN THE ENERGY METABOLISM OF ARSENIC

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Arsenic metabolism is proposed to be an ancient mechanism in microbial life. In fact, some Bacteria and Archaea are able to exploit arsenic as a bioenergetic substrate in either anaerobic arsenate respiration or chemolithotrophic growth on arsenite as an electron donor. The high-altitude Andean lakes (HAAL) consist of several shallow lakes located in a high-altitude desert known as Puna, and these placed are distributed through Argentina, Chile, Bolivia, and Peru along the Central Andes region in South America. This environment host unexplored ecosystems of shallow lakes and salt flats at altitudes of 3700 meters above sea level (masl). In these places high concentrations of arsenic were found in the water and these was attributed to the high Andean volcanism phenomenon which provides the geoenvironmental conditions and determine the availability of arsenic. Recently, our group has reported a metagenomics analysis of a microbialite from Diamante Lake, Catamarca, which was widely dominated by Archaea (96%), assigned to the class Halobacteria (commonly called as haloarchaea). Moreover, these authors strongly suggest that the prevalent haloarchaeal part of the biofilm have all the genes necessary for anaerobic arsenate respiration and arsenite oxidation, suggesting that these haloarchaea use arsenic compounds as bioenergetics substrates to sustain growth. The objective of our study was to investigate the presence and expression of genes (*aioA* and *arrA*) involved in obtaining energy from arsenic compounds in environmental samples and isolated strains. The effect of As [V] and As [III] during isolates growth and the possible role of As as bioenergetic substrate in two selected strains was also evaluated. The presence of *aioA* and *arrA* genes was confirmed in total community DNA from Diamante and Tebenquiche lakes, and the expression of these genes was confirmed by metatranscriptomic RNA samples, suggesting an active expression of both genes in the studied samples. Using selective isolation techniques, eighteen microorganisms belonging to the *Halorubrum* genera (phylum Euryarchaeota) were isolated. The genes enconding for *aioA* and *arrA* were detected in most of the isolates and their expression was verified in two selected strains. The physiological assays using a Chemically Defined Medium (CDM) showed a positive effect of As[III] and As[V] on cell growth. Moreover *Halorubrum* sp. DM2 was able to oxidize and reduce As. The confirmation of oxidation/reduction of arsenic and the transcriptional expression of these genes by RT-PCR in the strain DM2, support the previously raised hypothesis that the arsenic could be used in bioenergetics processes by the microorganisms inhabiting these environments.

MM-015

CHARACTERIZATION OF AN ANTIMICROBIAL PEPTIDE PRODUCED BY A CLINICAL ISOLATE AC172 OF *Shigella flexneri 2*

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The objective of this work was to characterize the antimicrobial peptide produced by AC172 strain. This strain was isolated in the summer period 2016-2017 from a pediatric patient with enterocolitis, recovered in the Centro Provincial de Salud Infantil Eva Perón (CePSI-Santiago del Estero province).The biochemical and serology tests allowed us to classify this strain as a member of the *Shigella flexneri* 2 serotype. In this study, we analyzed the antibiotic resistance profile of this strain using the antibiotics-disc technique. We also analyzed the AC172 plasmid profile, detecting the presences of a large number of these extrachromosomal elements. In addition, we determined that AC172 is able to produce a growth inhibitory substance, using the plate diffusion method and the *E. coli* AB1133 strain as sensitive. This inhibitory compound was characterized using a cell free supernatant obtained after the overnight culture of AC172 in LB. The supernatant's treatment with proteases showed that this inhibitory substance is of protein nature, since it maintained its ability to inhibit bacterial growth after 2 hours of incubation at 37°C. Moreover, it compound was presented as resistant to high temperatures degradation, even after being incubated at 100°C for 20 min. We estimated the size of such substance by electrophoresis in a polyacrylamide gel (12%), where was compared with a molecular weight marker and developed by its growth inhibition activity. The net charge was determined by electrophoresis, running a sample into a 1% agarose gel, at pH 8. The results of this study allow us to characterized this compound as a like-bacteriocin peptide, of low molecular weight (3 kDa, approximately), negatively charged, stable at high temperatures and of protein nature.

MM-016

EXPRESSION OF THE SMALL REGULATORY RNA GENE *mmgR* IS REGULATED NEGATIVELY BY AniA AND POSITIVELY BY NtrC IN *Sinorhizobium meliloti* 2011

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In the N2 -fixing symbiont of alfalfa root nodules, *Sinorhizobium meliloti* 2011, the *mmgR* gene encodes a 77-nucleotide small untranslated RNA (sRNA) that negatively regulates the accumulation of polyhydroxybutyrate when the bacterium is grown under conditions of surplus of carbon (C) in relation to nitrogen (N). The *mmgR* gene is expressed at all stages of the symbiotic interaction of the rhizobium with alfalfa roots, but at higher levels in the N2 -fixing bacteroids. Our previous work revealed that the expression of *mmgR* is primarily controlled at the transcriptional level and that it depends on the cellular N status, although the regulatory mechanism and the factors involved were unknown. In this study, we provide novel experimental data supporting that: a) *mmgR* is induced upon N limitation with the maximum expression found at the lowest tested C/N ratio in the growth medium; b) a conserved heptamer TTGTGCA located in between the -35 and -10 elements of the *mmgR* promoter is necessary and sufficient for the induction of expression by N limitation; c) induction of *mmgR* requires the global N-status regulator NtrC; d) under C limitation, transcription of *mmgR* is repressed by AniA, a global regulator of C flow; e) the *mmgR* promoter contains a conserved dyadic motif (TGC[N3]GCA) partially overlapping the heptamer TTGTGCA, that was also found in the promoter regions of the PHB-related genes *phaP1*, *phaP2*, *phaZ* and *phaR* (*aniA*) of *S. meliloti* and other alpha-proteobacteria. Taken together, these results suggest that the *mmgR* promoter would integrate signals from the metabolism of C and N through –at least- the global regulators NtrC and AniA, in order to provide an optimal cellular level of the MmgR sRNA to fine tune gene expression at the posttranscriptional level according to varying C and N availability.

MM-017

IDENTIFICATION OF GENES INVOLVED IN PRODUCTION OF PHENAZINES BY THE ANTIFUNGAL ISOLATE *Pseudomonas chlororaphis* SMMP3

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Members of the *Pseudomonas* genus colonize the rhizosphere of different plant species and display plant-probiotic traits. Therefore, they are interesting candidates for biological agricultural inputs to stimulate plant development and/or promote crop health. We have generated a collection of pseudomonad isolates from either bulk soil or the rhizosphere of major extensive crops from different plots under no-till management located in Argentina. In particular, the native isolate SMMP3 was obtained from a bulk soil sample of an agricultural field located in Monte Buey, Córdoba. Based on the sequence of 16S rDNA, *rpoB* and *oprF* marker genes, SMMP3 was affiliated to the *P. chlororaphis* species. SMMP3 displayed a broad spectrum of antagonism to 12 fungal pathogens in co-culture in PDA plates. SMMP3 cultures show extracellular phospholipase and proteolytic activities, solubilization of tricalcium phosphate, production of hydrogen cyanide (HCN), the presence of quorum sensing signals of the AHL type, and a relatively good capacity to develop biofilms in polystyrene plates. A diffusible orange pigment present around SMMP3 colonies and in the supernatant of stationary phase cultures strongly suggests the ability to produce phenazines, which are known for their antifungal activity. In order to better understand the genetic basis behind the broad antifungal activity of isolate SMMP3 we carried out both genomic and genetic approaches. On the one hand, we obtained the SMMP3 genome draft by Illumina HiSeq 1500 sequencing and subsequent annotation with the RAST tool. The draft genome sequence confirmed the taxonomic status of this isolate as well as the genetic potential to synthesize the antifungal compounds phenazine, pyrrolnitrin; in addition it revealed the occurrence of at least three quorum sensing systems of the AHL type, and genes related to metabolism of the plant growth regulator auxin. On the other hand, we performed Tn*5* mutagenesis followed by a screening of mutants with altered pigment (phenazines) production. In a first round of mutant selection, we identified 5 mutants with reduced pigment production (-50 to -95%). The mutants with the strongest reduction in pigment production but without significant impact in their growth rate, had Tn*5* insertions within the following loci: a *phzF* homolog involved in biosynthesis of phenazines (mutant 26; >90% reduction); a gene encoding a TonB-dependent receptor (mutant 25; 40% reduction); and a *gacS* homolog (mutant 9; >90% reduction), encoding the inner membrane autokinase that is part of the post-transcriptional regulatory cascade Gac/Rsm that has been characterized in several pseudomonads. The global nature of the *gacS* mutation in clone 9 was confirmed by the concomitant loss of extracellular protease activity. The whole set of genes required for a functional Gac/Rsm system was identified in the draft genome. Further genome mining and physiological characterization of the Tn*5* mutants is underway.

MM-018

STUDY OF THE SYNERGIC INTERACTION EFFECT BETWEEN NISIN AND THE *Shigella flexneri* 2'S ANTIMICROBIAL PEPTIDE, ON FOODBORNE BACTERIAL PATHOGENS.

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Bacteriocins are antimicrobial peptides that have antagonistic effects against other organisms. Nisin is the most studied bacteriocin able to inhibit a broad spectrum of food spoilage. *Shigella flexneri* 2 AC172 produce a bacteriocin like peptide that displays antimicrobial activity against *E. coli* AB1133. In this work we evaluated the synergistic effect produced by the combination of nisin with cell-free supernatant of *Shigella flexneri* 2 AC172 on the sensitive growth. The antimicrobial activity of these peptides, alone or in combination, against foodborne bacterial pathogens was determined by the minimal inhibitory concentration (MIC) method, followed by the optical density determination at 600 nm, using a microplate reader. For these purpose, a 96-well microplate containing serial double dilutions of nisin, the cell-free supernatant of *Shigella flexneri* 2 AC172 or different nisin/cell-free supernatant amount combination, were inoculated with a sensitive bacterial suspension containing 104–105 CFU/ml, and incubated for 24 h at 37°C. The MIC values were used to determinate the Fractional inhibitory concentration (FIC) and the FIC index (FICI), which finally defined the synergistic effect exert by them. These natural antimicrobial combinations represent a useful biotechnological strategy applicable to preservation of the food industry, in order to combat foodborne pathogens that can affect the human health.

MM-019

BIOFILM PRODUCING LACTIC ACID BACTERIA AS AN ALTERNATIVE TO CONTROL FOOD CONTAMINATIONS

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Biofilms are complex structures of microorganisms that colonize various biotic or abiotic surfaces. These organized communities are formed by bacteria embedded in a highly hydrated extracellular matrix that is composed mainly of polysaccharides, proteins, DNA and other substances. Several bacterial surface structures are necessary for this surface adhesion and an environment that favors the formation of biofilm against planktonic growth is required. The presence of biofilms is common in food industry and represents a concern because bacteria can adhere to almost any type of surface, such as plastic, metal, glass, soil particles and wood. Lactic bacteria are generally recognized as safe (GRAS) and could be an alternative for the biocontrol of pathogenic microorganisms biofilms forming in the food production chain. Use of probiotic biofilms can be an alternative approach for reducing the formation of pathogenic biofilms in food industries. The objective of this work was to study the biofilm production of lactic acid bacteria in different substrates and in different surfaces such as plastic, glass and stainless steel, commonly used in food industry. Biofilm formation was measured by violet crystal and plaque counts of seven strains of lactobacilli: *Lb. kefiri* CIDCA 83113, CIDCA 8321, CIDCA 8344 and CIDCA 5818, *Lb. plantarum* CIDCA 83114 and CIDCA 8327 and *Lb. delbrueckii subsp. lactis* CIDCA 133. These have different characteristics such as presence of S-layer, exopolysaccharide or glucan production, autoaggregation and hydrophobicity. The percentage of autoaggregation by decreasing OD at 600 nm and the percentage of hydrophobicity by partition in hexadecane and xylene (MATH method) was measured. For measurement of biofilm formation, 1 ml of medium (MRS and BHI) was inoculated with lactobacilli in 24 wells culture plates. For viable counts, the adhered bacteria were resuspended in PBS and counted in MRS agar plates. Only *Lb. kefiri* CIDCA 8321 and CIDCA 8344 strains presented a percentage of autoaggregation greater than 50%. Both strains also showed the highest hydrophobicity. None of the lactobacilli tested form biofilm in BHI medium. On the other hand, except for CIDCA 8321 strain that did not develop biofilm on any probed condition, the lactobacilli can form biofilm in MRS medium. The lowest biofilm production was observed in plastic, whereas in glass and stainless steel lactobacilli present high capacity of biofilm formation. It is remarkable that *Lb. plantarum* CIDCA 83114 is the one with the highest capacity for biofilm formation in the three surfaces studied, being stainless steel the most suitable surface for biofilm production. We observed that both hydrophobic and hydrophilic strains are capable of producing biofilm. We demonstrated that lactobacilli can form biofilm and this property depends on surface and growth media. This could be used as an alternative control of food pathogens

MM-020

SURFACE PROTEINS OF LACTIC ACID BACTERIA INTERFERE WITH THE BIOFILM FORMATION OF *Salmonella*

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Biofilms are composed of microorganisms attached to a substratum in which bacteria form structured communities immersed in a matrix composed mainly of polysaccharides, proteins and other macromolecules. The bacteria present in biofilms are more resistant to unfavorable environmental conditions and confer resistance to disinfectants, antibiotics and the host immune system. *Salmonella* is commonly associated with foodborne diseases, mainly related to the poultry industry, which produces the disease called salmonellosis. Since this pathogen is capable of forming biofilm, it is necessary to develop a biocontrol strategy to reduce the impact on public health and avian production. Lactic acid bacteria (LAB) may be used as an alternative to reduce the formation of biofilms of pathogens in the food industries. In this work we propose to study the surface proteins of lactic acid bacteria for the inhibition of the formation of *Salmonella* biofilm. The extraction of surface proteins from the *Lb. kefiri* CIDCA 83113, CIDCA 8321 and CIDCA 8344 and *Lb. plantarum* 83114 strains were used. For biofilm formation assays, *Salmonella enterica* serovar Gallinarum CIDCA 115 isolated from a poultry farm was used. Surface proteins of the LAB cultures were extracted with LiCl 5M. Biofilm formation assays were performed in 24-well plates. The surface proteins were pre-incubated on the plate for two hours before inoculation of *Salmonella* or co-incubated with pathogen. Plate was incubated for 48 hours at 28 °C. Detection of the surface proteins extracted was performed by electrophoretic run on polyacrylamide gel and the quantification was performed by the Bradford method. The majority of protein in the extracts of *Lb. kefiri* CIDCA 83113, CIDCA 8321 and CIDCA 8344 was the S-layer protein, while *Lb. plantarum* 83114 showed several protein bands without presence of S-layer protein. Pre-incubation with lactobacilli surface proteins decreased in all cases the formation of *Salmonella* biofilm determined by nutrient agar count. Co-incubation of different concentrations (10, 30 and 60 mg/ml) of *Lb. kefiri* CIDCA 8321 S-layer on the ability of *Salmonella* to form biofilm was also determined. It was observed that the presence of S-layer proteins decreases one or two orders of magnitude *Salmonella* counts in the biofilm and it was observed that this effect was dose- response. The surface proteins of lactic acid bacteria inhibit the development of *Salmonella* biofilm and could be an alternative to avoid possible contamination in poultry environments.

MM-021

STABILIZATION OF PHYTOENE SYNTHASE AFFECTS GROWTH RATE BUT DOES NOT ACCOUNT FOR THE LETHALITY OF THE *lonB* MUTATION IN THE ARCHAEON *Haloferax volcanii*

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Haloarchaea thrive in hypersaline lakes and solar salterns (> 2 M NaCl). Most strains display pink/red colonies due to the presence of carotenoid pigments (mainly bacterioruberins) that protect the cells from UV light irradiation. Energy-dependent proteolysis is a key process in cell physiology as it maintains the quality of proteins and regulates numerous cellular functions. The ATP-dependent Lon protease is conserved among the three domains of Life, however, its biological relevance in archaeal cells has not been elucidated. We have previously investigated the function of LonB in a conditional *lonB* mutant of the haloarchaeon *Haloferax volcanii* (HVLON3) and concluded that this protease is essential for cell viability; moreover, suboptimal Lon levels affected the growth rate, cell shape and produced hyperpigmentation. Proteomic analysis showed that LonB deficiency causes a dramatic accumulation of phytoene synthase (PSY), the key enzyme in carotenoid biosynthesis and that PSY degradation is LonB-dependent. The increase in carotenoids content likely affects the functionality of the cell membrane which is reflected by a decrease in growth rate in HVLON3 and may be responsible for the lethality of the *lon* mutation. To test this hypothesis, in this work we constructed a *H. volcanii* strain with regulatable PSY levels and observed that overexpression of this enzyme led to increased bacterioruberins cell content (4-fold) with a concomitant decrease in growth rate (μ 0.075 vs 0.033 h-1). To investigate whether stabilization of PSY impaired viability of *lon* KO mutants in *H. volcanii*, we attempted to delete the *lon* gene from a delta*crtB* (PSY) background. We were unable to detect clones that had completely deleted the *lon* gene, suggesting that PSY stabilization is not the sole/main reason for the indispensability of LonB in *H. volcanii.* On the other hand, the *crtB* gene was deleted from the chromosome of HVLON3 rendering almost all the resulting clones unpigmented. The high efficiency of *psy* KO mutation (nearly 100%) in addition to the fact that these mutants showed a higher growth rate than the parental strain HVLON3 suggests that even though PSY stabilization may not be the sole reason for the lethality of the *lon* mutation, it severely affects *H. volcanii* growth performance. Key words: *Archaea*, Lon protease, phytoene synthase. Ferrari and Cerletti contributed equally to this work Supported by CONICET, UNMdP, ANPCyT and MINCyT-BMBF.

MM-022

CONSTRUCTION AND STUDY OF *Pseudomonas putida* KT2440 MUTANTS, WITH REDUCED SALT TOLERANCE

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The aim of this work is to delve into stress salt tolerant mechanisms of *Pseudomonas putida* KT2440, which is a well known PGPR (Plant Growth Promoting Rhizobacteria). In order to evaluate this, transposon mutagenesis with mini-Tn5 (Km) was performed by triparental mating. The mutants obtained were screened for their reduced tolerance to salinity in solid media. After evaluating 1500 transposon mutants, only four showed less growth under saline conditions in solid media, in the preliminary screening: Mut10, Mut11, Mut50 and Mut59. Growth curves in different liquid culture media and spots assay, with and without saline conditions, were carried out. The curves showed that the mutants Mut11 and Mut59 grew markedly less with respect to the wild type, in saline conditions. Given these results, transposon insertion sites were determined by arbitrary PCR, followed by sequencing. The obtained sequences were analyzed and compared with genome databases. After analyzing the sequences for the Mut11, it was determined that the transposon was inserted into the gene PP_0024, coding for a membrane-associated metal-dependent hydrolase, involved in the synthesis of lipopolysaccharides. For the Mut59, the transposon was inserted into the gene PP_0003, which encodes a 16S RNA methyltransferase, whereby its growth is affected not only in saline conditions. For a better understanding of the importance of the correct synthesis of lipopolysaccharides in the stress salt tolerance, we studied the behavior of mutant bacteria (mus-40). This mutant is affected in *galU*, encoding UDP-glucose pyrophosphorylase and presents deficiencies in the synthesis of intact lipopolysaccharide. Growth curves and spots assay, in different media and in saline and non saline conditions, were carried out. As expected both mutants, Mut11 and mus-40 showed reduce stress salt tolerance, compared to the wild type. Also Congo red binding assay in saline and non saline conditions was performed, and the results showed rough and less red colonies for Mut11 and darker colonies for mus-40, compared to the wild type. Congo red is a dye with cellulose fibers affinity, therefore the more links of this type there are, the more red the colony will be. To evaluate EPS, calcofluor assay with and without saline stress was carried out, then calcofluor stainable EPS were visualized under UV light; mus-40 colonies were more fluorescent and Mut11 less fluorescent compared with the wild type colonies; the more fluorescence is an indicative for more EPS presence. Finally LPS extraction with phenol-chloroform technique, running in SDS-PAGE and visualized after silver stain; was performed. In the LPS profile the incomplete lipopolysaccharide structure can be observed, especially under saline conditions. The results presented in this work give an idea of the great importance of the study of polysaccharides to improve bacteria tolerance to saline stress.

MM-023

BIOINFORMATIC CHARACTERIZATION OF GENES ENCODING XYLAN DEGRADING ENZYMES IN THE *Paenibacillus* sp. AR247 AND *Cohnella* sp. AR92 GENOMES.

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The increasing interest to renewable lignocellulosic materials for the production of environment-friendly chemicals and biofuels boosts the search of new carbohydrate-active enzymes (Cazymes) and microbial strains. In this study, we analyze the draft genomes of two highly hemicellulolytic bacteria isolated from industrial liquor samples from the local paper industry to unravel their xylan degrading pathways. Gene annotations were carried out using Rapid Annotations Subsystems Technology (RAST) 2.0. BlastP was employed to find orthologous genes between the translated proteins from the predicted open reading frames of both genomes and a local database consisting on the translated proteins of the141 genomes of the *Paenibacillaceae* family members available on NCBI database up to date. Only bidirectional matches were considered. To identify potential Cazymes, translated proteins were submitted to the dbCAN database. The genome sizes were 7.1 Mb (*Paenibacillus* sp. AR247) and 6.0 Mb (*Cohnella* sp. AR92), which contained 7159 and 5439 coding sequences, respectively. 51 orthologous genes were found by the BlastP analysis, most of which corresponded to ribosomal genes. The phylogenetic tree built on the basis of those concatenated gene sequences showed *Cohnella* genus (including the strain AR92) as a monophyletic group within the paraphyletic group of *Paenibacillus* spp., while the strain AR247 was found to be related to *Paenibacillus* sp. P1XP2, *Paenibacillus* piniJCM 16418, *Paenibacillus* sp. IHBB 10380, forming a well supported clade. Both genomes displayed multiple genes encoding a broad variety of extracellular and cell-wall (SLH domians) of endo-b-1,4-xylanases (GHs 10; 11; 30 and 43), some of which also showed CBM domains (mainly CBM9; 22; 6). Sequences encoding potential intracellular exo-oligoxylanase (GH8) and b-xylosidases (GHs 39; 43; 51and 52) were identified, which might be responsible for processing the products released by the extracellular enzymes. Finally, the overall assimilation could beperformed by intracellular debranching enzymes a-glucuronidase (GH67), a-arabinofuranosidase (GHs 43 and 51) and acetylxylanesterase (mainly CEs 1 and 4). The redundancy of GH genes observed in the analyzed genomes, the predicted enzyme architectures and their cellular localization are in agreement with other well descripted *Paenibacillus* species. Therefore, the strains AR247 and AR92 might display similar strategies for the degradation of xylan.

MM-024

RESPONSE OF *Candida tropicalis* BIOFILMS TO OXIDATIVE STRESS: IMPLICATION OF PERSISTER CELLS

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Persisters cells (PCs) are defined as phenotypic variants of the wild type that display tolerance to killing by high doses of antimicrobial (ATM) drugs. Upon removal of the ATM pressure, these cells switch back to a growing state, thereby giving rise to a new population genetically identical to the original one. PCs are distinguished from resistant mutants because do not exhibit an increased minimal inhibitory concentration (MIC), and represents about 0.1 to 1% of the population. PCs play an important role in recalcitrance of chronic infections. The aim of this work was to study the oxidative stress and antioxidant response of *Candida tropicalis* biofilm formed from PCs fraction upon antifungal (ATF) treatment. *C. tropicalis* NCPF 3111 was used. Biofilm formation was assayed by adhesion to 96-well plate and crystal violet stain (0.1OD595nm=1BBU) and PCs fraction was determined by colony forming units counting. Biofilm was also analyzed by Scanning Confocal Laser Microscopy (SCLM) by Calcofluor White stain. Extracellular reactive oxygen species (ROS) were measuredby the reduction of the nitro-blue tetrazolium (NBT) reaction, while probe 2¢,7¢-Dichlorodihydrofluorescein diacetate was used for intracellular ROS measurement by SCLM. Reactive nitrogen intermediates (RNI) were measured by Griess assay. Superoxide dismutase (SOD) activitywas assayed based on the inhibition of NBT reduction and total antioxidant capacity was measured by FRAP assay. The experimental design proposed allowed comparing oxidative stress and antioxidant response of two different biofilms. "Biofilms 1" obtained from planktonic cells and exposed to 200 µg/ml of AmB. A second biofilm, derived from PCs that survived drug treatment ("Biofilm 2"), was again treated with 200 µg/ml AmB. *A classic biphasic killing curve indicative of PCs presence was obtained.* The equal MIC confirmed that they were PCs. A greater ATF effect -higher BBU reduction- was observed in "Biofilms 1". Both biofilms showed similar basal ROS levels. An increase was observed upon AmB treatment, being it greater in "Biofilms 1". RNI measurements showed similar profiles as ROS after AmB treatment in both biofilms. In relation to antioxidant system, specifically SOD enzyme, a higher activation was observed in "Biofilm 2" when treated with AmB. Same effect was observed for total antioxidant capacity of biofilm. The last also showed significant differences of basal levels. Result obtained by SCLM agreed with BBU assay. These results demonstrate that "Biofilm 2" shows a major capacity to respond to the stress generated upon ATF treatment. It could due to the fact that the cells giving rise to "Biofilm 2" had been previously exposed to AmB. The finding of a CPs subpopulation with different oxidative status would help to solve the puzzle of biofilm resistance to ATFs indicating that the oxidative misbalance may be important.

MM-025

EFFECT OF THE ESSENTIAL OIL OF MINTHOSTACHYS VERTICILLATA (GRISEB.) EPLING AND LIMONENE ON BIOFILM PRODUCTION IN BOVINE MASTITIS PATHOGENS

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Bovine mastitis is a disease that causes large annual economic losses. Different microorganisms are associated with the disease, as well as their ability to form biofilm. The development and establishment of the biofilm depend on the ability of the pathogen to adhere to bovine mammary epithelial cells and leads to antibiotic resistance. The intensive administration of antibiotics for prevention or treatment, leads to the emergence of resistant strains. Research is now leading to the search for alternative control methods, and medicinal plants can be a natural, safe, effective and inexpensive option for the treatment of this disease. *Minthostachys verticillata* is an autochthonous medicinal plant with multiple ethnobotanical properties. In a previous study, we demonstrated that the essential oil (EO) of this species and limonene, one of its compounds, inhibited the growth of pathogens causing bovine mastitis. The objective of the present work was to determine the inhibitory effect of the essential oil of *M. verticillata* and limonene, before and after biofilm formation produced by pathogens isolated from bovine mastitis. Time kill assay and bacterial lysis were also determined. Six samples were used in the present study. The bacterial isolates were identified by conventional bacteriological methods and later confirmed by molecular methods. Strains were identified as *Escherichia coli*, *Bacillus pumilus* and *Enterococcus faecium*. It was observed that MIC values for EO were 3.6 mg/ml for *Enterococcus faecium*; 3.6 to 14.5 mg/ml for *Escherichia coli* strains; 3.6 to 29.03 for *Bacillus pumilus* strains. Whereas, MIC values for limonene were 52.5 mg/ml for *E. faecium*; 105 to 210 mg/ml for *E. coli* strains; 52.5 to 210 mg/ml for *Bacillus pumilus* strains. These results demonstrated that the EO was more effective than limonene against the isolated strains, since it requires lower concentrations exert an inhibitory effect. However, EO demonstrated bactericidal action against *E. faecium* and MBC was 29.03 mg/ml. This result was corroborated by time of death assays in which was observed a significant decreased of cells since 6 h and then by bacterial lysis assay. Both EO and limonene were able to inhibit biofilm after formation in *E. faecium* ($p<0.01$ for EO y $p<0.05$ for limonene), *E.coli* strains ($p<0.01$ for both EO and limonene) and *Bacillus pumilus* strains ($p<0.001$ for EO and $p<0.05$ for limonene). The activity of the EO against the bacteria tested was assayed over the time without arriving at the absence of viable forms, except for *E. faecium*. This result was probably due to the bacteriostatic effect of the EO. Lysis assay showed that the incubation of the bacterial strains with a concentration corresponding to the MICs, did not give a significant decrease in the absorbance, suggesting that the oil may acts on the bacterial membrane. The results contribute to the study of EO and limonene which may serve as a therapy against bovine mastitis pathogens inhibiting the development of pathogenic bacteria.

MM-026

ANTIBIOFILM COMBINATION OF USNIC ACID WITH FLUCONAZOLE ON RESISTANT*Candida albicans*

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Treatment of *Candida* infections is often difficult due to, between others factors, the ability of *Candida* species to form biofilms. These highly resistant structures exhibit resistance to a variety of antifungal agents with clinical use. Therefore, combining them with compounds obtained from natural sources seems to be one of the strategies in order to restore the sensitivity of the microorganism to conventional antifungals such as azole drugs. In previous works, we reported inhibitory activity of usnic acid (UA), a natural compound obtained from lichens, against azole-resistant *Candida albicans* biofilm. The biofilms inhibitory concentration (BIC) was 4 mg/ml compared to fluconazole (FLZ, BIC, 2 mg/ml) with inhibitions percentages of about 70%. The present study investigated the sensitization (restoring the sensitivity of the microorganism to azole drugs used in the clinic) of azole-resistant *C. albicans* biofilms to FLZ by combining it with an active compound (UA) obtained from Argentinean native flora (*Usnea amblyclada*). UA was purified from the benzene extract of lichen *U. amblyoclada*. An azole-resistant strain of *C. albicans* isolated from the oral cavity (RCa) that overexpresses efflux transporters genes of type CDR1, CDR2 and MDR1 was used. Biofilm formation was measured by adhesion to a 96-well plate and quantified by Crystal Violet (CV) staining and spectrophotometric reading of Optical Density (OD) at 595 nm. The biofilm biomass unit (BBU) was defined as 0.1DO595nm = 1UBB. For antifungal activity determination different concentrations of UA (1 to 4 mg/ml) dissolved in dimethyl sulfoxide (DMSO), FLZ (0.5 to 2 mg/ml) or their combinations were added to each well containing the mature biofilm and incubated at 37 °C for 48h. The counts of Colony Forming Units (CFU/ml) were performed for BBU correlation studies. For Scanning Confocal Laser Microscopy (SCLM), the samples were stained with Calcofluor White (0.05% v/v). UA and FLZ combined at concentrations four-fold lower than their BICs had a greater inhibitory effect on biofilms. In fact, the combination of UA (1 mg/ml) and FLZ (0.5 mg/ml) achieved an inhibition of 79, 82% while UA and FLZ combined at 1 mg/ml and 0.5 mg/ml respectively, almost eradicated de mature biofilm with an inhibition of 97.69% (*$p < 0.01$). Analysis by SCLM showed a considerable decrease in biomass of biofilms treated with the combination of UA (1 mg/ml) and FLZ (0.5 mg/ml), compared to untreated RCa biofilms (* $p < 0.01$). These results suggest that the combination of UA with FLZ effect enhanced the activity of FLZ in the treatment of azole-resistant of *C. albicans* biofilms. This promising action would imply an improvement of the therapeutics due to the decrease of the concentrations used of antifungal drugs.

MM-027

PFGE PATTERNS OF *Escherichia coli* ISOLATED FROM DAIRY CATTLE PRODUCTION ENVIRONMENT IN BRAZIL

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Escherichia coli has been described as prevalent and highly pathogenic in environmental mastitis etiology. Molecular typing is a powerful tool that can provide information about genetic characteristics of microorganism responsible for this disease. The objective of this work was to evaluate the genetic diversity of *E. coli* present in the dairy cattle production environment through the molecular typing technique *Pulsed Field Gel Electrophoresis* (PFGE). Additionally, circulating clones causing bovine mastitis present in the milk and feces of the bovine as well as in the water used in the management of these animals was determined. 282 milk samples were collected from 94 lactating cows in three consecutive weeks in summer, winter, spring and autumn. We also collected 94 samples of fecal material from these animals and water samples from nineteen different points related to the milk production line. Thus, 152 strains of *E. coli* were obtained through the phenotypic analysis. These strains were investigated by virulence genes such as Intimin (*eae*A), Shiga toxin (*stx*I and *stx*II), thermolabile (*LT*) and thermostable (*ST*) enterotoxins, invasiveness (*ial*) and enteroaggregative *E. coli* (*eagg*). Furthermore, genes associated with adherence were analyzed as fimbria F1 (*fim*H), fimbria curli (*csg*A) and antigen 43 (*flu*). According to the genes evaluated, the *fimh*, *csgA* and *flu* genes were prevalent in strains isolated within the dairy cattle production environment, being *fimh* the most detected gene in the 72.2% of the strains. The *flu* gene was not detected in water samples. On the other hand, percentage of genes related to the production of toxins *eaeA*, LT and *stxI* were detected in the 11.1% of the strains. It was not possible to detect other genes related to the production of toxins *stxII*, ST, *ial*, *eagg* in the strains tested. Profiles based on the presence virulence genes were determined to select 18 strains, which were assayed by PFGE. Among the 18 isolates, 16 different PFGE patterns were observed. Two clusters with 100% of homology, grouping 2 strains each other were found. One of them clustered two strains isolated from milk that share the same virulence genes. The present study describes *E coli* genotypes associated with the milk production environment and shows that there is no predominant PFGE pattern and no association between virulence genes analyzed and PFGE patterns was found.

MM-028

ANTIBIOFILM EFFICACY OF BACTERIOCINS AGAINST THE EMERGENT PATHOGEN *Listeria monocytogenes*

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Listeria monocytogenes is a foodborne pathogen able to survive in a wide range of environments even at refrigerated conditions. Moreover, some strains of *L. monocytogenes* can form biofilm facilitating their persistence in the food processing environments. Recently, major advances have been made in the prevention and control of pathogens biofilm by lactic acid bacteria (LAB) or their bacteriocins. The aim of this work was to investigate the ability of bacteriocins produced by *Lactobacillus curvatus* CRL705 and *L. curvatus* CRL1532 to compete with *L. monocytogenes* FBUNT during biofilm formation on polystyrene microplate. Biofilm formation of Listeria strains (*L. monocytogenes* FBUNT, CECT 4031T, Scott A) with and ithout bacteriocins using crystal violet method after 6 days of incubation at 10 °C was determined. The strain *L. monocytogenes* FBUNT was selected due to its high biofilm-forming capacity in the control samples. Both bacteriocins inhibited biofilm development of pathogen microorganism being the bacteriocin produced by *L. curvatus* CRL705 the most effective. In addition, the presence and expression of genes related to *L. monocytogenes* biofilm formation were studied by PCR and real time-PCR, respectively. *L. monocytogenes* FBUNT showed to harbor *luxS* and *pfs* genes encoding enzymes that catalyze S-ribosyl homocysteine and genes of *agrBDCA* system. During sessile growth, the expression levels of *agrB* and *luxS* genes were 2-fold higher than under planktonic growth. In presence of both bacteriocins a higher expression of *agrD* gene was observed. Expression levels of *agrB* and *luxS* genes decreased at higher concentrations of bacteriocins assayed which could indicate that bacteriocin addition affected expression of key genes involved in biofilm formation. These results evidence the potential use of the bacteriocins produced by *L. curvatus* CRL705 and CRL1532 as inhibitors of *L. monocytogenes* biofilm formation in refrigerating conditions.

MM-029

DIFFERENTIAL EXTRACELLULAR ENZYMES EXPRESSION BY THREE *Paenibacillus* STRAINS USING GEL-FREE PROTEOMICS ANALYSIS

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Three *Paenibacillus* strains, identified according to their 16S rDNA gene sequence and named as AR247, AR460-1 AR489, were selected due to their ability to produce glycoside hydrolases (GH) for second generation ethanol and other biotechnological applications. The assessment of extracellular enzyme production was previously approached by utilizing a mineral-based medium, MM0.2, added with agricultural by-products. These substrates are low-cost and abundantly available carbon sources for biotechnological purposes. Among the tested carbon sources, an alkali pretreated sugarcane bagasse (OH-SCB) was the one that better promoted the production of extracellular xylanases for all strains. The aim of this work was to study the differential extracellular enzymes expression by these strains through gel-free proteomic analysis method. Firstly, crude extracts were obtained by centrifugation after 72 h of cultivation in MM0.2 - OH-SCB 1% medium. Then, samples were concentrated by lyophilization and digested by using trypsin for further mass spectrometry analysis. Tryptic peptides obtained were analyzed using 2D nano-Ultra Performance Liquid Chromatography, coupled to tandem mass spectrometry. Bioinformatics analysis for protein identification was performed by searching against Swiss-Prot database, using Mascot server and ProteoIQ v2.8. Peptide summary report provided by Mascot evidenced hemicellulases which are active not only over b-1,4-linkages of xylose units but also on substituents of xylan. An endo-b-1,4-xylanase with 20.1 kDa molecular weight and 9.2 isoelectric point (pI) was found exclusively in crude extract samples of strain AR247. This enzyme, identified as a GH11, was also detected as a band between 18-20 kDa in zymograms and showed a pairwise similarity of ³ 99 with an homologous from *Paenibacillus* sp. Y412MC10. In addition, a second b-1,4-xylanase was identified in the secretome samples, yet not detectable through zymography, from both AR247 and AR489 strains. It was of 140.9 kDa, 4.7 pI (potential GH10), and showed to be closely related to a b-1,4-xylanase from *P. glucanolyticus*. Moreover, additional extracellular xylanase were found, which were related to a GH30 detected in *P. favisporus* and to two GH25 from *P. polymyxa*. Other proteins related to xylan utilization identified were: S–layer protein; sugar ABC transporters and sequences from substrate-binding proteins towards beta glucans. On the other hand, strains AR460-1 and AR489, revealed sequences corresponding to a carbohydrate binding module type CBM54 and an ABC transporter protein. Finally, a chitosanase belonging to GH8 family was only found in strain AR489. In conclusion gel-free proteomics analysis proved to be a useful methodology to achieve a widespread knowledge of the enzymatic repetory contributing to better quality and quantity of results.

MM-030

INSIGHTS OF ALKANE DEGRADATION IN MICROAEROBIOSIS BY *Pseudomonas extremaustralis* USING TRANSCRIPTOMIC AND PHYSIOLOGICAL APPROACH

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Hydrocarbon contamination has become a tough problem worldwide. One of the most widely distributed of these compounds is diesel, a complex mixture of n-alkanes, branched alkanes, and small amounts of aromatic moieties. *Pseudomonas* species are capable to use n-alkanes as carbon source by activating the hydrocarbon as a key first step using the enzyme 1-alkane monooxygenase encoded by *alkB*. Diesel degradation has been studied mostly under aerobic conditions in this genus, however in the environment uneven distribution of water flow, nutrients, and microbial populations creates a dynamic spectrum of aerobic, microaerobic, and anaerobic conditions. *Pseudomonas extremaustralis* is a bacterium isolated from Antarctica that shows high stress resistance and a wide microaerobic metabolism. *P. extremaustralis* is also capable to grow using diesel as sole carbon source only when cultured in biofilm condition. In this work we analyzed RNA-deep sequence experiments comparing the expression profile in aerobic and microaerobic planktonic cultures. Surprising, genes involved in n-alkane degradation presented differential expression in microaerobic conditions in comparison with aerobic cultures. The *alkB* gene, encoding the key enzyme for alkane degradation, alkane 1-monoxygenase, *praA* and *praB* encoding hydrocarbon facilitating proteins, and other genes related with this pathway such as those coding an alcohol and aldehyde dehydrogenase that were found up-regulated under low oxygen conditions. Additionally, genes encoding for some steps of fatty acid b-oxidation were also up-regulated while rubredoxin coding genes necessaries for the oxidation reaction of alkanes presented a non-differential expression between aerobic and microaerobic conditions. *In-silico* analysis of the promotor zone of *alkB* gene showed a putative Anr-box upstream the ATG, suggesting a regulation driven by oxygen availability. Cultures in minimal medium showed that *P.extremaustralis* was able to grow under microaerobic condition using diesel as sole carbon source in presence or absence of KNO3 as secondary electron acceptor. Degradation of n-alkanes (C13 to C19 fraction) after 7 days reached 20.5 % and 22.87% when KNO3 was present or absent, respectively, indicating that the remnant oxygen present in this culture condition was the responsible of alkane oxidation step. Under aerobic conditions *P. extremaustralis* was able to grow only in a biofilm structure tightly attached to the bottle glass at the culture-air interface but no alkane degradation was observed, in line with *alkB* expression experiments. This study showed a novel effect of microaerobiosis on alkane degradation pathway in a *Pseudomonas* species.

MM-031

PRODUCTION OF QUORUM-SENSING SIGNALING MOLECULES BY BACTERIA ISOLATED FROM RHIZOSPHERE OF CHICKPEA

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Chickpea (*Cicer arietinum* L.), a important grain legume, forms a Nitrogen-fixing symbiosis with soil bacteria (rhizobia) that can be harnessed in agriculture to enable chickpea to be grown without nitrogen fertilizer. When cultivated in soil lacking compatible rhizobia, chickpea can be inoculated with a symbiotically effective strain of *Mesorhizobium*. In Argentina, *Mesorhizobium ciceri* has been the commercial inoculant for this crop. Many bacteria, including rhizobia, use a molecular communication system, referred to as quorum sensing (QS), to synchronize the expression of certain genes and adopt a group behavior. Specifically QS communication via AHLs in rhizobia affects many metabolic and physiological process, including motility, exopolysaccharide production, biofilm formation, plasmid transfer, root nodulation efficiency, and nitrogen fixing efficiency. Previous studies in our laboratory demonstrated the existence of cell communication mechanisms among bradyrhizobial strains symbiotic of peanut. In this work, we investigated efficiency of inoculation of chickpea with M. ciceri in an assay carried out in the experimental field of the Facultad de Agronomia y Zootecnia – Universidad Nacional de Tucumán, located in the town of El Manantial. We used Norteño variety seeds treated with fungicide products and inoculated with commercial liquid inoculation at the doses suggested by the manufacturer. In addition the QS signals produced by bacterial strains isolated from root nodule and rhizosphere was tested. Our study indicated that there was a positive response to inoculation, which suggests that it is a recommended practice for this important legume crop. On the other way, detection of AHLs in bacteria strains was performed using the biosensor strains *Agrobacterium tumefaciens* NTL4 (pZLR4) and *Chromobacterium violaceum* CV026 for AHLs with long and short acyl chains, respectively. Total of 100 strains isolated from the rhizosphere, 31% showed production for short chain (8 strains) and long chain (27 strains) AHL. Qs activity was detected in one recommended strains for inoculation this crop. Our results demonstrate the existence of cell communication mechanisms among bacterial strains interacting with chickpea roots. Further characterizations of the phenotypes regulated by quorum-sensing signaling molecules are underway.

MM-032

QUORUM SENSING INHIBITORS IN LEGUME-ASSOCIATED BACTERIA

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Quorum sensing (QS) systems use N-acyl-homoserine lactones (AHLs) as signaling molecules, commonly found in Gram-negative bacteria that live in association with plants. QS system allows bacteria to function as multicellular organisms, because the extracellular concentration of autoinducer increases with bacteria population growth, after attaining a determinate number. Presently, QS mechanisms are considered as a potential novel target for the study of bacteria-plant interactions. *Arachis hypogaea* L. (peanut) constitutes an important legume crop with high relevance at different agroecological areas worldwide. Peanut establishes a nitrogen-fixing symbiosis with genetically diverse rhizobia grouped as *Bradyrhizobium* sp.. In this work, we analyzed the QS mechanisms used by rhizobia with special emphasis on the advances related to QS inhibitors produced by legume and bacteria. For that purpose, the bacterial biosensor strains, were employed for the different assays. In order to determine QS inhibitors molecules in bacteria and peanuts materials, inverse assays were carried out. Inhibition test of short and long acyl chain were performed by adding C_6-HSL or C_{12}-AHL, respectively. QS inhibition assay with biosensor strains demonstrated that legume and bacteria inhibited the expression of short and long homoserine lactones mediated phenotypes, suggesting a possible degradative activity for AHL in these extracts. In addition, *Bradyrhizobium* sp. P8A, a native strain isolated from peanut nodules, in contact with the biosensor strain and in absence of the plant was capable of producing AHLs with long acyl chains. Since that the synthesis of these AHLs by the P8A strain is only reached at the interface medium-air and not in the entire medium, we were able to demonstrate the production of inhibitors of QS molecules in strains capable of moving to the air interface would be linked to the aerobic behavior of this bacteria. We are currently in the process of identifying of AHL-lactonase activity. So far, our results indicate that in our experimental work model, legume and rhizobacteria are capable of communicating among themselves to coordinate group responses in order to adapt their physiology to environmental factors.

EDUCACIÓN EN MICROBIOLOGÍA

MODALIDAD ORAL

EM-001

LEARNING EXPERIENCE WITH VIRTUAL ACTIVITIES FOR TEACHING MICROBIAL BIOTECHNOLOGY

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La enseñanza basada en las TIC incluye desde el uso de pequeñas estrategias en aulas tradicionales hasta cursos completos a distancia. En disciplinas experimentales como la Microbiología no es fácil implementar actividades virtuales. Un "curso híbrido" es aquel que utiliza una combinación entre un formato tradicional de aula y un proceso de enseñanza-aprendizaje utilizando entornos virtuales. Se implementó una nueva experiencia de aprendizaje combinando estrategias tradicionales e innovaciones virtuales en la enseñanza de la Biotecnología Microbiana. El objetivo fue que los estudiantes de Biología puedan complementar y enriquecer la construcción de conocimientos, permitir optimizar el proceso de enseñanza–aprendizaje a partir del intercambio de ideas y experiencias, realizar trabajos colaborativos, presentaciones interactivas, conexiones sincrónicas y asincrónicas, entre otras. Del mismo modo superar desafíos tales como limitaciones en tiempos, recursos y feedback que ocurren a menudo en un laboratorio tradicional. La asignatura (opcional de 4 ó 5 año) duró 2 meses; incluyó clases teóricas (8), seminarios (2), trabajos prácticos (5) y visita a una industria. Participaron veinte estudiantes. Se utilizaron módulos dentro de un aula virtual (Moodle) como cierre da cada clase teórica y seminarios, con diferentes aplicaciones interactivas como búsqueda de nuevas investigaciones, interpretación y presentación de resultados, confrontaciones científicas y producciones personales. Las actividades virtuales se centraron en el análisis de diferentes problemas biotecnológicos reales (industriales, ambientales, farmacéuticos y agronómicos). Los ejercicios de laboratorio virtual se dividieron en 8 módulos dependiendo del objetivo educativo: (1) identificación de microorganismos con interés biotecnológico, (2) metodologías microbiológicas avanzadas, (3) análisis de fermentaciones microbianas (4) técnicas moleculares (5) análisis microbiológico de alimentos (Código Alimentario Argentino - Producción cerveza artesanal) y (6) métodos microbiológicos de biorremediación. Finalmente, 2 actividades con análisis de artículos científicos para su presentación oral donde debieron realizar interpretaciones de procesos biotecnológicos. Al final del estudio, los participantes evaluaron el aula y entorno virtual, su diseño y los ejercicios virtuales basados en su propia percepción a través de una encuesta. Como docentes consideramos que la combinación de diferentes escenarios de enseñanza-aprendizaje con "enfoque híbrido" mejoró el proceso que ocurre en el aula. Fue posible aumentar aprendizaje autónomo de los estudiantes, mejorar la satisfacción general, la adquisición de habilidades de investigación y el rendimiento del curso. Los estudiantes lograron calificaciones más altas y los conocimientos construidos resultaron muy satisfactorios valorando positivamente la experiencia.

EM-002

CIENCIA (RE) CREATIVA: SCIENCE IS LEARNED BY PLAY

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Ciencia (Re) Creativa es una plataforma para la enseñanza de la Microbiología en el nivel inicial, en el cual los niños aprenden jugando. Desde 2014 nuestro objetivo es transmitir nuestro conocimiento sobre la existencia microorganismos, como las bacterias y los virus, su relación con las enfermedades y cómo prevenirlas. Todo esto de un modo creativo y entretenido. Dado que la prevención es un pilar importante cuando se trata de afrontar problemas ambientales o de salud, y la educación a edades tempranas tiene un gran impacto en la población, un grupo de docentes, investigadores y alumnos de la FCEyN-UBA trabajaron se enfocaron en la enseñanza de la implicancia de la Microbiología a niños de entre 3 y 8 años, a través de obras de títeres, talleres y juegos. Las obras de títeres se centraron principalmente en la la prevención de enfermedades, la importancia del lavado de manos y dientes y la existencia de bacterias nocivas y benéficas. Fueron creadas desde cero por el grupo de trabajo e incluyó el libreto, la música, la iluminación y el diseño y elaboración de las marionetas y un teatro itinerante. El taller de "Creación de Microbios", realizado con materiales reciclados, les muestra conceptos de Microbiología capaces de fascinar a los niños y llevarlos a hacer preguntas. Los juegos y las imágenes de microorganismos para colorear les permiten relajarse y entretenerse, asi como reforzar los conceptos impartidos en las otras tareas. Todas estas actividades se llevaron a cabo en lugares públicos como Feria Internacional del Libro de Buenos Aires, La Noche de los Museos y jardines de infantes. Con el apoyo un subsidio UBANEX, llevamos durante un año, nuestra propuesta educativa no convencional a diferentes instituciones sociales y educativas, dando especial relevancia a aquellos a los que asistían niños en condición de vulnerabilidad socioeconómica. Pudimos llegar a 300 niños que asisten a guarderías, comedores populares y otras instituciones de la comunidad en 7 distritos C.A.B.A y el conurbano. Nuestras actividades fueron evaluadas a través de una encuesta diseñada a tal fin, recibiendo siempre comentarios completamente positivos y sugerencias por parte de los adultos responsables de los sitios visitados. El objetivo de las diferentes actividades es crear una continuidad de manera que los niños puedan aprender y consolidar las enseñanzas y mostrar una modificación en las conductas de higiene. Hemos visto que incluso los niños muy pequeños mostraron un gran interés en los temas y que alcanzar este segmento de edad requiere de actividades lúdicas que corresponden a la creatividad ya la imaginación.

EDUCACIÓN EN MICROBIOLOGÍA

MODALIDAD POSTER

EM-003

SERVICE-LEARNING PROJECTS AS TOOLS FOR TRAINING ENGINEERING STUDENTS IN MICROBIOLOGICAL ASSAYS

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El programa UBANEX de la Universidad de Buenos Aires promueve, estimula y fortalece su vinculación con la sociedad financiando proyectos de extensión en sus unidades académicas. Estos proyectos interdisciplinarios se desarrollan en conjunto entre facultades y permite la interacción con otras universidades nacionales. Además incentivan la participación de estudiantes, siendo herramientas muy valiosas para el mejoramiento del aprendizaje. El involucramiento de docentes y estudiantes en este programa genera un compromiso social quedando los conocimientos a disposición de la comunidad para contribuir al mejoramiento de su calidad de vida. El proyecto *Plan de acción a corto plazo para la prevención de riesgo sobre la salud por consumo de aguas de pozo* fue presentado por docentes de la Facultad de Ingeniería, con la colaboración de la Facultad de Arquitectura y la Universidad Nacional de General Sarmiento. El objetivo fue efectuar la evaluación de la calidad fisicoquímica y microbiológica de aguas habitualmente de consumo en el Barrio San Agustín de San Francisco Solano, Almirante Brown, Buenos Aires, surgido por expreso pedido de las autoridades del Jardín de Infantes de la zona, ante problemas de salud de su población. Se reclutaron estudiantes voluntarios de Ingeniería Química y Civil con diferentes grados de avance en sus carreras y con profunda sensibilidad social. Luego de reuniones previas de organización e intercambio de ideas, se realizó la distribución de tareas. Los estudiantes se dedicaron a realizar encuestas en el Barrio, al diseño del muestreo y toma de muestras, a evaluar el sistema de distribución del agua y a los análisis de laboratorio. En particular aquéllos que realizaron los ensayos microbiológicos no tenían conocimientos previos en Microbiología ni habilidades de laboratorio en esta disciplina. Se realizó un entrenamiento en técnicas microbiológicas básicas y, luego de preparar todo el material, procedieron al tratamiento de las muestras. Los resultados obtenidos de estos análisis no fueron nada alentadores desde que ninguna muestra de agua de consumo resultó potable acorde a lo establecido en el Código Alimentario Argentino y además el agua del canal pluvial del Barrio resultó con un alto grado de contaminación microbiana asociada a desechos cloacales. Los estudiantes, guiados por los docentes, pudieron recopilar e interpretar estos resultados realizando un informe completo para elevarlo a las autoridades del Jardín de Infantes. A partir de una encuesta final, los estudiantes expresaron su desconocimiento previo de la problemática del agua, el haber aprendido nuevas técnicas de laboratorio aplicables en su desempeño profesional y su interés por el desarrollo de sistemas de tratamiento no sólo para eliminar la contaminación microbiana sino también química, poniendo en evidencia el alto grado de involucramiento. La continuidad con el diseño de un proceso de potabilización será hará efectiva en un nuevo proyecto UBANEX.

EM-004

NATURAL ANTIFUNGAL FOR WOODS: AN APPROACH OF SECONDARY STUDENTS TO MICROBIOLOGY

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Uno de los problemas más preocupantes del sistema educativo es el fracaso escolar, particularmente en el área de las ciencias duras. Por ello, en este proyecto se ha trabajado sobre algunos de los aspectos esenciales para avanzar en la solución de este problema: la motivación del alumnado y su actitud hacia las disciplinas científicas. Nos centramos en fomentar su interés, concretamente en el estudio de Física y Química durante el ciclo de enseñanza secundaria obligatoria, introduciendo al alumno al área de Microbiología aplicada, la cual es sólo incorporada en términos conceptuales en el área de Biología. Mediante un proceso de interrelación disciplinario de las cátedras de Física y Química, se logró incorporar conceptos de Microbiología en estudiantes secundarios de educación técnica de la modalidad Maestro Mayor de Obra (MMO), diseñando un experimento con una posible aplicación en el área de la construcción. A través de esta experiencia los alumnos abordaron la temática de la formación de hongos en maderas, evaluando la utilización de extractos de origen vegetal como protectores frente a estos microorganismos que dañan este material usado en construcción. En el marco del programa nacional Los Científicos Van a la Escuela (LCVE 2016), se conformó un grupo de trabajo Docente-Científico, mediante el cual se exploró la viabilidad de experimentos, relacionando conceptos claves como productos naturales, materiales de la construcción y microorganismos. Tras la revisión bibliográfica con el equipo docente del 3er. año de la modalidad MMO, se estableció la metodología experimental acorde al material, equipo e instalaciones disponibles en la Esc. Técnica N°12 (Ciudad de Fernández-Santiago del Estero). Los alumnos construyeron en el taller una "caja" de cultivo, y posteriormente se optimizaron las condiciones de crecimiento microbiano en muestras de madera de algarrobo. Se preparó el "bioprotector" utilizando ajo (*Allium sativum*) como principio activo en medio hidro-alcohólico. Se evaluó la inhibición del crecimiento fúngico, aplicando el extracto de ajo sobre una muestra y dejándola incubar bajo las condiciones anteriormente optimizadas. Se observó la aparición de hongos (coloración en maderas) tras 10 días de ser colocadas las muestras en la "caja" de cultivo a 25-30°C, la cual fue rellenada con tierra hasta la mitad de su volumen e hidratada con 500 mL de agua cada 2 días. Comparada a una muestra de madera control, no se observó aparición de coloración en la muestra tratada con el "bioprotector", persistiendo la protección aún a los 20 días de iniciado el experimento. Los alumnos realizaron una exposición para la comunidad educativa, y el par docente-científico diseñó un plan de clases docente y una guía para el estudiante. Los alumnos lograron vincular conceptos teóricos abarcados en las cátedras de Física y Química, como así también conocer de las propiedades fisicoquímicas de las maderas e incorporar los conceptos básicos de Microbiología.

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