SAMIGE

Sociedad Argentina de Microbiología General

X CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL SAMIGE

2 al 4 de Julio de 2014
Hotel 13 de Julio
Mar del Plata, Argentina

www.samige.org.ar
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Comisión de Investigaciones Científicas

AGENCIA

AMERICAN SOCIETY MICROBIOLOGY

CONICET MAR DEL PLATA

AGRADECEMOS

A todas las instituciones que apoyaron el desarrollo del X Congreso de Microbiología General y el Taller "Microbiología Ambiental, una mirada actual"

Evaluadores

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Y a todos los estudiantes, becarios e investigadores que comparten sus resultados y materiales en el espacio de SAMiGe.
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Presidente

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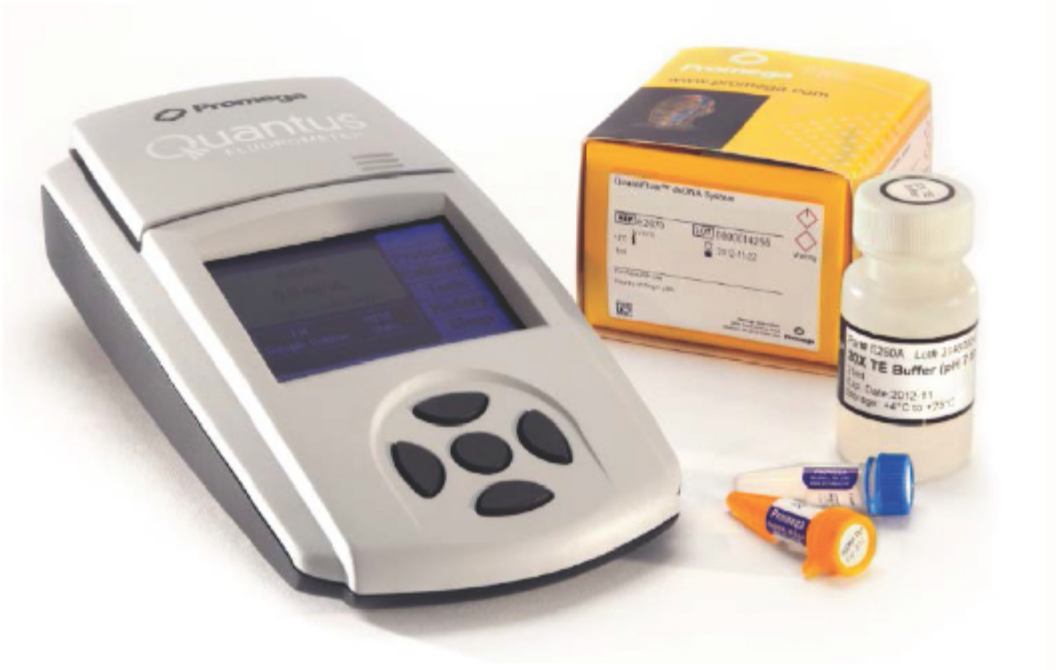

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X Congreso Argentino de Microbiología General SAMIGE 2014

Miércoles 2 de Julio	Simposio-Taller Microbiología Ambiental, una mirada actual	
8:00 - 9:00 hs	Inscripción	
9:00 - 9:30 hs	Ceremonia Apertura Taller	
9:30 - 10:00 hs	Simposio I: Microbiología y Biodegradación-Biotransformación	Marcela Ferrero *PROIMI. San Miguel de Tucumán, Argentina* Diversidad y fisiología de bacterias que transforman arsénico
10:00 -10:30 hs		Edgardo Donati *CINDEFI-UNLP. La Plata, Argentina* Microorganismos extremófilos para aplicaciones ambientales
10:30 -11:00 hs	Café	
11:00 -11:15 hs	Comunicaciones Orales MS-001 Análisis funcional y bioinformático de un conjunto de plásmidos obtenidos de aislamientos bacterianos extraídos de un sistema de biopurificación utilizado para la eliminación de pesticidas María C Martini, Francisco J Albicoro ,José L López ,María E Salas, Mariano Pistorio, Andreas Schlüter, Antonio Lagares, María F Del Papa *IBBM, Fac. Cs Exactas, Universidad Nacional de La Plata, CONICET.*	
11:15 -11:30 hs	MS-002 Multicelularidad de *Bacillus* formadores de esporas aislados de ambientes extremos Marco Bartolini ,Cecilia Leñini ,Verónica Donato ,Diego Tonieti ,María E Farías, Roberto R Grau *Universidad Nacional de Rosario, Facultad de Bioquímica y Farmacia, CONICET*	
11:30 -11:45 hs	MS-003 Análisis de la composición de las comunidades microbianas de lagunas de estabilización de efluentes de pequeñas industrias lácteas usando secuenciación de amplicones del gen 16s María M Adjad, Erica Schmidt, Miguel Taverna, Ariel F Amadio *INTA EEA Rafaela*	
11:45 -12:00 hs	MS-004 Desnitrificación de efluentes nucleares provenientes de la industria nuclear utilizando soportes obtenidos por irradiación gamma Mariano Venturini, Marisela Perez, Ramiro Martín Perez Caracotche, Gustavo Curutchet, Ramón Augusto Pizarro *CNEA - División Biominería y Biotec. Ambiental*	

12:00 -12:15 hs 12:15 -12:30 hs	MS-005 Estudio de megaplásmidos en *Sphingomonas* degradadoras de hidrocarburos policíclicos aromáticos Viviana Ayelén Starevich, Laura Madueño, Ileana Paula Salto, Mariano Pistorio, Irma Susana Morelli *CINDEFI-CONICET UNLP* MS-006 Promoción de la dominancia de cianobacterias filamentosas formadoras de floraciones por factores ambientales Anabella Aguilera, Ricardo Echenique, Graciela Salerno, Luis Aubriot *INBIOTEC-CONICET y CIB-FIBA*	
12:30 -14:00 hs	Almuerzo	
14:00 -15:00 hs	Posters	
15:00 -15:30 hs	Simposio II: Microbiología y Biorremediación	Howard Junca *Asociación Latinoamérica de Microbiología. Colombia* Ecología microbiana de la biodegradación de aromáticos: ejemplos de nuevas integraciones técnicas y conceptuales
15:30 -16:30 hs	Mesa Redonda – Actividad Integradora Silvia Murialdo *Grupo de Ingeniería Bioquímica Facultad de Ingeniería UNMdP* Alternativas de procesos y reducción de contaminantes orgánicos en el puerto de Mar del Plata	
16:30 -17:00hs	Café	
Miércoles 2 de Julio	X Reunión Anual de la SAMIGE	
8:00 - 17:00hs	Inscripción	
9:00 - 9:30hs	Ceremonia de Apertura Congreso	
18:00 - 18:45hs	Conferencia Plenaria Mecky Polschroder *Universidad de Pennsylvania. Philadelphia, USA* Nuevas perspectivas en la biosíntesis y función de diversas estructuras presentes en la superficie celular de arqueas	
18:45 - 19:00hs	Coro	
19:00 - 20:30hs	Brindis de Bienvenida	
Jueves 3 de Julio	X Reunión Anual de la SAMIGE	
9:00 - 9:30hs	Conferencia Virtual (auspiciada por la ASM) Roberto Kolter, *Harvard Medical School. Boston, USA* Ecología química microbiana	

Jueves 3 de Julio	X Reunión Anual de la SAMIGE
9:30 - 10:00 hs	Mini Conferencia Alfonso Soler Bistué *Instituto Pasteur. París, Francia* La relación entre la posición del locus *s10-spc-alpha*, la tasa de crecimiento y la capacidad de *Vibrio cholerae* de invadir al hospedador ayuda a comprender la organización genómica de la bacteria
10:00 - 11:00hs	Café
11:00- 13:00hs	Posters (presentan número impar)
13:00 - 14:30hs	Almuerzo
14:30 - 14:45 hs	Comunicaciones Orales MM-001 Señalización independiente a través de complejos de quimiorreceptores separados en *Escherichia coli* Karina Herrera Seitz, Vered Frank, Diego A Massazza, Ady Vaknin, Claudia A Studdert *Universidad Nacional de Mar del Plata, Mar del Plata, Argentina*
14:45 - 15:00 hs	MM-002 Caracterización funcional del trasnportador CDF SMc02724 (smyiip) en *Sinorhizobium meliloti*: roles en la homeostasis de manganeso y nodulación Daniel Raimunda, Graciela Elso-Berberián *Instituto de Investigación Médica M y M Ferreyra INIMEC-CONICET-Univ. Nac. de Córdoba.*
15:00 - 15:15 hs	MM-003 Viviendo sobre una superficie: desarrollo de biofilms y comunidades multicelulares deslizantes en *Bacillus subtilis*. Paula De Oña, Cecilia Leñini, Akos Kóvacs, Oscar Kuipers, Roberto R Grau *Universidad Nacional de Rosario, Facultad de Bioquímica y Farmacia, CONICET*
15:15 - 15:30 hs	MM-004 Análisis genómico del cluster biosintético de vitamina b12 (cobalamina) en *Lactobacillus coryniformis* CRL 1001 A.C. Torres, V. Vannini, F. Sesma, G. Font, L. Saavedra, M.P. Taranto *Centro de Referencia para Lactobacilos CERELA-CONICET*
15:30 - 15:45 hs	FM-001 Preservación de alimentos: efectos bactericidas y anti-colonización de polifenoles del vino sobre bacterias patógenas Darío Vileta, Verónica Donato, Valentín Torres, Héctor Lucero, Roque Masciarelli, Roberto R Grau *Universidad Nacional de Rosario, Facultad de Bioquímica y Farmacia, CONICET*

Jueves 3 de Julio	X Reunión Anual de la SAMIGE
15:45 - 16:00 hs	FM-002 Impacto de las señales de *quorum sensing* y hierro en la arquitectura del biofilm y la virulencia de *Stenotrophomonas maltophilia* Carlos García, Eliana Alcaraz, Yamila Figueroa, Rocío Romero, Beatriz Passerini de Rossi, Mirta Franco *Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, UBA*
16:00 - 16:15 hs	FM-003 Propiedades probióticas y anti-*Helicobacter pylori* de la cepa ácido-tolerante *Lactobacillus salivarius* UCO-979C Enrique A Sanhueza , Juan P Inostroza, Silvia A Rojas-Caro, José A Freire, Carlos L González, Apolinaria García *Depto de Microbiología, Facultad de Cs Biológicas. Universidad de Concepción. Concepción, Chile*
16:15 - 16:30 hs	FM-004 Perfiles redox en biofilms de *G. sulfurreducens* determinados mediante microscopía raman confocal Luciana Robuschi, J. Pablo Tomba, German D Schrott, P. Sebastián Bonanni, P. Mariela Desimone, Juan P Busamen *Lab. de Bioelectroquímica, División Electroquímica y Corrosión, INTEMA-CONICET*
16:30 - 18:00 hs	Café y Posters (presentan número impar)
18:00 - 18:45 hs	Conferencia plenaria Alejandro Buschiazzo *Instituto Pasteur. Montevideo, Uruguay.* Dos componentes, dos sistemas: cambios conformacionales de proteínas de señalización en bacterias
19:00 hs	Asamblea de la SAMIGE

Viernes 4 de Julio	X Annual Meeting of the SAMIGE
9:00 - 9:45 hs	Conferencia Plenaria Patricia Juarez *UNLP. La Plata, Argentina.* Interacción de hongos patógenos y su huésped: aspectos bioquímicos y moleculares
9:45 - 10:00 hs	Comunicaciones Orales EM-001 Biotecnología industrial y microbiología aplicada (bima): un enfoque diferente para el dictado de un curso de grado Laura J Raiger Iustman, Nancy I Lopez, Sandra M Ruzal, Diana L Vullo *Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires*
10:00 - 10:15 hs	EM-002 Clase experimental para ilustrar daño mutagénico letal por radiación UV, fotoreparación y reparación mutagénica en una cepa doble auxotrófica de *Pseudomonas aeruginosa* Patricio Sobrero, Claudio Valverde *Área Microbiología e Inmunología, LBMIBS, Depto. Ciencia y Tecnología, U N. Quilmes*

10:15 -11:00 hs	Café y Posters (presentan número par)
11:00 - 11:15 hs	Comunicaciones Orales BB-001 Contribución de la proteína S-layer en la actividad mosquitocida de *Lysinibacillus sphaericus* Mariana C Allievi, María Mercedes Palomino, Mariano Prado Acosta, Joaquina Fina Martin, Pablo M Waehner, Sandra M Ruzal, Carmen Sanchez Rivas *Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales-UBA. IQUIBICEN-CONICET*
11:15 - 11:30 hs	BB-002 *Bacillus* formadores de esporas como agentes naturales de biocontrol de enfermedades de plantas Marco Bartolini, Cecilia Leñini, Sebastián Buelva, Matías Ábalos, Gustavo Gonzalez Anta, Roberto R Grau *Universidad Nacional de Rosario, Facultad de Bioquímica y Farmacia, CONICET*
11:30 - 11:45 hs	BB-003 *Rosmarinus officinalis*: Una fuente potencial de drogas anti-infecciosas Silvia Moreno, Adriana M Ojeda-Sana, Paulo A Cáceres Guido, Catalina H van Baren *Fundación Instituto Leloir-Instituto de Investigaciones Bioquímicas, Buenos Aires-CONICET (IIBBA).*
11:45 - 12:00 hs	BB-002 Efecto del surfactante no-iónico triton x-100 sobre la biodegradación de PAH y la comunidad microbiana de un suelo crónicamente contaminado Martina Cecotti, Verónica C Mora, Marisa Viera, Irma S Morelli *CINDEFI, UNLP, CONICET*
12:00 - 12:15 hs	IN-001 Estudio de asociación a células thp-1 de cepas potencialmente probióticas de *Bifidobacterium* Jessica Minnaard, Ivanna S Rolny, Sabrina E Assad, Pablo F Perez *Centro de Investigación y Desarrollo en Criotecnología de Alimentos-CONICET*
12:15 - 12:30 hs	IN-002 Levaduras endofíticas de caña de azúcar presentan actividad *quorum quenching* Elisa V Bertini, Ana C Leguina, Lucía I Castellanos de Figueroa, Carlos G Nieto Peñalver *Planta Piloto de Procesos Industriales Microbiológicos PROIMI-Biotecnología CONICET*
12:30 - 14:30 hs	Almuerzo
14:30 - 14:45 hs	Comunicaciones Orales BF-001 Clones transformantes de la levadura P*ichia pastoris* con mayor nivel de expresión de quimosina bovina recombinante Diego G Noseda, Joaquín Bozzo, Matías Recúpero, Martín Blasco, Miguel A Galvagno *Ins. Inves. Biotecnológicas, Univ. Nac. de San Martín (CONICET).*

14:45 - 15:00 hs	BF-002 Fábricas microbianas multiespecie formadas por microalgas oleaginosas y bacterias modificadas genéticamente para la excreción de amonio Juan Cesar F Ortiz Marquez, Mauro Do Nascimento, Rafael Ambrosio, Leonardo Curatti *INBIOTEC-CONICET y FIBA*
15:00 - 15:15 hs	BF-003 Producción de electricidad en lechos acuáticos con bacterias electro-activas Guido Fier, Sebastian Bonanni, Germán Schrott, Juan Pablo Busalmen *INTEMA (CONICET) - Universidad Nacional de Mar del Plata*
15:15 - 15:30 hs	BF-004 Screening de poligalacturonasas bacterianas con actividad en medio alcalino con potencial uso en la industria ambiental Yuly A Ramirez-Tapias, Claudia N Britos , Cintia W Rivero, Jorge A Trelles *Universidad Nacional de Quilmes. CONICET*
15:30 - 15:45 hs	BD-001 Biodiversidad y dinámica microbiana durante la elaboración de vinos chilenos y su rol en la producción de aminas biógenas Silvia A Rojas-Caro, Carolina P Herrera, José A Freire, Enrique A Sanhueza, Rodrigo J Andler, Mario A Aranda, Apolinaria García *Depto de Microbiología, Facultad de Cs Biológicas. Universidad de Concepción. Concepción, Chile*
15:45 - 18:00 hs	Café y Posters (presentan número par) Votación de mejores comunicaciones orales
18:00 - 18:45 hs	Conferencia Plenaria Fabiano Thompson *Universidad de Rio de Janeiro. Rio de Janeiro, Brasil.* Microbiología de sistemas de arrecifes
21:30 hs	Cena de Clausura

ÁREAS TEMÁTICAS:

BD	Biodiversidad	FM	Fisiología Microbiana
BB	Biorremediación y Biocontrol	IN	Interacciones procariota-eucariota
BF	Biotecnología y Fermentaciones	MS	Microbiología del suelo y el ambiente
EM	Educación en Microbiología	MM	Microbiología Molecular

CONFERENCIAS PLENARIAS

2 al 4 de Julio de 2014
Hotel 13 de Julio
Mar del Plata, Argentina

Código de Resumen: CP-001

Sección: Conferencias Plenarias

Modalidad: Oral

MICROBIOLOGIA DE SISTEMAS CORALINOS

MICROBIOLOGY OF REEF SYSTEMS

Fabiano Thompson[1]

[1] *Universidad de Río de Janeiro, Brasil.*

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The Abrolhos reef system (ARS) is the most important system of the Southwestern Atlantic Ocean, comprise a mosaic of megahabitats, including coral reefs, rhodolith beds, sink-hole like structures, and unconsolidated beds. The ARS harbors the world's largest continuous rhodolith bed (~21,000 km^2) and has one of the largest marine $CaCO_3$ deposits. We observed that rhodoliths contained a specific microbiome that displayed a significant enrichment in microbes suggested to have important roles in biomineralization of carbonates and in the genesis of the rhodoliths. High rates of photosynthesis were measured for Abrolhos rhodoliths, allowing the entire Abrolhos rhodolith bed to produce 5.65 × 105 tons Carbon per day. This estimate illustrates the great importance of the Abrolhos rhodolith beds for dissolved carbon production in the Southwestern Atlantic Ocean. The rhodolith also serves as nursery place for larvae of different types of invertebrates (e.g. crustaceans, mollusks, cnidaria), suggesting that the rodholiths also play a significant role in fertilizing the ocean with marine life.

Código de Resumen: CP-002

Sección: Conferencias Plenarias

Modalidad: Oral

DOS COMPONENTES, DOS SISTEMAS: CAMBIOS CONFORMACIONALES DE PROTEÍNAS DE SEÑALIZACIÓN EN BACTERIAS

TWO COMPONENTS, TWO SYSTEMS: PROTEIN CONFORMATIONAL SWITCHES IN BACTERIAL SIGNALING

Alejandro Buschiazzo[1]

[1] *Instituto Pasteur, Uruguay.*

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Two-component systems (TCSs) are key players in bacterial signaling. Nevertheless, sensing and signal-transduction mechanisms are still poorly understood at the molecular level. This knowledge has not only fundamental importance for Biology, but also extends to a better understanding of virulence and pathogenesis in bacteria that cause disease. I will present results on two different bacterial species. The TCS DesK/DesR controls fatty acid desaturation in *Bacillus subtilis* in response to cold shock. Through a combined approach using crystallography, biochemistry and microbiology, we have gathered experimental evidence supporting a model of signal-dependent allosteric modulation of the sensor histidine kinase (HK) catalytic activity.

Focusing on detailed structural/functional traits of these key protein constituents, we have also discovered a novel and non-canonical activation pathway for the cognate response regulator (RR). By solving the crystal structures of DesR in both, active and inactive configurations, molecular details of the activation switch have been unraveled. The $\alpha1\alpha5$ surface is shown to be essential for a non-canonical, phosphorylation-dependent dimerization and activation mechanism. We show that this surface is further involved in cognate HK binding, disclosing a novel view of the HK/RR interaction, ensuring signaling pathway specificity. I shall also present recent work on signaling systems in Leptospira spp., the etiologic agent of leptospirosis. This disease is a major zoonosis, with high impact in animal health in Uruguay, being one of the main causes of abortions in cattle.

Protein science has been instrumental in deciphering heme metabolism regulation controlled by the TCS HemK/HemR. We show that HemR simultaneously acts as a transcriptional activator

and repressor of key heme metabolism-related genes. A systematic effort to isolate and type the prevalent *Leptospira serovars* in Uruguay has been launched at our Institute. Genome- and proteome-based information thereof, will allow us to pursue vaccine optimization goals, linking protein science with field applications.

Código de Resumen: CP-003

Sección: Conferencias Plenarias

Modalidad: Oral

INTERACCIÓN DE HONGOS PATÓGENOS Y SU HUÉSPED: ASPECTOS BIOQUÍMICOS Y MOLECULARES

INTERACTIONS BETWEEN PATHOGENIC FUNGI AND THEIR HOSTS. BIOCHEMICAL AND MOLECULAR FEATURES

M. Patricia Juárez[1]

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Both animal and plant pathogenic fungi that reside in soils and vegetation must withstand major environmental challenges. Hence, many of the virulence factors appear to allow survival in the environment and establishment in the host. Entomopathogenic fungi mostly attack their insect hosts by penetrating through the cuticle, covered by a lipid-rich layer, usually containing large amounts of very long-chain hydrocarbons, that are essential to prevent lethal desiccation in the insect. Here we will review how the hydrocarbon barrier can be efficiently used as carbon source for fungal growth. An initial hydroxylation step by a microsomal P450 monooxigenase system, followed by successive transformations, will eventually provide the appropriate fatty acyl-CoAs for complete degradation in the peroxisomes, the site of β-oxidation in fungi. Concurrently, an oxidative stress scenario is established to counter this situation. After the cuticle is breached, the fungi reach the internal cavity rich in more easily available substrates; thus, a correlation between fungal ability to catabolize hydrocarbons and virulence can be established. Among plan pathogenic fungi, the genus Fusarium is one of the most important genera affecting cereal crops in many areas of the world. Many Fusarium species are usual pathogens of wheat and barley; one of the most serious diseases produced is Fusarium head blight (FHB). Infection can affect the quantity, quality, and marketability of the grain and contaminate the grains with trichothecenes and other mycotoxins. Both during primary and secondary metabolism, fungi produce volatile organic compounds (VOC), intermediate and end products of various metabolic pathways; some of them can be used for their detection and identification. Trichodiene is the volatile precursor of deoxynivalenol, the most toxigenic trichotecene mycotoxin released by Fusarium graminearum. We will review the utility of solid phase microextraction (SPME) coupled to capillary gas chromatography (CGC) and mass spectrometry (MS) to differentiate trichotecene-producer and non-trichotecene producer *Fusarium* spp. We will further show the utility of these techniques on the early detection of *F. graminearum* in wheat cultivars, before FHB development.

Código de Resumen: CP-004

Sección: Conferencias Plenarias

Modalidad: Oral

LA RELACIÓN ENTRE LA POSICIÓN DEL LOCUS S10-spc-alpha, LA TASA DE CRECIMIENTO Y LA CAPACIDAD DE Vibrio cholerae DE INVADIR AL HOSPEDADOR AYUDA A COMPRENDER LA ORGANIZACIÓN GENÓMICA DE LA BACTERIA

INTERPLAY BETWEEN POSITION OF *S10-spc-alpha* LOCUS, GROWTH RATE AND HOST-INVASION CAPACITY OF *Vibrio cholerae* GIVES INSIGHT INTO BACTERIAL GENOME ORGANIZATION

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Comparative genomics revealed a conserved trend in genome organization of fast-growing bacteria: position of ribosomal and RNA polymerase genes are biased towards the origin of replication (*oriC*). It has been speculated that this bias would allow increased gene dosage during exponential phase. Experimental evidence is scarce though. In bacteria, a single locus *S10-spc-*α (S10) encodes half of ribosomal protein genes. *Vibrio cholerae*, a bichromosomal fast-growing pathogen, was tested for precise relocation of this highly conserved locus. S10 repositioning was achieved using lamboid phage recombinases through transient excision followed by targeted reintegration. A set of isogenic mutants where S10 was moved next to its original position, to the middle of the replichore, to the terminal region of Chromosome 1 and to the secondary chromosome was obtained. Analysis of growth kinetics and time-lapse microscopy experiments showed that, in fast-growing conditions, the further away S10 was moved the lower growth rate (GR) was obtained. Interestingly, this phenotype correlated with a reduction of S10-dosage and S10 mRNA abundance. Importantly, upon the returning the locus to its original position GR is recovered.

Although S10 could be easily moved to several positions within the genome, infection tests on the model organism *Drosophila melanogaster* showed that mutants are highly impaired in host invasion. This fact suggests that, in nature, strong selective pressure drove this locus near *oriC*. Overall, our results support the idea that fitness advantage of positional bias is to provide higher dose during fast growth. S10 positioning plays a key role linking genome structure with cell physiology as it is involved in growth global control and in host invasion that may have a role in the ecological success of *V. cholerae*.

Código de Resumen: CP-005

Sección: Conferencias Plenarias

Modalidad: Oral

NUEVAS PERSPECTIVAS EN LA BIOSINTESIS Y FUNCION DE DIVERSAS ESTRUCTURAS PRESENTES EN LA SUPERFICIE CELULAR DE ARQUEAS

NOVEL INSIGHTS INTO THE BIOSYNTHESIS AND FUNCTION OF DIVERSE ARCHAEAL CELL SURFACE STRUCTURES

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Archaeal cell surfaces, like those of bacteria, are decorated by a variety of protein complexes that play important roles in allowing cells to resist the deleterious effects of environmental stresses. We have recently focused on characterizing three types of archaeal cell surface structure: the cell wall, which, in archaea is composed entirely of the S-layer glycoprotein; type IV pili, which play important roles in a multiplicity of biological processes, including surface adhesion and biofilm formation; and flagella, which allow cells to swim toward attractants and escape toxic conditions. Our recent characterizations of these structures in *Haloferax volcanii* have allowed us to identify several key features of the molecular mechanisms that support the biosynthesis, function and regulation of these structures in this model archaeon. Results from these studies led to the identification of: 1) a novel cell-surface anchoring mechanism, present in a broad range of archaea and bacteria, that is catalyzed by an enzyme analogous to the sortases found in gram-positive bacteria, but which, upon processing anchors proteins, such as the S-layer glycoprotein, to the cell membrane rather than the cell wall; 2) a unique class of adhesion pilins, including a subset that are involved in microcolony formation, and another subset that appears to inhibit this early step in biofilm formation; and 3) a mechanism that post-translationally regulates flagella-dependent motility, which requires the presence of the adhesion pilins and is specifically dependent upon the conserved H-domain of these pilus-subunits. These intriguing results have led to novel insights into the functional roles played by surface filaments in the processes that initiate biofilm formation, as well as the mechanisms that regulate these functions. Given past experience, it is likely that, in the near future, similar results will be reported for other organisms, including bacterial species, as well as additional species of archaea.

MICROBIOLOGÍA AMBIENTAL Y DEL SUELO

2 al 4 de Julio de 2014
Hotel 13 de Julio
Mar del Plata, Argentina

Código de Resumen: CPMS-001

Sección: Microbiología Ambiental y del Suelo

Charla Plenaria

MICROORGANISMOS EXTREMOFILOS PARA APLICACIONES AMBIENTALES

EXTREMOPHILIC MICROORGANISMS FOR ENVIRONMENTAL APPLICATIONS

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Microorganisms that thrive in extreme conditions possess properties suitable for biotechnological applications. This has opened a new era in biotechnology as it is shown through their use in numerous commercial processes. Enzymes and other biomolecules isolated from extremophiles have a wide variety of uses from additives to biomedical applications. In addition, several applications to clean up the environment and also innovative clean technologies have been developed based on microbial strategies of extremophiles. Biomining and biofuels are two of those clean technologies which can be enhanced using extremophiles. Biomining comprising bioleaching and biooxidation processes is used for metal solubilization and metal beneficiation, respectively, through the activity of iron- and sulfur oxidizing microorganisms. These processes are much cleaner than other traditional extractive techniques like pyrometallurgy. Biofuels are produced by the activity of living organisms. Many extremophilic microorganisms have proved to be great producer of lipids for biodiesel production. From the environmental point of view, the use of biofuels is carbon neutral since the carbon produced when burning them is offset by the one consumed by the microorganisms. Environmental contamination can be mitigated using microbial remediation; the strategies of extremophiles to survive in such hard environments can be converted in methodologies to treat and remediate contaminated sites. Besides the mineralization of polluting organic matter, extremophiles can be used to remove heavy metals. Biosorption and bioprecipitation are two of the main heavy metal detoxification methodologies. Living and non-living microorganisms have been used to concentrate and remove heavy metals present into industrial effluents. Biosorption utilizing non-living biomass to accumulate heavy metals from wastewaters seems to be a more competitive, effective and economically attractive treatment method but bioaccumulation using living microorganisms, and especially those able to develop in the presence of heavy metals, is successful under many field conditions. Due to its ability to reduce sulfate to sulfide, sulfate-reducing microorganisms (SRM) can be used as an inexpensive sulfide source to precipitate heavy metals. SRM are heterotrophic microorganisms which require anaerobic conditions using sulfate as the terminal electron acceptor. From a biotechnological point of view, this reaction implies important applications: not only the precipitation of metal as sulfides but also the decrease of sulfate concentration and the increase of pH (through bicarbonate formation). These facts are especially important if SRM are used to remediate metal-rich effluents like acid mine drainage. This presentation discusses the use of extremophiles in the development of some clean technologies along with the description of different bioremediation technologies employed to remove heavy metals.

Código de Resumen: CPMS-002

Sección: Microbiología Ambiental y del Suelo

Charla Plenaria

ECOLOGÍA MICROBIANA DE LA BIODEGRADACIÓN DE AROMÁTICOS: EJEMPLOS DE NUEVAS INTEGRACIONES TÉCNICAS Y CONCEPTUALES

MICROBIAL ECOLOGY OF AROMATIC BIODEGRADATION: PINPOINTED EXAMPLES OF RECENT TECHNICAL AND CONCEPTUAL INTEGRATIONS

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Similarly to many daily aspects of our present life, the field of microbial ecology has been experienced stronger technological and conceptual impacts in the last two decades derived from the advances and availability of tools to produce high throughput data, to handle such information, and to discover new connections by exploratory or by hypothesis-based research. These strategies, relying in interpretation of data produced by novel devices and techniques, are allowing the retrieval of genomic, transcriptomic, metabolic and proteomic information from complex microbial communities in the environments under study. In a path exemplifying a tool-driven revolution as one major source of knowledge and conceptual innovation, in this talk I will present selected examples of our previous and current research on how different developments and applications of such recent techniques or on its interdisciplinary integrations had been applied and interpreted on the frame of known genes and functions of hydrocarbon contaminants microbial metabolism, thus, helping to gain a better ecogenomic understanding of complex microbial communities thriving or acting on pollutants at open environments or even in microbiomes associated to organisms under multiple selections, with consequences evidenced in diversity, adaptation or evolutionary processes. Out of this basic information there are applications for biocatalyst/bioactive biotechnological explorations and for accurate and effective bioremediation regimes.

Código de Resumen: CPMS-003

Sección: Microbiología Ambiental y del Suelo

Charla Plenaria

DIVERSIDAD Y FISIOLOGÍA DE BACTERIAS QUE TRANSFORMAN ARSÉNICO

ARSENIC TRANSFORMING BACTERIA: DIVERSITY AND PHYSIOLOGY

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Arsenic is widely present in water, rocks, and soils in many different areas of the World. It is generally assumed that arsenic has mainly a magmatic genesis trough volcanic activity and ore deposits with high arsenic. Endogenous and exogenous agents, such as meteorological effects on the soil, and the circulation of subterranean water and wind, distribute arsenic through the atmosphere, soil and water. Arsenic is a highly toxic element that supports a surprising range of biogeochemical transformations. The biochemical basis of these microbial interactions lies in energy yielding redox biotransformations that cycle between the As(V) and As(III) oxidation states. Specialist bacteria able to obtain energy for growth through redox transformations of arsenic have been evidenced in many environments. Those biotransformations have a subsequent impact on the chemistry and the microbiology of studied environments. Microbes

primarily metabolize inorganic arsenic either for resistance or for energy generation. The transformations may involve oxidation, reduction, methylation or demethylation. These transformations greatly contributes to the arsenic mobility and bioavailability in several environments.

Código de Resumen: MS-001

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

ANÁLISIS FUNCIONAL Y BIOINFORMÁTICO DE UN CONJUNTO DE PLÁSMIDOS OBTENIDOS DE AISLAMIENTOS BACTERIANOS EXTRAÍDOS DE UN SISTEMA DE BIOPURIFICACIÓN UTILIZADO PARA LA ELIMINACIÓN DE PESTICIDAS

BIOINFORMATIC AND FUNCTIONAL ANALYSIS OF A PLASMID POOL FROM BACTERIAL ISOLATES FROM AN ON-FARM BIOPURIFICATION SYSTEM USED FOR PESTICIDE REMOVAL

María C Martini[1] , Francisco J Albicoro[1] , José L López[1] , María E Salas[1] , Mariano Pistorio[1] , Andreas Schlüter[2] , Antonio Lagares[1] , María F Del Papa[1] .

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Background. Biopurification systems (BPSs) are used in farms as pollution control technique to treat pesticide contaminated water. BPSs are efficient systems containing a biological active matrix that retains pesticides and enhances their degradation.
Considering that BPSs are used for the pesticide removal, they are target sites for finding genes associated with degradation of these compounds. It is assumed that mobile genetic elements (MGEs) carrying genes coding for enzymes involved in degradation might contribute to the degradation of pesticide and other xenobiotic compounds. In particular, plasmids play an essential role in the evolution and adaptation of bacteria as they encode accessory genes that increase bacterial fitness in a given environment, making them a target for studying the presence of degradative functions.
Objectives. The aim of this work was to analyze the information acquired from the sequencing of a plasmid pool obtained from a collection of bacteria recovered with and without selection pressure from a BPS contaminated with several pesticides. **Results.** In order to investigate the type and diversity of the information encoded in the plasmid pool present in a BPS enriched with several pesticides, a plasmid search was performed using *in situ*-lysis gel electrophoresis. Out of 1,400 isolates screened, a collection of 75 plasmid-containing bacteria was generated. Isolates were classified in 35 groups according to their plasmid profiles. Plasmid sizes ranged from 30 to 300 Kpb, being almost 50% of them more than 100 Kbp in length. Also, a phenotypic characterization of the BPS collection comprising *i)* antibiotic and metal tolerance of isolates, *ii)* evaluation of the presence of broad host range (BHR) plasmid incompatibility groups and *iii)* ability of these plasmids to be mobilized to an *E. coli* host stain, was carried out.
In order to go deeper in the characterization of these plasmids, 35 representative isolates comprising at least 50 plasmids were pooled for high throughput sequencing using Illumina MySeq technology. Preliminary *in silico* analysis of the obtained data showed a high abundance of plasmid-related functions such as replication, stabilization, partition and mobilization. The variety of these genes gives an idea of the great diversity of the plasmids present in the BPS collection. As expected, the presence of different antibiotic and metal resistance types were detected, in agreement with the multi-resistance phenotypes observed for most of the isolates. In addition, the assembled data allowed us to obtain six complete replicons ranging between 4 and 40 Kbp in length.
Finally, the established database that contains the collective genes and operons with novel activities will be used for further bioinformatics and functional analysis. The presence of putative proteins with potential biotechnological applications justifies further work on gene cassette reservoirs as sources of functionally diverse proteins.

Código de Resumen: MS-002

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

MULTICELULARIDAD DE BACILLUS FORMADORES DE ESPORAS AISLADOS DE AMBIENTES EXTREMOS

MULTICELLULARITY OF SPORE FORMING *BACILLUS* ISOLATED FROM EXTREME ENVIRONMENTS

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Modern agriculture techniques tend to expand into inhospitable regions, which can be characterized by having saline and poor soils, climates with extreme temperatures and scarcity of available water and nitrogen. Although genetically modified crops can be adapted to these conditions their performance will be affected in the absence of a plant-beneficial rizhosphere bacterial community (i.e. root biofilms) adapted to that extreme environmental conditions. Furthermore, it might be postulated that current inoculants and plant growth promoting rhizobacteria (PGPR), of great performance in good agricultural soils, could fail when applied in poor soils of unhealthy environments such as lands of extreme climate, extreme salinity, water scarcity, high irradiation incidence, etc. Located at altitudes of more than 4,000 above sea level the Andean Puna constitutes an almost unexplored ecosystem of great biodiversity and extreme environmental conditions that resemble the initial Earth.Our hypothesis suggests that the bacterial communities in these (and similar) extreme environments should possess a greater ability to adapt to stresses than other wild strains of the same genus and species isolated from non-extreme environments. The aim of this work was to characterize several *Bacillus* strains isolated from the Puna for their ability to form biofilms and to sporulate at high temperature. Based on it, we examined their capacity to grow, move and colonize solid substrates and form solid and liquid (pellicle) biofilms in the presence of different salt concentrations (up to 20% NaCl); different temperatures of incubation (up to 60°C) and UV-tolerance (both UV-B and UV-C). The selected *Bacillus* strains were genetically analyzed and genotypically identified as novel isolates of *B.amyloliquifaciens*, *B. licheniformis* and *B. subtilis*. Our results indicate that the wild Andean isolates were able to grow and colonize surfaces at temperatures above the maximum temperatures of reference *Bacillus* strains isolated from more moderate environments. In addition, these Andean isolates had a higher tolerance to UV radiation. These results confirm the initial hypothesis and point out the necessity to isolate and characterize wild bacterial isolates with potential PGPR phenotypes, specially the ability to form robust biofilms, from extreme environments to be used at the near-future when the actual agriculture frontier reaches unexploited poor-soils.

Código de Resumen: MS-003

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

ANÁLISIS DE LA COMPOSICIÓN DE LAS COMUNIDADES MICROBIANAS DE LAGUNAS DE ESTABILIZACIÓN DE EFLUENTES DE PEQUEÑAS INDUSTRIAS LÁCTEAS USANDO SECUENCIACIÓN DE AMPLICONES DEL gen 16s

MICROBIAL COMMUNITY COMPOSITION ANALYSIS OF WASTEWATER STABILIZATION PONDS FROM SMALL CHEESE INDUSTRIES USING 16S GENE AMPLICON SEQUENCING

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Whey disposal is an important problem for small cheese industries. When is not possible to use it downstream, a large volume of whey is discarded to the wastewater treatment system. 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. In this work, we analyzed the bacterial and archaeal community composition in two stabilization ponds systems of dairy industries using amplicon sequencing of 16S gene fragments. Samples were collected from six full-scale stabilization ponds belonging to two cheese 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. Physicochemical parameters were recorded and samples were collected by triplicate on each pond. DNA was extracted using physical shearing and phenol-chloroform extraction. Amplification of the V4 region of 16S gene was done using universal primers F515 and R806 thus a total of 18 sequencing libraries were constructed. Sequencing was done in a Genome Sequencer FLX pyrosequencer using Titanium chemistry in quarter of a plate. A total of 178,200 raw sequences were obtained. Sequences were demultiplexed, denoised and quality trimmed using QIIME v1.7. Quality checked sequences (142,974, 80.23%) varied from each sample between 2531 and 14723, and were clustered into OTUs using a 97% pairwise sequence similarity with UCLUST.
Alpha-diversity was assessed using different parameters and rarefaction analysis. All samples showed a limited number of
OTUs compared to other environmental samples as soil or sea, since the higher Chao1 value was 217. Taxonomic classification of OTUs was done using the implementation of RDP classifier in QIIME against Greengenes database v13_5 with a confidence threshold 0.8. A general overview showed that the distribution of bacterial and archaeal species resemble the community composition of anaerobic digesters but with substantial changes in the bacterial composition. Bacterial phyla accounts from 95.21% to 99.51% of phyla identified. In five ponds, the most abundant phylum was Synergistetes, ranging from 43.79% to 69.73%. Other phyla identified were Fimicutes, Proteobacteria, Thermotogae, Bacteroidetes and Verrucomicrobia. Archaeal composition is different for each industry, while abundance in AUR was in the range of 0.22% to 1.02%, in CYC was from 0.76% to 4.70%. Beta-diversity analysis was done to compare the composition of the ponds. Using PCoA of calculated Bray-Curtis distances showed that samples from both industries are well separated. Also, different samples from the same pond grouped together, showing that each pond had a stable and independent composition. This knowledge about the community composition is key to improve and maintain the efficiency and stability of the process.

Código de Resumen: MS-004

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

DESNITRIFICACIÓN DE EFLUENTES NUCLEARES PROVENIENTES DE LA INDUSTRIA NUCLEAR UTILIZANDO SOPORTES OBTENIDOS POR IRRADIACIÓN GAMMA

DENITRIFICATION OF EFFLUENT FROM THE NUCLEAR INDUSTRY USING CARRIER OBTAINED BY GAMMA IRRADIATION

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In the nuclear industry, nitrate and methanol are used to get uranium precipitates and concentrates which are used as fuel elements, at nuclear plants. For this reason the effluent of this process contain high quantities of nitrogen and methanol, meaning high levels of DQO. Therefore these high concentrations must be diminishing for complying locals' environment law.
Nitrate is a very soluble ion and then it is difficult to precipitate, so the EPA recommends applying biological process as best treatment for reducing these kinds of elements.
In the present work we study and apply the denitrification process (anaerobic reduction of NO_3 to N_2). A key point in this process is the immobilization of the biomass, avoiding the washing and enhancing the performance in kinetic and effectiveness terms, by retaining biomass. Taken this into account, we work with a hydrogel which were obtained by gamma irradiation for retaining biomass. As co-polymer we added Acrilamide and silica within the hydrogel solution which allow us to get a material with diverse density or more harshly, depending on the needs. The hydrogel obtained was found to be a biocompatible, resilient, elastic and flexible material. This proved to be effective for use in fluidized bed bioreactors because the collisions that take place constantly renew the biofilm, left over without dead zones along continuous assays.
As a result, working at a laboratory scale of 1 liter the nitrate levels of the initial effluent decrease in a Kinetic rates of 4g/l.day and the final DQO level declined significantly. For this reason this hydrogel will be assayed in other treatments of effluent of the nuclear industry.

Código de Resumen: MS-005

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

ESTUDIO DE MEGAPLÁSMIDOS EN *Sphingomonas* DEGRADADORAS DE HIDROCARBUROS POLICÍCLICOS AROMÁTICOS

MEGAPLASMIDS STUDY IN POLYCYCLIC AROMATIC HYDROCARBONS DEGRADING *Sphingomonas*

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Bioremediation is a methodology that provides an economical and effective solution for the treatment of contaminated soils with polycyclic aromatic hydrocarbons (PAH). Inoculation with degrading microorganisms in contaminated environments (bioaugmentation) is one of the strategies used in last years. The genus *Sphingomonas* (sensu latu) has been intensively

studied because of their large catabolic diversity. It was shown in the last years that many sphingomonads possess (often several) plasmids and especially large plasmids commonly designated as megaplasmids. Furthermore, there is increasing evidence for the existence of plasmids that only can be disseminated among sphingomonads and which undergo after conjugative transfer pronounced rearrangements.
The aim of this work was to demonstrate the presence of megaplasmids in our collection of PAH-degrading *Sphingomonas* strains (1A, 22A, 22B, 20006FA and AM) isolated from soils of different regions with distinct pollution histories; to characterize and to find evidence of the presence of PAH-degrading genes located in megaplasmids. The presence of megaplasmids in PAH-degrading strains was demonstrated using lysis in situ technique. The same electrophoresis mobility bands in all megaplasmid preparations were observed, showing that the PAH-degrading strains would have at least one megaplasmid. In order to show differences in sequence and molecular weight, the restrictions pattern of the megaplasmids were obtained from purified plasmids by Kieser protocol. The same restrictions profiles were visualized for the strains tested with EcoRI, XbaI, HindIII, and molecular weight was calculated in 40-50 kpb. These results suggest that despite having been isolated from different soils, the studied PAH-degrading *Sphingomonas* strains could have closer related megaplasmids. The location in the megaplasmids of a gene implicated in PAH-degradation pathway was evidenced from purified plasmids, by PCR using genus-specific primer sets targeted at the catechol 2,3-dioxygenase gene of proteobacterias. The expected molecular weight band was sequenced showing 100% identity and 97% of coverage with catechol 2,3 dioxygenase of PAH-degrading *Sphingomonas* PNB and *Sphingobium* BNQ31, confirming the probably location of the gene.
Different mating assays were made to determine if a complete degradation pathway are located in the plasmid. For this purpose, mobilization functions were incorporated in megaplasmid by transposition mutagenesis, then the megaplasmid was forced to move using a triparental mating assay. Mobilization of the megaplasmid was not achieved. This result could be due to a system failure in mating strategy or absence in replication capacity of the megaplasmid in gammaproteobacteria. The conjugation failure, together with the high similarity found between megaplasmids from PAH-degrading *Sphingomonas* phenotypically diverse, suggest that the degrading property could be specifically transferred among this bacterial genus.

Código de Resumen: MS-006

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

PROMOCIÓN DE LA DOMINANCIA DE CIANOBACTERIAS FILAMENTOSAS FORMADORAS DE FLORACIONES POR FACTORES AMBIENTALES

PROMOTION OF THE DOMINANCE OF FILAMENTOUS BLOOM-FORMING CYANOBACTERIA BY ENVIRONMENTAL FACTORS

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Raphidiopsis mediterranea and *Planktothrix agardhii* are planktonic, filamentous cyanobacteria. Both are of great concern due to their ability to produce toxins. In eutrophic freshwater bodies, these two bloom-forming cyanobacteria regularly occur, but it is not completely known what leads to the dominance of either species. Studies have pointed out that *P. agardhii* is favored by turbid conditions with elevated phosphorus concentrations, while the abundance of *R. mediterranea* is related to less turbid warmer waters.
The aim of the present work was to investigate the response of *P. agardhii* and *R. mediterranea* to changes in light and phosphate availability.
A part of the study was carried out in Los Patos shallow lake (Buenos Aires, Argentina) a small eutrophic water body where dense blooms of both species have been observed during the past

decades. *In situ* environmental measurements, nutrient and phytoplankton samples were taken over two years.
In field, *R. mediterranea* and *P. agardhii* co-occurred and dominated the phytoplankton during the study but their abundances were negatively related: *R. mediterranea* abundance increased when *P. agardhii* population decreased. Regression analyses showed that these species responded differently to environmental variables.
The second approach was carried out at the laboratory, investigating the coexistence of *P. agardhii* and *R. mediterranea* in co-cultures growing in MLA media. Two light intensities (40 and 80 µmol photon m^{-2} s^{-1}) and two phosphate concentrations (5 µM and 0.1 mM K_2HPO_4) were tested in batch cultures over 18 days. The variation in morphology, abundance and biomass of the two strains growing together were evaluated daily by optical density and trichome counting by optical microscope.
Before starting the experiment, the co-culture was dominated by *P. agardhii* (83%). After 7 days, there was a shift in the dominances under high light, which was more pronounced in the 5 µM treatment. After 18 days, *P. agardhii* population collapsed and the co-culture was dominated by *R. mediterranea*, accounting for the 98% and 96% in the 5 µM and 0.1 mM treatment, respectively. Under low light, *R. mediterranea* population increased, but at the end of the experiment the species co-dominated in both phosphorus conditions. Although most of the trichomes of *R. mediterranea* were straight, a higher proportion of coiled trichomes (approximately 15%) were observed under high light and 0,1 mM K_2HPO_4.
These results support the conclusion that both species have different environmental preferences. A better understanding of the conditions that promote the dominance of *R. mediterranea* or *P. agardhii* is needed to characterize their niches, which will useful to improve water management practices.
Supported by CONICET, UNMdP and FIBA.

Código de Resumen: MS-007

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

CARACTERIZACIÓN MICROBIOLÓGICA Y MOLECULAR DE UN CONSORCIO BACTERIANO CELULÍTICO

MICROBIOLOGICAL AND MOLECULAR CHARACTERIZATION OF A BACTERIAL CELLULOLYTIC CONSORTIUM

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Lignocellulosic biomass is highly recalcitrant and its bioconversion to fermentable sugars is considered the key step in second generation bioethanol production. In nature, several microorganisms carry out this degradation in a synergistic manner, with a combination of different cellulases, hemicellulases and auxiliary enzymes, sometimes establishing microbial consortia.
Therefore, the aim of this study was to characterize a cellulose-degrading bacterial consortium CB1-2, isolate its pure components and evaluate their different roles in enzymatic activity. CB1-2 consortium was previously isolated from a forest soil sample by enrichment on carboxymethylcellulose as sole carbon source and their bacterial components were identified by 16S rRNA sequence. The isolation of pure strains from CB1-2 was attempted by enrichment on different cellulosic substrates such as: solka floc, cellobiose, carboxymethylcellulose, avicel and xylan from birchwood, by successive enrichment on liquid culture, followed by culture on solid media. After that, four different strains were obtained and identified, by direct 16S rRNA gene sequencing, as *Pseudomonas sp, Stenotrophomonas sp, Bacillus sp* and *Paenibacillus sp*, which correlated with the results of 16S rRNA gene clone analysis of CB1-2.These results were also confirmed by 16S-DGGE. Cell free supernatants of all strains were tested for cellulase and

xylanase activity and only *Paenibacillus sp* showed measurable values. However they were lower than CB1-2 consortium, which indicates the need for another member of the consortium.

Código de Resumen: MS-008

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

ESTRUCTURA DEL BACTERIOPLANCTON A LO LARGO DEL SISTEMA COSTERO ATLÁNTICO SUDOCCIDENTAL (SCAS, 34 - 41°S)

BACTERIOPLANKTON STRUCTURE ALONG SOUTHWESTERN ATLANTIC COASTAL SYSTEM (SACS, 34 - 41°S)

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Planktonic bacteria are recognized as important drivers of biogeochemical processes and energy flow in all aquatic ecosystems, however, the taxa that make up these communities are poorly known. The aim of this study was to investigate the structure of the dominant bacterial communities along a highly productive area which sustains important artisanal and coastal fisheries in Uruguay and Argentina. To this end, on the Southwestern Atlantic Coastal System a sectioned-sampling design (37 samples distributed in 4 transects) was implemented to provide a rapid and reliable survey of bacterioplankton distribution in relation to the physical environment .The bacterioplankton biomass content in each sample was determined by using epifluorescence microscopy with dual- staining procedures (4´,6-diamidino-2-phenyllindole and acridine orange). For bacterial composition analysis we used a 16S rDNA based PCR –DGGE approach followed by DGGE bands phylogenetic sequence analysis. The results obtained showed that abundance, bacterial biomass and cell volume values ranged from 0.48 to 5.42 x 10^6 cells ml^{-1} , 2 and 120.5 mg C l^{-1} and 0.004 to 0.156 μm^3 respectively, showing a predominance of coccoid forms. *Bacteriodetes* and α- *Proteobacteria* accounted for approximately 70 % of the total bacteria taxa. Community fingerprint analysis by PCR-DGGE revealed that, in general, there was no apparent difference of bacterioplankton community composition among sampling locations in the SACS. On the DGGE gel, a total of 150 OTUs were observed and 68 were successfully sequenced. They were affiliated to different bacterial phylogenetic assemblages in the GenBank with 81–100 % similarities, including *Bacteroidetes* (23 sequences), α-*Proteobacteria* (24 sequences), β-*Proteobacteria* (1 sequence), δ-*Proteobacteria* (2 sequences), γ-*Proteobacteria* (6 sequences), *Cyanobacteria* (3 sequences), *Sphingobacteriales* (1 sequence) and *Actinobacteria* (3 sequences). Within their corresponding assemblages*S*,*y nechococcus (Cyanobacteria), Ruegeria, Rhodobacteraceae (α-Proteobacteria); Nereida Rhodobacteraceae, (α-Proteobacteria); Roseobacter, Rhodobacteraceae (α-Proteobacteria); Flavobacteriia (Bacteroidetes); Krokinobacter, Flavobacteriia (Bacteroidetes)* and *Corynebacterium (Actinobacteria)* were also found. In transect 3 (marine environment) we determined the highest phylogenetic diversity. Some of these phylotipes matched with those found in a previous study, performed with the 16S pyrosequencing technique, from samples taken in only two representative stations of this area.

Thus, our results show that PCR-DGGE and band sequencing provide a robust and cost effective exploratory approach, and are effective in distinguishing among dominant bacterial groups. Interestingly, we found the maximum bacterial concentrations in the

same zone where high densities of fish eggs and larvae were previously observed, demonstrating the important role of bacterioplankton in this productive area.

Código de Resumen: MS-009

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

EFECTO DE LA HUMEDAD DEL SUELO Y DE LA CALIDAD DEL MANTILLO VEGETAL SOBRE LA ACTIVIDAD NITRIFICADORA EN SUELOS ÁRIDOS DEL MONTE PATAGÓNICO

EFFECT OF SOIL MOISTURE AND PLANT LITTERFALL QUALITY ON THE NITRIFYING ACTIVITY OF ARID SOILS FROM THE PATAGONIAN MONTE

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Water availability has a major influence on nutrient cycling; particularly in arid and semiarid ecosystems were rainfall events are scarce. In addition, the composition of plant cover through the quantity and chemical quality of its litterfall, which is altered by selective grazing, influences the input of nutrients into the soil and the nutrient cycling. The aim of this study was to analyze the effect of both soil moisture and plant litter quality from sites with different grazing disturbances on the nitrifying activity of arid soils from the Patagonian Monte.
Upper soil samples associated to vegetated patches were collected at two sites with different signs of grazing disturbance (DS: disturbed site; CS: conserved site) within the Wildlife Refuge “La Esperanza” (42°12’S, 64°58’W). Then, samples of each site were pooled in a composite sample. Aliquots of the composite sample were used to assess soil moisture, pH and texture. Also, the concentration of organic C was measured by wet combustion (8.69±0.79 mg/g dry soil) and the concentration of total N by the semi-micro Kjeldahl technique (0.71±0.03 mg/g dry soil). In addition, plant litterfall samples were collected at each site. Both litter samples differed significantly ($p<0.01$) in their concentrations of total N (DS: 7.95±0.36; CS: 10.02±0.52 mg/g dry soil) and soluble phenolics determined by the Folin-Ciocalteu method (DS: 5.20±0.26; CS: 3.64±0.24 mg/g dry soil).
One hundred and forty four soil microcosms containing composite soil covered by plant litter from DS or CS and control
microcosms without litterfall (treatments DL, CL and CTRL, respectively) were subjected to two different moisture conditions (5 and 15% humidity). Microcosms were incubated at room temperature and three replicates of each treatment (combination of soil moisture and litter quality) were withdrawn at different times to analyze the evolution of nitrifying activity in soil. During the first week of incubation, the highest nitrification activity was in the 5% soil moisture treatments, suggesting that the slow growing-nitrifying community could be in a lag phase and would still have not responded to the watering treatment. However, this effect was reversed in the following weeks, were nitrification was in average 16% higher at the highest soil moisture.
Nitrification in CL was higher or equal to DL, but never lower. The chemical composition of plant litter from DS (high phenolic concentration) may be negatively affecting the rate of nitrification in soils from this site. Finally, at the end of the incubation period there was interaction between the effects of soil moisture and litter amendment.
In this study, high soil moisture and plant litterfall quality exerted a positive effect over the nitrifying activity. Further studies are being performed to estimate the abundance of the nitrifying microbial populations in these microcosms by means of qPCR.

Código de Resumen: MS-010

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

CALIDAD BACTERIOLÓGICA Y FISICOQUÍMICA DE AGUA EMBOTELLADA PRODUCIDA EN TANDIL, BUENOS AIRES

BACTERIOLOGICAL AND PHYSICOCHEMICAL QUALITY OF BOTTLED WATER PRODUCED IN TANDIL, BUENOS AIRES

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The consumption of bottled water is becoming increasing in Tandil mainly because tap water exceeds the recommended level of nitrate concentration for human consumption. There are four factories in Tandil that produce bottled water. The source is tap water obtained of groundwater and they apply reverse osmosis. This study is aimed to assess the bacteriological and physicochemical qualities for human consumption of four bottled water produced in Tandil. Bacteriological parameters analyzed included aerobic mesophilic bacteria, total coliforms, *Escherichia coli, Pseudomonas aeruginosa,* sulphite-reducing bacteria and *Enterococcus sp*. Physicochemical parameters included pH, electrical conductivity, total dissolved solids, hardness, bicarbonate, sulfate, chloride, nitrate, sodium, calcium, potassium and magnesium. The results were compared with Argentinean Food Code which regulates bottled water quality for human consumption. Also, labels of bottles were analyzed in order to evaluate the fulfillment of required information and to compare with the results of quality analysis. Results showed that aerobic mesophilic bacteria were detected in four samples; but lower than legal levels. The others bacteria were not found in any case, however
Klebsiella sp was isolated in one sample. The pH values were from 6.9 to 7.6. Electrical conductivity ranged from 160 to 370 µS/cm. Total dissolved solids varied from 150.6 to 230.22 mg/L and hardness were lower than 83.2 mg CO_3 Ca/L. All the ions analyzed were within the recommended limits. Considering that nitrate in groundwater of Tandil tap water exceeds 45 mg/L, we can determine that the four samples have low values due the application of treatments. Nevertheless, labels of bottles showed contents of nitrate below we determined. In conclusion, the four bottled water produced in Tandil were suitable for human consumption. However, it is necessary to improve the information for consumers presented in labels including other parameters as bacteriological information.

Código de Resumen: MS-011

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

EVALUACIÓN DE LA BIODEGRADACIÓN DE FENANTRENO POR BACTERIAS INDÍGENAS AISLADAS DE LA ZONA ESTUARIAL DEL RÍO DE LA PLATA

EVALUATION OF PHENANTHRENE BIODEGRADATION BY INDIGENOUS BACTERIA ISOLATED FROM THE RÍO DE LA PLATA ESTUARINE ZONE

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Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants produced by industrial operations using petroleum products. Many of these compounds have toxic, mutagenic and carcinogenic properties. The Río de la Plata estuarine zone is a highly productive environment both in the ecological and the economic aspect, with a relevant importance on fisheries. This zone is exposed to chronic pollution of hydrocarbons, mainly associated with chemical industry, oil refining and port activities. Microbial degradation is the

main process to eliminate these compounds from the environment. The aim of this work was to investigate the growth kinetics in phenanthrene of indigenous bacteria to survey their potential degradation ability and elucidate the degradation pathway by detection of naphthalene dioxygenase gene (*ndoB*). We isolated 41 strains from water samples collected along this estuarine system by enrichment on salt mineral medium with phenanthrene. Restriction Fragment Length Polymorphism (RFLP) analysis of their 16S rRNA gene resulted in 35 unique RFLP pattern. Sequence analysis of the 16s RNA gene of these 35 strains indicated that 16 genera (*Halomonas, Cobetia, Acinetobacter, Psychrobacter, Marinobacter, Pseudomonas, Pseudoalteromonas, Vibrios, Rhizobium, Celeribacter, Thalassospira, Micrococcus, Microbacterium, Mycobacterium, Rhodococcus y Stenotrophomonas*) were present. The kinetics of bacterial growth of these 35 strains was examined in mineral medium culture supplemented with 50 mg/l phenanthrene. According to the result of growth, the strains identified as *Rhizobium naphthalenivorans, Vibrio azureus, Rhodococcus erytrhopolis, Pseudomonas sp., Halomonas eurihalina,* showed the highest growth after 48 h and *Mycobacterium sp., Micrococcus luteus, Microbacterium laevaniformans* and *Celeribacter sp*. had the maximum growth after 72 h. The screening for the presence of *ndoB* by using PCR method showed that 5 isolates were positive, suggesting the presence of different metabolic pathways to transform PAHs.
The results of the present work show that there are some bacteria with potential for PAHs utilization as sole source of carbon and energy; being able to play an important role in the removal and degradation of PAHs from the Río de la Plata estuarine zone.

Código de Resumen: MS-012

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

EFECTO DE LA LUZ UV Y LA SALINIDAD SOBRE EL DESARROLLO, EN CONDICIONES DE LABORATORIO, DE TRES CEPAS BACTERIANAS AISLADAS DE AGUA DE MAR

THE UV RADIATION AND SALINITY EFFECT ON THREE BACTERIAL STRAINS ISOLATED FROM SEAWATER AND DEVELOPED UNDER LABORATORY CONDITIONS

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The main purpose of the present study was to determine, under UV radiation and different saline concentrations, the growth and survival of *Escherichia coli*, *Staphylococcus* spp. and *Enterococcus* spp. frequently used as indicators of recreational water quality. *E. coli, Staphylococcus* spp. *and Enterococcus* spp. were isolated from seawaters of Mar del Plata beaches. For the assays, suspensions of each strain were done in growth exponential phase, with a concentration determined by Mac Farland N° 3 scale, and by spectrophotometry.
UV assay: suspensions were UV irradiated (26 cm distant) at 0, 10, 20, 30, 60, and 120 secondes. A 3 ml aliquot was taken from each irradiated suspension, and from controls (0 secondes), and the optic density was measured. Simultaneously, the bacterial count of these suspensions was carried out by means of pour plate technique. After 20 secondes of UV exposure, a total inhibition of *E. coli* and *Staphylococcus* spp. development was registered. Meanwhile, *Enterococcus* spp. decreased its development slightly and inversely to exposure time, being viable up to 120 secondes.
Salinity assay: the three bacterial strains growth in nutritive broth and at 0%, 5% and 10% ClNa concentrations was evaluated.
One of the replicates was used as assay control, and the others were inoculated with 5 ml of bacterial suspension. The incubation was carried out at environmental temperature during 144 hours. Aliquots were daily removed, the absorbancy was measured, and bacterial count by pour plate technique was done, employing an appropriate culture medium, and incubating at 37°C during 24 or 48 h, according to the strain under study. At 0% ClNa assay, an increase in the counts of all strains, and then, a slight decrease, more evident in *Enterococcus* spp., were

registered. This decrease could be attributable to the culture medium depletion. At 5% ClNa assay, the growth was similar to that registered at 0% ClNa one, but lower for all bacterial strains. At 10% ClNa assay, *Staphylococcus* spp. counts increased, while those of the other strains decreased. According to the results obtained from both assays it can be concluded that: a) the UV radiation was the most effective factor inhibiting *E. coli* and *Staphylococcus* spp. growth, while *Enterococcus* spp. instead, decreased its counts but persisted up to the end of the assay; b) the three strains developed at low ClNa concentrations, and c) at 10% ClNa concentration, *Staphylococcus* was the only genus enable to develop.
As *Escherichia coli*, *Staphylococcus* spp. and *Enterococcus* spp. are frequently mentioned as indicators of bacteriological quality for recreational waters, the present study will bring information about the effects of the herein assayed factors upon the development and survival of these microorganisms. This information could be then employed to improve depuration actions in different aquatic systems, and to select the more efficient indicator, according to the local environmental conditions.

Código de Resumen: MS-013

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

DESARROLLO DE INOCULANTES BACTERIANOS CON ACTIVIDAD ANTIFÚNGICA Y CAPACIDAD DESHIDRATANTE DE PLANTAS USADOS EN LA FORMACIÓN DE FORRAJES CONSERVADOS DE ALTA CALIDAD PARA ALIMENTACIÓN ANIMAL

DEVELOPMENT OF BACTERIAL INOCULANTS WITH ANTIFUNGAL ACTIVITY AND PLANT-DEHYDRATION CAPACITY USED IN THE FORMATION OF HIGH-QUALITY CONSERVED FODDER FOR ANIMAL FEED

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In our country there are between 5 and 6 million hectares implanted with alfalfa. The first destination of the forage produced is grazing or fresh supply and about 35% of it goes to the development of conserved forages for hay and silages utilization. The hay is the main way of preserving alfalfa. Many problems associated with hay-making would be solved if hay drying time could be reduced or if hay could be stored at high moisture (HM) without fungal spoilage. When hay is baled at greater humidity than 20% moisture, molds and fungi grow, and excessive heating can occur; this phenomena result in reduced nutritive value of the conserved hay (or silage). Only about 5% of fungi produce mycotoxins, however, they can adversely affect palatability and feed intake. Feeding moldy hay to animals would result in significantly lower dry matter intake and reduced weight gains. Bacterial inoculants would be an alternative for improving hay (and silage) preservation. *Bacilli* are bacterial species that offer several advantages over other bacteria for protection against pathogens because of their ability to form endospores, and because of the broad-spectrum activity of their antibiotics. The aim of this study was to identify, isolate and select the best spore-forming *Bacillus* strains capable of inhibiting the growth of undesirable fungus and accelerate the dehydration of forage in order to conserve its nutritional value. In this research, we isolated more than 500 strains of Bacillus spp. from hay samples that were taken from the provinces of Buenos Aires, Santa Fe and Córdoba. Each isolate was characterized by antifungal activity and dehydration of alfalfa under laboratory conditions and open-field trials. From the 500 tested isolates, five were potentially identified as able to control a broad spectrum of fungal diversity and with excellent dehydrating capacity comparing with commercial acids. These isolates could be adopted for future applications to control fungal diseases and accelerate dehydration of conserved forage (hay and silages) improving the productivity and quality of ruminant livestock farms.

Código de Resumen: MS-014

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

ESTUDIO MEDIANTE PIROSECUENCIACIÓN DE UN CONSORCIO BACTERIANO DEGRADADOR DE PAH Y DE LOS CAMBIOS PRODUCIDOS EN SU COMPOSICIÓN POR LA INOCULACIÓN DE UNA CEPA BACTERIANA COMPETENTE

PYROSEQUENCING STUDY OF A BACTERIAL PAH-DEGRADING CONSORTIUM AND THE SHIFTS PRODUCED IN ITS COMPOSITION BY INOCULATION WITH A COMPETITIVE STRAIN

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Bioaugmentation requires different species of introduced degrading microorganisms, which can compete with the indigenous microbial community in PAH-contaminated soil, in order to participate in the main carbon and energy flux processes and enhance PAH removal. PAH-degrading bacterial consortia, usually obtained by enrichment techniques from of environmental samples, have been proven to be diverse and complex providing a unique system to challenge the new molecular tools allowing the characterization of molecular interactions and microbial activities on a global level never before achieved. A consortium (CON) was obtained from a chronically contaminated soil with polycyclic aromatic hydrocarbons (PAH), five strains were isolated and identified as *Sphingobium sp*. (AM), *Enterobacter sp*. (B and B1) and *Pseudomonas sp*. (T and Bc). The CON was inoculated with *Sphingomonas paucimobilis* 20006FA, a strain able to degrade a wide range of PAH, generating a new consortium (CON+I). Both consortia were characterized in terms of structure, diversity and functionality. The CON and CON+I consortia were cultivated in LMM with 200 mg/l of phenanthrene for 15 days. The CON showed a degradation of 59 % with the accumulation of 1-hydroxy 2-naphtoic acid, the CON+I showed a degradation of 78% without accumulation of this intermediary.
Population dynamic between the two consortia was observed at cultivable (counts) and non cultivable level (PCR-DGGE). This work aims to study shifts in diversity and composition of CON, by pyrosequencing technique, when is inoculated with a competent bacterial inoculant. The phylogenic composition of the consortia was profiled using pyrosequencing of
PCR-amplified bacterial 16S rRNA gene fragments with the posterior analysis with EstimateS (Version 9). All representative sequences of each OTU were classified into the domain *Bacteria*, class *Proteobacteria* (Alpha, Beta and Gamma with 89.3%, 2.93% and 7.73% of the reads respectively). Relative phylotype frequency at the genus level revealed the presence of *Inquilinus* 1.1%, *Achromobacter* 2.9%, *Sphingobium* 87.7%, *Pseudomonas* 0.5%, *Enterobacter 6,6%, Luteibacter* 0.6%.,and *Sphingomonas* 0.5%. A correlation of some of the sequences with the isolated strains was observed. Surprisingly in the CON+I the genera belonging to the family Sphingomonadaceae remained constant, while a marked increase at the level of *Achromobacter* 12.2% and *Pseudomonas* 1.7% was observed, suggesting a syntrophic interaction between the inoculated strain and these two genera. Additionally it could be observed a decrease in diversity with inoculation, being the Shannon index (H) 1.51 for CON and 1.29 for CON+I. Using a high throughput technique it was possible to observe that the relative composition of bacterial genera within the consortium are strongly affected by the introduction of an inoculant, giving clues that complex interactions between bacterial species could influence the biodegrading potential.

Código de Resumen: MS-015

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

DIVERSIDAD DE LOS RIZOBIOS SIMBIONTES DE *Desmodium incanum* QUE CRECEN EN ARGENTINA

DIVERSITY OF THE RHIZOBIAL SYMBIONTS OF THE *Desmodium incanum* THAT GROW IN ARGENTINA

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Desmodium incanum is an herbaceous legume native to Argentina. Its perennial characteristics and good adaptability make this legume interesting for animal nutrition and introduction in agricultural management practices. Research on native legumes species well adapted to diverse ecological conditions is an important subject for the agronomic potential that many species show.
Little information is available about the symbionts of *Desmodium* species. To study the genetic diversity of rhizobia associated with *Desmodium incanum* grown in our country, isolates from temperate and subtropical regions were collected and analysed. A collection of *Desmodium* nodulating isolates from Argentina was obtained and characterized. A total of 75 rhizobial isolates from 4 *Desmodium incanun* populations from different geographic locations (Chaco, Santa Fe, Tucuman and Corrientes) were selected and analyzed.
The phenotypical characterization included the determination of growth ability under different stressed conditions, whereas the genetic and phylogenetic diversity was assessed through MBOREP, BOXA-PCR, *nifH* and *nodC* amplification as well as sequencing of the 16SRNA in selected isolates. Most microsymbionts of *Desmodium* species belonged to *Bradyrhizobium* closely related to *Bradyrhizobium elkanii*, *Bradyrhizobium japonicum*, *Bradyrhizobium liaoningense* and *Bradyrhizobium*
yuanmingense. Some small groups or single strain were related to *Rhizobium sp*. The results indicated high diversity among symbiotic rhizobia.
These results offered the first systematic information about the microsymbionts of *Desmodium incanum* grown in the temperate regions of Argentina and they open a new line of research on native rhizobia which could be useful for the forage inoculants industry.

Código de Resumen: MS-016

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

AISLAMIENTO DE MICROORGANISMOS TOLERANTES A SALES A PARTIR DE MUESTRAS DE AGUA HIPERSALINAS

ISOLATION OF SALT-TOLERANT MICROORGANISMS FROM HYPERSALINE WATER SAMPLES

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Mining is one of the most polluting activities, dangerous for the environment and also for the living beings. Therefore, some sustainable alternatives that allow the extraction of metals with microorganisms have been developed. These processes are known as ‘biolixiviation’ and they

consist in having microorganisms ‘attack’ the minerals, so that it is finally solubilized. The main characteristic of the microorganisms that develop these capacities is their ability to survive and grow in hostile environments with high temperature, exposure to UV radiation, extreme pH, among other factors.
The aim of this work is to isolate and characterize microorganisms from hypersaline water samples and to study their behavior in presence of a mixture of salts. In this work, we used water samples obtained from Salar del Hombre Muerto, in Catamarca, Argentina. These samples have high salts concentration and were analyzed to determine the presence and concentration of elements such as Li, Na, Ca, As, S, Si. The Na concentration in the water samples was about 98,000 ppm, Mg 1,000 ppm and Li about 600 ppm. These high salinity values are known to be a very limiting factor for the growth of microorganisms.
Microbial growth was studied by inoculating an aliquot of the water sample in nutritive media (YPD and APC). Two experiments were carried out: first, the microorganisms were grown in media without the addition of salt. Under this condition 37 bacteria were isolated. Grown bacteria were then inoculated on salt-added media and incubated till growth. Finally, 30 different bacteria and 3 fungal strains were isolated in media containing 12,250 ppm of Na and 98.5 ppm of Li. Filamentous fungi were grown on solid Sabouraud medium with and without the addition of salt.
The isolated bacteria were characterized according to their cellular wall structure and microscopic morphology through Gram staining. Out of the 30 strains, 17 were found to be Gram positive and the remaining strains (13) were Gram negative. Most of the isolated strains grew rod shaped, however cocci were found as well. The cells were seen to have different size in most of the isolates.
Filamentous fungi were detected to show different growth morphology when growing in salt-added media in comparison with the growth in media without any addition of salt. When growing in a salt-added media, fungi were more colored and folded than when grown on media without salt.
Finally, through optical and electronic microscopy, bacteria and algae were detected in the water samples. Algae were found in different colors (brown and green) and morphology (for example, some of them were covered by a shell, similar to that of Diatoms). Salt crystals were also observed to be in the water samples.
Further investigation will be conducted to identify and select the microorganisms that have the greatest salt tolerance. Those selected microorganisms will be studied to reveal the mechanism used by microbes to survive and grow in hypersaline environments.

Código de Resumen: MS-017

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

DEGRADACIÓN DE FENANTRENO POR MICROORGANISMOS HALOFÍLICOS AISLADOS DE SALINAS PAMPEANAS

PHENANTHRENE DEGRADATION BY HALOPHILIC MICROORGANISMS ISOLATED FROM LA PAMPA SALTERN PONDS.

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Polycyclic Aromatic Hydrocarbons (PHAs) are compounds with two or more fused aromatic rings that are highly toxic because of their mutagenic and carcinogenic effects on living organisms. Bioremediation technology employs microorganisms which are able to metabolize toxic compounds and transform them into harmless ones. In this direction, much has been studied about bacterial remediation, but the knowledge about metabolic pathways and enzymes involved in hydrocarbon degradation in hypersaline environments is scarce. One important source of PHAs pollution is waste water of oil production process, which is indeed generated in

large amount. Because of its high saline content, microorganisms conventionally employed in PHAs remediation cannot be effective in biological treatment of this waste water, which took researchers to consider extremophilic microorganisms, as halophilic archaea. We isolated and identified nine microorganisms (seven Archaea and two Bacteria) of La Colorada Grande, Salitral Negro and Guatraché saltern ponds located in La Pampa province. They were tested, along with other halophilic microorganisms and consortia, to evaluate their hydrocarbon degrading capacity. For this, degradation assays were performed in liquid medium under low oxygen concentration in the presence of 0.02% phenanthrene, for four weeks.
Degradation products and the remaining phenanthrene were extracted from the extracellular medium with ethyl acetate and analyzed by High Resolution Liquid Chromatography (HPLC). Chromatograms evidenced complete phenanthrene degradation by eight of the tested strains, to different byproducts, while an additional strain showed only partial degradation (about 70%).
Degradation by halophilic consortia was greater than 50%. Three of the more effective microorganisms (*Halobacterium*
piscisalsi, Haloarcula argentinensis and *Salicola* sp.) were selected for further analysis of the degradation pathways by gas chromatography, which allowed the assessment of both aromatic and aliphatic hydrocarbons and the identification of the byproducts.
Supported by CONICET, ANPCyT and UNMdP.

Código de Resumen: MS-018

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

UN MÉTODO CROMATOGRÁFICO PARA DETECCIÓN TEMPRANA DE *Fusarium graminearum* EN CULTIVOS DE TRIGO

A CHROMATOGRAPHIC METHOD FOR EARLY DETECTION OF *Fusarium graminearum* IN WHEAT CULTIVARS.

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In Argentina, Fusarium head blight (FHB) is a destructive disease of cereal grain crops, mainly in wheat crops. *Fusarium* species infect wheat during the flowering period. In addition to losses of yield, these fungi can also synthesize toxic components (mycotoxins) in suitable environmental conditions, thus threatening animal and human health.
Fungi produce volatile organic compounds (VOC), during both primary and secondary metabolism, VOC appear as intermediate and end products of various metabolic pathways and belong to numerous structure classes such as mono- and sesquiterpenes, alcohols, ketones, lactones, esters or C8 compounds, which can be used for detection and identification.
We investigated the potential of solid phase microextraction (SPME) coupled to capillary gas chromatography (CGC) and mass spectrometry (MS) to detect volatile precursors of mycotoxins released by *Fusarium* species.
Fusarium graminearum was differentiated from *F. poae*, *F. equiseti*, *F. verticillioides* and *F.oxysporum* by comparison of the VOC profiles. Within trichotecene-producers, *F. graminearum* and group A fungi differed on the structure of their volatile sesquiterpenes.
This methodology was also useful to predict the presence of *F. graminearum* in wheat cultivars, based on the detection of trichodiene (TRI), the volatile precursor of trichothecenes. TRI is a useful marker to detect toxigenic Fusarium in wheat spikes from live plants, regardless the actual development of Fusarium head blight (FHB) disease.

Código de Resumen: MS-019

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

DIVERSIDAD MICROBIANA Y PROCESOS DE CALCIFICACION EN MATAS MICROBIANAS PUSTULARES EN UNA LAGUNA DE ALTURA DE LOS ANDES (CATAMARCA, ARGENTINA)

MICROBIAL DIVERSITY AND CALCIFICATION PROCESSES IN A PUSTULAR MICROBIAL MAT IN A HIGH-ALTITUDE ANDEAN LAKE (CATAMARCA, ARGENTINA)

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Laguna Negra, in the Puna region of Argentina is a high-altitude hypersaline lake with extreme environmental conditions (high UV-radiation, temperature extremes, and salinity). High rates of evaporation result in mineral precipitation within an extensive microbial mat system. Microbial mats in the Laguna Negra display a variety of stratiform, pustular, and pinnacle morphologies. Biofilms are also present, coating both the subaqueous sedimentary substrate and mineralized components.

This study is focused on the dark colored pustular microbial mats. These mats are usually located in areas where the substrate is partially exposed, growing close to the air-water interface or partially subaerial, where desiccation and a high UV radiation influx are common. In order to record and understand calcification processes, we analyzed microbialites and microbial mats samples with epifluorecent microscopy, scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) in fresh and resin embedded samples. In addition we analyzed microbial diversity to recognize the main bacterial groups present in this site in order to evaluate their potential influence on carbonate precipitation. To achieve this we performed 16S rDNA pyrosequencing. Most of the environmental sequences obtained were affiliated to Proteobacteria, Verrucomicrobia, Bacteroidetes, Thermi, Cyanobacteria and to a lesser extent, Spirochaetes and Firmicutes. Between the bacterial groups here recorded, and those that are known to have some impact on carbonate precipitation, we can mention abundant Cyanobacteria and specially the filamentous Cyanobacteria *Rivularia* whichis predominant.

Rivularia can be encrusted by calcium carbonate (calcite) and can also be associated with sub-spherical aggregates. These aggregates are composed of Cyanobacteria and Diatoms (*Achnanthes brevipes, Halamphora, Navicula, Surirella Striatula*, etc), together with other bacterial groups, and are usually embedded in exopolymeric substances (EPS) where carbonate precipitation also takes place. Carbonate precipitation inside these aggregates is represented by nano-meter sized globular to spherical particles inside the EPS matrix.

It is still not clear if *Rivularia* filaments are active participants in the precipitation process or just passively entombed. Ongoing studies are focused on understanding this microbial diversity, specific metabolisms in active microbial mats, with an special focus on *Rivularia* Cyanobacteria and its potential impact on carbonate precipitation. This will allow us to better understand the geological record of *Rivularia*-like filaments in ancient examples.

Código de Resumen: MS-020

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

RECUPERACIÓN DE COBRE DE UN CONCENTRADO DE CALCOPIRITA USANDO LA ARQUEA TERMOACIDOFILA *Acidianus copahuensis*

COPPER RECOVERY FROM CHALCOPYRITE CONCENTRATE BY THE THERMOACIDOPHILIC ARCHAEON *Acidianus copahuensis*

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Bioleaching means the dissolution of metal sulfides (MS), such as pyrite (FeS_2) and chalcopyrite ($CuFeS_2$), catalyzed by microbial oxidation processes. Metals like copper, cobalt, zinc and nickel can be recovered from low-grade ores by bioleaching which is less contaminating and less expensive than the traditional alternatives in metallurgy. Chalcopyrite is the most abundant copper sulfide, but due to the refractory nature of this mineral bioleaching using mesophilic microorganisms shows low dissolution rates. This process can be strongly enhanced using thermophilic microorganisms.

Acidianus copahuensis is a new species of archaeon recently isolated from Copahue geothermal zone. One of the most striking characteristics of *Acidianus copahuensis* is its metabolic versatility. It grows on sulfur, tetrathionate, iron (II) and glucose under aerobic conditions, but it can also develop under anaerobic conditions. Due to the extremely metabolic versatility it is interesting to study its potential applications in bioleaching processes especially in the case of refractory sulfides like chalcopyrite. Precisely in this work we have studied the solubilization of metals (mainly copper) from a chalcopyrite concentrate by an *Acidianus copahuensis* strain.

With the aim of study the effects of different energy sources on the mineral bioleaching, several systems varying the culture medium composition were designed. Alternatively, one or more of the following compounds were used as energy source: sulfur, tetrathionate, iron (II), glucose and yeast extract. All cultures contained 2% (w/v) pulp density of mineral and were inoculated with cells of *Acidianus copahuensis*. Flasks were incubated at 65°C with shaking at 120 rpm during 110 days. Samples from supernatants were taken periodically and pH, copper, ferric and ferrous iron concentrations were determined. All inoculated systems tested in this study successfully leached the copper concentrate. In controls the amount of copper released from the mineral was very low and did not increase significantly over the time of the experiment. An enhanced oxidation activity of these microorganisms over the ore was detected in autotrophic conditions. After 110 days of the experiment, copper recoveries of 100% and 84% were obtained in the cultures without any additional energy source and with the supplement of tetrathionate, respectively.

Código de Resumen: MS-021

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

MICROORGANISMOS DEGRADADORES DE HIDROCARBUROS PRESENTES EN AGUAS DE SENTINAS DE BARCOS DEL PUERTO DE MAR DEL PLATA Y SU CAPACIDAD DE CRECIMIENTO EN DISTINTOS EFLUENTES

HYDROCARBON DEGRADING MICROORGANISMS FROM BILGE OIL WATERS OF MAR DEL PLATA PORT SHIPS AND THEIR GROWTH CAPACITY IN DIFFERENT SHIP WASTEWATERS

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Bilge oil water is a toxic effluent continuously generated by the ship operations. It has a high organic pollutant load, which mainly consists of hydrocarbons with different properties. Due to its toxicity and the lack of specific treatment in the port of Mar del Plata, the achievement of a suitable biodegradation process for in situ disposal is of fundamental importance. Therefore the development of a proper microbial flora able to degrade this waste is very important for environmental engineering and wastewaters treatment. The native consortiums of bilge oil water are presented as an appropriate source of microorganisms able to tolerate and degrade these compounds. To date, there are no previous studies in the city related to this type of liquid waste. Therefore, the objective of this work consisted of studying the microbial ecology of different bilge oil wastewaters to evaluate the diversity, abundance and distribution of microorganisms.

Bilge samples were taken from different vessels: marine dredger, coastal vessels and seagoing vessels. An enrichment of native microbial consortia was done in sterilized sea water with 0.5% of bilge oil water as a sole carbon source. For determination of the cultivable bacterial diversity, the surface spread technique was done in mineral medium with 30 μl of a 1:1 gas oil mixture (Pucci and Pucci, 2003). Plates were incubated at 25 °C for 21 days. The bacterial counts values were analyzed according to Pucci et *al.* (2009).

The bilge from seagoing vessels present the most abundance of the microorganisms with 70% of the total colony count, possibly because this residue contains more sea water and waste from fish. Applying the diversity index by Shannon-Wiener, the bilge of the marine dredge presented the highest diversity of organisms (H = 0.85), followed by the bilges from coastal vessels. Relative to the species presented, the relative abundance index showed differences in the presence of species for each of the bilges.

The residue from the marine dredge provided 72% of D1, the sea going vessels pressented 93% of D4 and the coastal vessels showed similar abundances for species A2 and A3. The 7 isolated species presented in the plates were sent to be sequenced and is waiting for the corresponding results. A batch reactor was stabilized to study de degradation rate. A DNA extraction of the stabilized microflora in the stationary phase was performed with the corresponding amplification and sequencing of 16S by the Sanger method. The presence of strains of *Pseudomonas stutzeri*, *Kocuria rosea Kocuria sp*. and *Dietzia maris sp* was observed.

Código de Resumen: MS-022

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

DIVERSIDAD MICROBIANA EN UN SEDIMENTO ANAERÓBICO EXTREMO DEL SISTEMA GEOTERMAL DE CAVIAHUE-COPAHUE, NEUQUEN, ARGENTINA

MICROBIAL DIVERSITY IN AN EXTREME ANAEROBIC SEDIMENT FROM GEOTHERMAL CAVIAHUE–COPAHUE SYSTEM, NEUQUÉN, ARGENTINA

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The geothermal Copahue system is an extreme environment located in the north-west of Neuquén province, Argentina, at the foot of Copahue volcano. The area is characterized by a wide range of temperatures and pH values and the presence of heavy metals. Copahue is receiving an increasing amount of research within different areas like biogeochemistry, biotechnology (including bioleaching and bioremediation processes) and microbiology. Most work has been focused on aerobic, acidophilic sulfur- and iron-oxidizing bacteria and archaea. Relatively few studies have investigated the anaerobic zones of this environment. In this work we have examined the bacterial diversity focused on anaerobic prokaryotes in a sample taken in February 2012 in the hot spring sediment placed in Las Maquinitas (LMi) using a combination of molecular and cultivation techniques. Physicochemical parameters of Las Maquinitas sample were measured in situ: 90°C, pH 5, and a redox potential (EH) of -126 mV.

The bacterial diversity of the sample was analyzed by a 16S rRNA gene bacterial clone library. The sequences obtained were compared with those in NCBI database using BLAST, and the phylogenetic affiliations of the clones determined by the ARB software package and using the Classifier tool of the Ribosomal Database Project (RDP). Several thermotolerant mesophilic and acidophilic bacteria were identified in this clone library and most of them are involved in the sulphur and iron cycles.

Although PCR- based methods are not quantitative, *Acidithiobacillus caldus* seems to be the most abundant species found in the sample. These microorganisms can grow autotrophically – and mixotrophically- using different sulphur compounds. This microorganism has been isolated from other geothermal sites and acid mine drainages. *Thiomonas intermedia* was also identified in LMi sample. Members of the genus *Thiomonas* are moderate acidophiles that oxidize reduced sulphur compounds.

Other clones represented by *Alicyclobacillus tolerans* and *Sulfobacillus thermotolerans* were closely related to *Firmicutes* phylum. Among other characteristics, these bacteria are able to grow lithotrophically using iron (II) ions and/or sulphur compounds and/or heterotrophically using different organic substances. Most of them can form endospores which contribute to their resistant to high temperature values. Members of the genus *Alicyclobacillu*s can also grow under facultative anaerobic conditions through iron (III) reduction. Other genera like *Acidiphilum*, *Erythrobacter*, and *Thermoanaerobacterium* were represented by only one clone. Although the negative EH of the environmental sample suggested the presence of

sulphate-reducing bacteria, culture-independent techniques did not detect them. However, a mesophilic, spore-forming sulphate-reducerrelated to *"Desulfobacillus acidavidus"* strain CL4 (99% sequence similarity) could be isolated from enrichment cultures at 30°C.

TALLER: MICROBIOLOGÍA AMBIENTAL, UNA MIRADA ACTUAL

ALTERNATIVAS DE PROCESOS Y REDUCCIÓN DE CONTAMINANTES ORGÁNICOS EN EL PUERTO DE MAR DEL PLATA

Dra. Silvia E. Murialdo

El puerto costero de la ciudad de Mar del Plata está ubicado en el sudeste de la Provincia de Buenos Aires, Argentina. Se empezó a diseñar a fines del siglo XIX y se inauguró en 1924. Es un puerto artificial encerrado por dos importantes escolleras, comprendiendo dos sectores: el sector Sur de carácter comercial y el sector Norte donde operan las flotas pesqueras. Si bien es un puerto netamente pesquero, el transporte de petróleo y cereales junto con actividades turísticas constituyen también actividades importantes.

El puerto de Mar del Plata está integrado a la ciudad afectando la vida urbana en forma positiva, como promotor del desarrollo del comercio, pero también es "víctima pasiva" de la contaminación urbana e industrial. Los principales contaminantes que han sido detectados en el puerto Marplatense son los derrames de sentina, los desechos industriales y el polvillo atmosférico. La industria de la harina de pescado genera desechos conteniendo un alto grado de proteínas, grasas y soda cáustica que terminan vertidos en las cloacas marplatenses y directamente en el mar. El límite de Sólidos Solubles en Éter Etílico (SSEE) permitidos en Kg/día son de ≤100 para el vertido en cloacas y de ≤ 50 para la descarga a mar abierto. Durante el 2010 se reportó un SSEE de 779,57 proveniente de las harineras de pescado en el puerto marplatense (*González et al., 2010*). Además existe una contaminación por Hidrocarburos Policíclicos Aromáticos (PAHs, por sus siglas en inglés), considerados de alto riesgo debido a su potencial efecto carcinogénico. Los niveles detectados de PAHs, superiores a 12 µg/l, constituyen un problema que se percibe en primera instancia en la absorción de estos hidrocarburos por animales marinos, especialmente en bivalvos que se crían en áreas próximas a la orilla (*Bernatené et al., 2012*). Además se han detectado hidrocarburos alifáticos, plaguicidas organoclorados y bifenilos policlorados tanto en el agua como en sedimentos (*Colombo et al., 2013*).

Dentro de las iniciativas desplegadas por el puerto de la región se destaca una preocupación por la mejora de la calidad de las aguas, de la calidad del aire, gestión de los residuos de operaciones portuarias, adecuación de los dragados, y fundamentalmente promoción de la importancia del tema en cada una de las comunidades portuarias.

En este taller se realizará un análisis de la problemática de la región, se focalizará en los problemas de impacto ambiental como son la carga orgánica pesquera y los hidrocarburos de barcos, y se analizarán probables técnicas de bioremediación para disminuir la contaminación, o evitarla directamente, aplicando la legislación vigente sobre residuos peligrosos.

MESA REDONDA-ACTIVIDAD INTEGRADORA

Temario de discusión

1. Identificación de puntos críticos actuales y futuros
2. Mitigación y prevención a corto y largo plazo
3. Técnicas de diagnóstico, monitoreo y remediación específicos
4. Extrapolación a situaciones similares
5. Implementación de un Sistema de Gestión Ambiental (SGA)

Código de Resumen: MM-001

Sección: Microbiología Molecular

Modalidad: Oral

SEÑALIZACIÓN INDEPENDIENTE A TRAVÉS DE COMPLEJOS DE QUIMIORECEPTORES SEPARADOS EN *Escherichia coli*

INDEPENDENT SIGNALING THROUGH SEPARATED CHEMORECEPTOR ARRAYS IN *Escherichia coli*

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Bacterial chemotaxis allows microorganisms to move following chemical gradients in order to find optimal conditions to live in. The information from the environment is transmitted through the bacterial chemoreceptors that, interacting with CheA kinase and CheW coupling protein, are able to translate the signals into changes in the rotation of flagellar motors.

Chemoreceptors usually have an extracellular ligand binding domain and a highly conserved cytoplasmic domain that consists in an alpha-helical hairpin and forms in the homodimer a coiled-coil four-helix bundle. *E. coli* chemoreceptors from different specificities form mixed trimers of dimers that are assembled together in a stable array and signal in a collaborative fashion. Symmetric insertions/deletions in both arms of the hairpin that occurred during evolution originated chemoreceptors that differ in the length of their cytoplasmic domain and can be grouped into seven different families.

Many bacterial species code for chemoreceptors that belong to two or more different classes, but how these receptors are organized in the cell remains as an open question. To analyze the behavior of cells expressing chemoreceptors of different families, we engineered Tsr, the serine chemoreceptor of E. coli, through the introduction of symmetric 14-residue insertions. After random mutagenesis of this construct we obtained two functional Tsr derivatives with a significantly longer cytoplasmic domain. The aim of this work was to analyze the organization and signaling abilities of two chemoreceptors of different classes when they are co-expressed in the same cell.

Functional analyses of the Tsr derivatives showed that they were able to control CheA in response to serine and localized to the poles of the cells as determined by fluorescent microscopy with CheZ-YFP and CheA-YFP analysis. Crosslinking experiments showed that the longer Tsr does not form mixed trimers of dimers with native Tar (aspartate sensing) *E. coli* chemoreceptor. Fluorescence anisotropy analysis further suggests that the longer Tsr does not incorporate efficiently in the same cluster with native Tar. The responses to serine and aspartate in cells co-expressing both receptors were analyzed by a FRET-based *in vivo* assay. Whereas cells expressing native Tar and Tsr signal collaboratively in response to aspartate and serine, cells expressing long Tsr and native Tar show independent responses to their ligands, suggesting that the two receptors of different lengths assemble into separate complexes that signal independently.

Our results indicate that bacteria coding receptors from different families might sort them into different complexes, spatial or temporally separated from each other.

Código de Resumen: MM-002

Sección: Microbiología Molecular

Modalidad: Oral

CARACTERIZACIÓN FUNCIONAL DEL TRASNPORTADOR CDF SMC02724 (SMYIIP) EN *Sinorhizobium meliloti*: ROLES EN LA HOMEOSTASIS DE MANGANESO Y NODULACIÓN

FUNCTIONAL CHARACTERIZATION OF THE CDF TRANSPORTER SMC02724 (SMYIIP) IN *Sinorhizobium meliloti*: ROLES IN MANGANESE HOMEOSTASIS AND NODULATION

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In bacteria membrane transporters of the cation diffusion facilitator (CDF) family participates in Zn^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} homeostasis. Functional roles during infection processes for CDFs have been shown and these are linked to the CDF specificity of transport. *Sinorhizobium meliloti* has two homologous CDF genes with unknown transport specificity. Here we evaluated the role played by the CDF SMc02724 (SmYiiP). The deletion mutant strain of SmYiiP (Δ*smyiiP*) showed a reduced *in vitro* growth fitness only in presence of Mn^{2+} .Incubation of Δ*smyiiP* and WT cells with sub-lethal Mn^{2+} concentrations resulted in 2-fold increase of the metal only in the mutant strain. Normal levels of resistance to Mn^{2+} were attained by complementation with the gene SMc02724 under regulation of its endogenous promoter. Supporting the role of SmYiiP as a Mn^{2+} exporter, liposomes with incorporated heterologous expressed pure protein accumulated Mn^{2+}. Additionally, Co^{2+} and Ni^{2+} transport was detected. Nodulation assays in alfalfa plants showed that the strain Δ*smyiiP* induced a lower number of nodules compared to plants infected with the WT strain. Our results indicate that Mn^{2+} homeostasis in *S. meliloti* is required for full infection capacity, or nodule function, and that the specificity of transport *in vivo* of SmYiiP is narrowed down to Mn^{2+} by an unidentified mechanism. Further studies are required to identify these processes.
This work was supported by the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET-G111220090100063)(Subsidio extraordinario).

Código de Resumen: MM-003

Sección: Microbiología Molecular

Modalidad: Oral

VIVIENDO SOBRE UNA SUPERFICIE: DESARROLLO DE BIOFILMS Y COMUNIDADES MULTICELULARES DESLIZANTES EN *Bacillus subtilis*

LIVING ON A SURFACE: BIOFILM DEVELOPMENT AND MULTICELLULAR SLIDING COMMUNITIES IN *Bacillus subtilis*

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Bacteria colonize surfaces in various ways. When growing on semi-solid surfaces, *Bacillus subtilis* shows swimming, swarming, and sliding motility depending on the agar concentrations. While swimming and swarming depend on the activity of flagella, sliding is a passive surface translocation and does not require an active motor. On medium with higher agar concentration *B. subtilis* forms architecturally complex colonies. Several regulators were described to affect swimming and swarming motility behaviors or biofilm formation in *B. subtilis*. Sliding motility and biofilm formation

of *B subtilis* Natto are affected by the agar concentration used and depends on Spo0A, the global transcription regulator of sporulation. Spo0A integrates environmental signals related to starvation or stress conditions and activates various developmental pathways. Examination of strains with reduced biofilm structure formation in *B. subtilis* resulted in the identification of *bslA* gene required for biofilm development. BslA is a small secreted protein that forms a hydrophobic layer on the surface of *B. subtilis* biofilms and increases liquid repellency. Transcription of *bslA* is regulated by several global regulators and shows a spatiotemporal expression pattern during the development of complex colonies. Interestingly, we detected altered expression of *bslA* gene next to the genes related to biofilm formation in our microarray experiments where we examined the sliding behaviour of *B. subtilis* Natto and B. subtilis Marburg strains under sliding restrictive compared to permissive conditions (using *spo0A* mutant strain or higher agar concentration). Introduction of the *bslA* mutation into *B. subtilis* reduced sliding motility. Further, our experiments show that the production of exopolysaccharide is also needed for sliding of *B. subtilis*, while the protein component of the biofilm matrix, the amyloid fibers and the presence of flagella are not required for sliding. BslA, therefore next to protecting the biofilm community against various stresses, contributes to surface spreading. Our results point to presence of shared regulators (some of them presented herein) and genes for distinct surface-dependent growth of *B. subtilis*.

Código de Resumen: MM-004

Sección: Microbiología Molecular

Modalidad: Oral

ANÁLISIS GENÓMICO DEL CLUSTER BIOSINTÉTICO DE VITAMINA B_{12} (COBALAMINA) EN *Lactobacillus coryniformis* CRL 1001

GENOMIC ANALYSIS OF VITAMIN B12 (COBALAMIN) BIOSYNTHETIC CLUSTER IN *Lactobacillus coryniformis* CRL 1001

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The vitamin B12 (cobalamin-CBL), a very complex non-polymeric macromolecule synthesized only by some bacteria and archaea, and essential to humans and animals. The highly complicated CBL biosynthesis involves about 30 synthesis steps.
We demonstrated that cell extract of *Lactobacillus coryniformis* CRL 1001 is able to correct the coenzyme B12 requirement of *Salmonella enterica* serovarTyphimuriumin minimal medium. The aim of this study was the sequencing of CRL1001 genome and the molecular characterization of CBL biosynthesis in this strain.
L. coryniformis CRL 1001 genome was sequenced by a whole-genome shotgun (WGS) strategy with an Ion Torrent personal genome machine based upon libraries created using NEBNext DNA library kits. Genomic analysis was done using the RAST annotation Server, Blast algorithms, ISGA and KEGG databases.The draft genome sequence consists of 2,829,178 bases with a mean GC content of 42%. A total of 3,341 coding sequences (CDS) and 82 structural RNAs (58 tRNAs) were predicted.
RAST analysis evidenced the presence of at least 30 genes (*cob* genes) involved in the CBL biosynthesis in CRL 1001 strain. This finding is the first evidence for cobalamin biosynthesis genes in this species.
Comparative studies among vitamin B12 producer strains demonstrated that the genetic organization of *cob* operon is conserved in this strain and these genes are adjacent to the *pdu* operon and *pocR* gene. The *hem* genes (*hem A, C, B y L*) present in *L. coryniformis* CRL 1001 genome are located among *cob* operon in similar way to anaerobic microorganisms. Interestingly, the *cblT* y *cblS* genes were identified in CRL1001 strain genome. These genes encode a putative protein kinase and a α-ribasol transporter, respectively. The *cblT* y *cblS* genes are present in CBL-producers *Listeria sp.* strains but absent in CBL-producers *L. reuteri* strains.
The knowledge of *cob* genes and their regulation in vitamin producer lactic strains constitutes an interesting biotechnological alternative for developing fortified foods.

Código de Resumen: MM-005

Sección: Microbiología Molecular

Modalidad: Poster

LA EXPRESIÓN DE PROTEÍNAS ÓXIDO NÍTRICO SINTASAS DE ORGANISMOS FOTOSINTÉTICOS CONFIERE UNA RÁPIDA ADAPTACIÓN A CONDICIONES DE DEFICIENCIA DE NITRÓGENO EN *Escherichia coli*

EXPRESSION OF NITRIC OXIDE SYNTHASES FROM PHOTOSYNTHETIC MICROORGANISMS CONFERS RAPID ADAPTATION TO LOW NITROGEN GROWTH CONDITIONS IN *Escherichia coli*

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Nitric oxide (NO) is a free radical involved in several physiological processes in all living organisms. NO is synthesized by nitric oxide synthases (NOS) from the substrate L-arginine (L-Arg). Our laboratory characterized a NOS from the marine unicellular algae *Ostreococcus tauri* (OtNOS, Foresi et al. 2010), the first NOS belonging to the plant kingdom. Later, through bioinformatics analysis we found a sequence coding for a putative NOS in another photosynthetic organism, the cyanobacteria *Synechococcus* PCC 7335 (SyNOS). The full length sequences of SyNOS and OtNOS were cloned and expressed in *Escherichia coli.* The recombinant proteins were detected in cells by western blotting after 2 h of induction with IPTG. Bacteria expressing the recombinant NOS in LB medium reach a higher OD at stationary phase than cells transformed with the empty vector (EV). The cell cultures expressing *OtNOS* and *SyNOS* genes show higher levels of NO production and the expression of the NO-sensor *hmp* gene confirmed the increased levels of NO in the transformed bacteria. Additionally, cultures of *E. coli* expressing the recombinant OtNOS and SyNOS growing in deficient nitrogen (N) media display similar growth rates to those attained in sufficient N conditions. It is postulated the participation of NOS activity and its product NO, in the N metabolism of the bacteria. The molecular mechanism responsible for the NOS ability of conferring enhanced adaptation to N starvation is currently under study.
Supported by UNMdP, CONICET and ANPCyT Foresi et al. (2010) Plant Cell 22:3816-3830

Código de Resumen: MM-006

Sección: Microbiología Molecular

Modalidad: Poster

MUTACIONES EN LA PROTEÍNA ACOPLADORA CheW APOYAN SU ROL ACTIVO EN LA TRANSMISIÓN DE LA SEÑAL PARA QUIMIOTAXIS

MUTATIONS IN THE COUPLING PROTEIN CheW SUPPORT ITS ACTIVE ROLE IN SIGNAL TRANSMISSION FOR CHEMOTAXIS

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The chemotaxis system in bacteria allows controlled movement in response to chemical gradients. Chemoreceptors transmit ligand-binding information to an associated histidine kinase, CheA, within a ternary complex that also contains the coupling protein CheW. CheA autophosphorylates and donates its phosphoryl group to the response regulator CheY, which then diffuses to the flagellar motors to control their mode of rotation. Attractants inhibit CheA phosphorylation, decreasing the levels of phosphorylated CheY.
CheW binding interactions with receptors and with CheA are important for the formation of ternary complexes that respond to chemotactic stimuli. However, it is not clear whether the role of CheW is

limited to bridge the two proteins and thus contribute to the assembly of the large chemoreceptor clusters at the cell poles, or whether it plays an active role in signal transmission through stimuli-mediated conformational changes.
In this work, we introduced mutational replacements in CheW, located at the contact surface with chemoreceptors, and analyzed the signaling consequences of such changes.
We identified five CheW mutant proteins that failed to mediate chemotaxis responses towards serine, as determined in soft agar swimming assays. Two of them coded for proteins that were unable to activate the CheA kinase, in spite of showing expression levels comparable to wild-type CheW. Two other replacements generated CheW proteins that activated the kinase irrespective to the presence of attractant, indicating that the signaling complex in these cells was locked in the "on" state. Moreover, one of the mutant proteins, that displayed a significant truncation of the protein, also behaved as a "lock-on" mutant, indicating that the missing portion of the protein was dispensable to form kinase-activating complexes.
Taken together, these results support an active role for the coupling protein CheW in signal transmission.

Código de Resumen: MM-007

Sección: Microbiología Molecular

Modalidad: Poster

DETERMINACIÓN DE LA ESPECIFICIDAD DE QUIMIORRECEPTORES DE *Rhodobacter sphaeroides* USANDO CÉLULAS DE *Escherichia coli*

DETERMINATION OF *Rhodobacter sphaeroides* CHEMORECEPTORS SPECIFICITY IN *Escherichia coli* CELLS

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Chemotaxis is an adaptive behavior that allows bacteria to find optimal conditions for survival. This is achieved through a signal transduction pathway that detects chemical gradients and transmits the information to the flagellar motors in order to control navigation in preferred directions. The pathway is conserved among Bacteria and Archaea and relies in the detection of stimuli by chemoreceptors that typically possess an extracellular ligand binding domain and a highly conserved cytoplasmic signaling domain. They form large polar complexes based in interactions between each other, the coupling protein CheW and the histidine kinase CheA, whose activity is controlled in response to ligand binding.
Bacterial genomes code for variable numbers of chemoreceptors, ranging from very few to up to fifty. In most cases, very little is known about individual receptors, which are expected to sense specific ligands through their highly variable extracellular domain. Given the conservation of the signaling domain we hypothesized that it might be possible to use *E. coli* as a reporter strain in order to study their specificity.
The aim of this work was to find out whether chemoreceptors from *Rhodobacter sphaeroides* are able to form complexes with *E. coli* chemotaxis proteins and control the activity of CheA in response to ligands.
Rhodobacter sphaeroides is a α-subgroup, purple nonsulfur, photosynthetic bacterium. It codes for thirteen chemoreceptors of unknown specificity. We chose two receptors from *R. sphaeroides*, McpH and McpB, and expressed them in an *E. coli* strain lacking native receptors.
Both receptors were able to form signaling complexes and drive polar localization of a CheA-associated phosphatase fused to YFP. Moreover, these complexes were functional, as they were able to generate clockwise rotation of flagella, which is dependent on CheA kinase activity. Using the in vivo flagellar rotation assay, the cells were challenged with different ligands. Complexes containing McpB or McpH as the only receptor did not show any response to aminoacids or sugars. However, they did respond to the addition of organic acids. Cells expressing McpB inhibited the kinase in response to all organic acids tested; including lactate, pyruvate, acetate, citrate and succinate, indicating that this receptor mediates attractant responses to these stimuli. In contrast, only lactate, pyruvate and acetate mediated responses in cells expressing McpH; and these responses were of repellent type.

Moreover, in cells co-expressing a serine receptor from *E. coli* and McpB, responses to the specific ligands showed collaborative signaling. These results indicate that foreign receptors are capable of assembling functional signaling complexes with *E. coli* proteins, providing a useful assay to identify their cognate ligands.

Código de Resumen: MM-008

Sección: Microbiología Molecular

Modalidad: Poster

IDENTIFICACIÓN DE NUCLEÓTIDOS RELEVANTES PARA LA REGULACIÓN POR NITRÓGENO DE LA EXPRESIÓN DEL sRNA SM8 EN *Sinorhizobium meliloti*

MUTATIONAL ANALYSIS IDENTIFIES CRITICAL PROMOTER NUCLEOTIDES INVOLVED IN NITROGEN CONTROL OF SM8 sRNA EXPRESSION IN *Sinorhizobium meliloti*

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Small regulatory non-coding RNAs (sRNAs) are key players in post-transcriptional regulation of gene expression in prokaryotes. Hundreds of transcripts and sRNA genes have been identified in *Sinorhizobium meliloti*, the nitrogen-fixing symbiont of alfalfa root nodules, but their biological function remains unknown for most of them. In our lab, we have identified the sRNA gene *sm8* encoding an 80-nt transcript that accumulates in stationary phase and that achieves higher cellular levels in minimal rhizobial defined medium (RDM) than in complex tryptone-yeast (TY) medium. In order to understand the factors controlling expression of the *sm8* gene, we constructed *S. meliloti* strains bearing a chromosomal P*sm8-gfp* transcriptional reporter fusion. For comparative purposes, transcriptional *gfp* fusions were also generated under the control of three other sRNA gene promoters (P*sm12,* P*sm26,* P*sm145*) identified in our lab, and under the control of an mRNA promoter (PSmc01852) regarded as a constitutive reference gene for microarray and qRT-PCR analyses. Our results showed differential regulation depending on the complexity and/or availability of the N source (activation by NO_3^- repression by NH_4^+ or amino acids), suggesting that these sRNAs would be involved in the regulation of nitrogen metabolism in *S. meliloti* strain 2011. Interestingly, we have detected the presence of a conserved sequence motif in all studied promoters which coincides with a novel *S. meliloti* promoter consensus recently identified by RNAseq (“Motif 1”; Schlüter et al 2013), which may explain their similar regulatory pattern. Additionally, multiple sequence analysis of these promoters and their homologues in other alpha-proteobacteria, allowed identification of nucleotide positions that are strictly conserved just downstream of the -35 element of the *sm8* gene (“-26 box”), but not within the rest of the studied promoters. Moreover, this conserved stretch is almost identical (6 out of 7 bases) to the recognition sequence reported for the Nitrogen regulator protein NtrC. Based on these sequence findings, we carried out point mutagenesis within either the -10 promoter element or the -26 box. Replacement of the conserved “-26 box” resulted in a complete loss of P*sm8* activation by the presence of NO3- as N source, suggesting that the conserved -26 box may be the binding site of a transcriptional activator. Complementary genetic approaches (i.e., the use of *S. meliloti* mutants affected in N-regulatory proteins and generalized random mutagenesis with Tn*5* to identify mutants that lost P*sm8* expression) are underway to provide additional support to our hypothesis.

Código de Resumen: MM-009

Sección: Microbiología Molecular

Modalidad: Poster

ANÁLISIS DE LA SECUENCIACIÓN PROFUNDA DEL RNA TOTAL EN LA BACTERIA ANTÁRTICA *Pseudomonas extremaustralis* EN RESPUESTA A BAJAS TEMPERATURAS

DEEP RNA-SEQUENCING ANALYSIS OF THE ANTARCTIC BACTERIUM *Pseudomonas extremaustralis* IN RESPONSE TO LOW TEMPERATURE

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In cold environments low temperatures and ice presence exerts severe constraints on living organisms and those able to survive present several adaptations to cope with these unfavorable conditions. Environmental adaptability involves different physiological and genetic strategies including the response of individual genes or operons and complex regulatory networks that coordinate the control of several genes. RNA-deep sequence is a powerful tool in bacterial species to analyze gene expression, discover previously unannotated genes and detect small regulatory RNA. We used RNA-seq technology in *Pseudomonas extremaustralis*, an Antarctic bacterium able to grow under low temperatures and survive to freezing. Total RNA was extracted from cultures grown at 30oC and 5oC and the rRNA was depleted from the samples to allow a better coverage. Directional libraries were prepared with ScriptSeq v2RNA-Seq Library Preparation Kit (Epicentre) and were sequenced using the Illumina X HiSeq2000 platform with a paired-end protocol and read lengths of 100 nt. For each condition duplicated independent RNA extraction and libraries were used. Bioinformatics analysis of around 6000 transcripts expression levels in both conditions using Rockhoper software showed that the majority of transcripts did not present a statistical significant change in the expression level. Down-regulated genes were around 750 and included cytochromes and enzymes belonging to different catabolic pathways. Interestingly, 76 genes were up-regulated under cold conditions including several lipoproteins, transcriptional regulators with unknown function, alginate biosynthesis activator, osmotic response elements and other cellular functions. We also detected several unnanotated transcripts in both conditions. Additionally, we have found around 170 putative non-coding small RNAs. Some of them presented altered expression levels under low temperatures with unknown function. The results showed a novel variety of transcripts and regulatory elements that could explain growth and survival under low temperatures in this psycrotolerant bacterium.

Código de Resumen: MM-010

Sección: Microbiología Molecular

Modalidad: Poster

DETECCIÓN DE *Paenibacillus larvae* MEDIANTE PCR EN TIEMPO REAL A PARTIR DE ESPORAS PROVENIENTES DE ESCAMAS

REAL-TIME PCR DETECTION OF *Paenibacillus larvae* DNA FROM SPORES OF SCALE SAMPLES

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The Gram-positive bacterium *Paenibacillus larvae* is a major pathogen of *Apis mellifera*, causing the disease known as American foulbrood. This condition affects the larval stage and causes, as a final step desiccation of the larvae leaving only a scale, which contains millions of bacterial spores. Spores of the microorganism initiate the infectious stage and are the major vectors for the spread of the disease. The delay in diagnosis causes the collapse of infected hives, therefore the development of a fast and reliable method of detection will be of great help to prevent the spread of the disease. The objective of this work was to develop a real-time PCR methodology for the detection of *P. larvae* DNA from spores from scales samples.
Methodology: Validation of real-time PCR reactions for the detection of *P. larvae* DNA was performed with DNA extracted from pure cultures from Arthropods Laboratory strain collection, with primers that amplify a 380 bp fragment of the bacterial 16S rRNA sequence. A methodology for DNA extraction from scales was optimized by using the commercial kit Multisource Genomic DNA Miniprep AxyPrep. To verify the success of DNA extraction from the samples and lack of inhibition in the PCR reactions DNA amplifications of *A. mellifera* beta actin gene were performed. In those suitable samples (beta actin Ct values < 35) PCR reactions for detection of *P. larvae* were carried out using EvaGreen as fluorescent intercalating dye, in a final volume of 20 µl. Detection of the amplified product was monitored on a Rotor Gene Q thermocycler. Results: It was possible to apply a real-time PCR method for the detection of *P. larvae* DNA from bacterial isolates and also in scale samples. Thus, the real-time PCR in a few hours could determine the presence or absence of *P. larvae* DNA in isolates and scale samples. This is the first report of *P. larvae* detection by real-time PCR directly from scales.

Código de Resumen: MM-011

Sección: Microbiología Molecular

Modalidad: Poster

CARACTERIZACIÓN DE UN MUTANTE EN LA GLUTAMINA SINTETASA II EN *Bradyrhizobium diazoefficiens* USDA110

CHARACTERIZATION OF A GLUTAMINE SYNTHETASE II MUTANT IN *Bradyrhizobium diazoefficiens* USDA110

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Bradyrhizobium diazoefficiens exists as a free-living organism, growing at the expense of soil nitrogen, or as a symbiont, reducing dinitrogen to ammonia for itself and its soybean host. Particularly, in free-living rhizobia,N assimilation proceeds through the high-affinity glutamine-synthetase (GS) - glutamate-synthase (GOGAT) cycle. Bacteria of the *Rhizobiaceae,* including *Bradyrhizobium diazoefficiens* USDA110, the subject of this study, are distinct from other bacteria in that they have at least two isoforms of GS: GSI y GSII. The most striking difference between GSI (encoded by *glnA*) and GSII (encoded by *glnII*) is how these enzymes are controlled. The activity of GSI is altered post-translationally by adenylylation but the level of the protein remains relatively constant. In contrast, GSII synthesis is under the control of the NtrBC system that responds to the nitrogen and carbon sources. Both control systems depend on the relative amounts of intracellular α-cetogutarate and glutamine. A high α-cetoglutarate/glutamine ratio, signal of N limitation, activates a Nitrogen Stress Response cascade promoting deadenylylation of GSI and the synthesis of GSII to increase NH_4^+ assimilation. These two control levels operating on different GS enzymes might help the rizobia become highly efficient for scavenging, in their natural environments, the low levels of N that are needed to establish a productive nitrogen-fixation symbiosis. In order to advance in this topic we generated a mutant in *glnII*, LP4169 strain, by a double homologous recombination strategy. The first step to characterize this mutant strain was analyzing the growth in Evans media with a low concentration of N source (0.1 µM of NH4Cl) and in Evans cero nitrogen (N_0,0.02 µM of NH4Cl). In both cases it was found that there was no significant difference in the OD500nm between LP4169 and the wild type (WT) strain. Nevertheless, when the colony forming unit per milliliter (cfu/ml) were analyzed, it was found that at stationary state (after 7 days of growth) LP4169 reduces drastically the cfu/ml and then (at the 11^{th} day approximately) the number of cfu/ml increases again. This happens both in low N and N_0 media so, taking this into account, it could be thought that the mutant is not able to survive in a proper way, as the WT strain does, during stationary state. To increase our results, the growth of LP4169 in different N sources like nitrate and glutamate (0.02%) was studied. Unfortunately, no differences could be noticed in the growth between the mutant and the WT strain. Finally, we studied GS activity through the g-glutamyl transferase assay. The results showed that total GS activity was the same for both WT and mutant strains but GSI activity was lower in LP4169 strain. These results could suggest that another enzyme is involved in this system but we need to carry on more studies to elucidate it.

Código de Resumen: MM-012

Sección: Microbiología Molecular

Modalidad: Poster

DESARROLLO DE BIOINSECTICIDAS BASADOS EN AISLAMIENTOS NUEVOS DE *Bacillus thurigiensis* PARA EL CONTROL DE INSECTOS PLAGA

DEVELOPMENT OF BIOINSECTICIDES BASED IN NEW ISOLATES OF *Bacillus thurigiensis* TO PLAGUE INSECT CONTROL

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The massive use of synthetic chemical agents of biocontrol (i.e. insecticides) with potential toxicity to the users and the environment pushes for the development of alternative (ecological and natural) compounds. To this respect, the use of natural and human-friendly bacteria with insecticide activities (biocontrol agents) represents an interesting alternative. The leading biorational pesticide, *Bacillus thuringiensis*, is a ubiquitous Gram-positive, spore-forming bacterium that forms a parasporal crystal during the stationary phase of its growth cycle. *B. thuringiensis* was initially characterized as an insect pathogen, and its insecticidal activity was attributed largely or completely (depending on the insect) to the parasporal crystals. This observation led to the development of bioinsecticides based on *B. thuringiensis* for the control of certain insect species among the orders Lepidoptera, Diptera, and Coleoptera. *B. thuringiensis* is already a useful alternative or supplement to synthetic chemical pesticide application in commercial agriculture, forest management, and mosquito control. In this research, we isolated 119 novel *B. thuringiensis* strains from 57 agricultural soils samples that were taken from the provinces of Buenos Aires, Santa Fe and Cordoba (major regions of soybean, corn and wheat explotations). Each isolate was characterized by crystal protein production with light/phase-contrast microscopy; evaluation of toxicity against Lepidopteran insect pests; and finally the 16S ribosomal subunit genes of the selected isolates were sequenced. The presence or absence of specific *cry* genes was associated with the observed average larval mortalities. From the 119 isolates, ten (8.4 %) were potentially able to control *Spodoptera frugiperda* larvae and five were potentially able to control *Rachiplusia ñu*. Some of these isolates display toxic potential and, therefore, could be adopted for future applications to control some agriculturally important insect pests in the scope of integrated pest management for sustainable agriculture.

Código de Resumen: MM-013

Sección: Microbiología Molecular

Modalidad: Poster

LOS GENES DE BIOSÍNTESIS DE PQQ RESULTAN ESENCIALES PARA EL CRECIMIENTO EN FRÍO EN *Pseudomonas extremaustralis*

PQQ BIOSYNTHESIS GENES ARE ESSENTIAL FOR GROWTH UNDER COLD CONDITIONS IN *Pseudomonas extremaustralis*

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Cold environments constitute stressing habitats that limit bacterial survival and colonization of new ecological niches due to the effect of unfavorable conditions. Exposure to cold conditions provokes changes in solubility, reaction kinetics, membrane fluidity, degradation, stability and conformation of proteins and gene expression so bacteria that live in such conditions must have some physiological adaptations. *Pseudomonas extremaustralis* is an Antarctic bacterium capable of

growing at low temperatures with high stress resistance in association with the accumulation of large amounts of polyhydroxybutyrate (PHB). Screening of a mini Tn5 library allowed the detection/identification of a clone carrying a mutation in *pqqB* gene that was unable to grow under cold conditions. Pyrroloquinoline quinone (PQQ) is as cofactor of several enzymes and the proteins involved in its biosynthesis are encoded in the *pqqABCDE* cluster. The *pqqB* mutant strain was unable to grow and survive when exposed to low temperatures and freezing conditions, but presented a high growth rate at 28°C in comparison with the wild type strain. In addition, a significant increase in oxygen consumption was observed in the *pqqB* strain. The expression of *pqqB* gene in the wild type strain did not show significant differences between 28°C and 10°C. PQQ has been also proposed as a reactive oxygen species (ROS) scavenger. As an increase of ROS has been reported at cold conditions, experiments were conducted in order to assess if the cold sensitive phenotype of the *pqqB* strain was related to oxidative stress. Sensitivity to H_2O_2 was measured by a disk inhibition assay for the wild type and the *pqqB* strain grown at 28°C. The resistance to H_2O_2 was similar in both strains. Moreover, we tested growth at 10°C for both strains under microaerobic conditions and in the presence of antioxidant compounds. None of the tested conditions suppressed the cold sensitive phenotype, as the mutant was unable to grow in the presence of the antioxidant compounds or low oxygen tension at 10°C. Our results demonstrate that *pqqB* gene is essential for growth and survival under low temperatures, and that this phenotype does not seem to be associated with an increase of oxidative stress. However, the molecular mechanism involved in this novel *pqq* phenotype associated with cold sensitivity remains to be elucidated.

Código de Resumen: MM-014

Sección: Microbiología Molecular

Modalidad: Poster

Serratia marcescens PUEDE INVADIR Y PERSISTIR DENTRO DE CÉLULAS FAGOCÍTICAS

Serratia marcescens IS ABLE TO INVADE AND PERSIST INSIDE PHAGOCYTIC CELLS

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Serratia marcescens is an opportunistic human pathogen that represents a growing problem for public health, particularly in hospitalized or immunocompromised patients. Despite its clinical prevalence, factors and mechanisms that contribute to *Serratia* pathogenesis remain unclear. *S. marcescens* produces numerous exoproteins with toxic effects, including ShlA hemolysin that has been catalogued as a potent cytotoxin. In our previous work, we have shown that *Serratia* is able to invade, persist, and multiply inside non-phagocytic cells, residing in nonacidic, nondegradative, autophagosome-like vacuoles. In this host cells *S. marcescens* elicits an autophagic response. We determine that the ShlA is responsible for the autophagic response that is promoted previous to the bacteria internalization in host epithelial cells. We have recently observed that *S. marcescens* is able to escape from infected non-phagocytic cells in a ShlA-dependent manner. In this work, we have determined that the mutant strain *shlB*, which is unable to activate and secrete the ShlA hemolysin, increases up to 2-fold intracellular counts and decreases egress to the extracellular media. Other bacterial pathogens can infect a range of phagocytic and non-phagocytic cells during their life cycles. In this work, we analyse the invasion process of *S. marcescens* in phagocytic cells. We here show that *S. marcescens* is able to invade and persist inside of the murine macrophages cells RAW 264.7. We here analyse *S. marcescens* escape from infected phagocytic cells. Our results show that the bacterial intracellular increase of a *shlB* strain in infected phagocytic cells in the same manner that in non-phagocytic cells. This result suggests that the ShlA-dependent escape of *S. marcescens* from infected cells is a conserved mechanism that guarantees the spread of bacteria into the extracellular medium.

Código de Resumen: MM-015

Sección: Microbiología Molecular

Modalidad: Poster

Escherichia coli VEROTOXIGÉNICO O157:H7 DE LA REGIÓN PAMPEANA: ISLAS DE PATOGENICIDAD Y GENES EFECTORES.

VEROTOXIGENIC *Escherichia coli* O157:H7 FROM ARGENTINEAN PAMPEAN REGION: PATHOGENICITY ISLANDS AND EFFECTOR GENES

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Verotoxigenic *Escherichia coli* (VTEC) are important foodborne pathogens associated with sporadic cases and outbreaks of diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). Cattle have been recognized as the main reservoir of VTEC worldwide and O157:H7, as the dominant serotype associated with severe diseases in different parts of the world. Single nucleotide polymorphisms (SNP) typing was previously used to differentiate VTEC O157:H7 into 9 distinct clades, being clade 8 the most virulent one. On the other hand, important virulence properties are carried by pathogenicity islands (PAIs) such as the “locus of enterocyte effacement” (LEE), which governs the capability of VTEC to colonize the intestinal mucosa of the host. Other PAIs, termed O islands, contain non-LEE (nle) effector genes that encode translocated substrates of type III secretion system. Some of these effectors contribute to the colonization and persistence of VTEC in cattle and interfere with the human inflammatory response.

The aim of this study was to evaluate the presence of clade 8 strains in our area and to analyze the distribution of *nle* genes encoded in different PAIs. A total of 33 VTEC O157:H7 strains isolated, mainly in Argentinean pampean region, from cattle, humans and food, were analyzed. They had been previously characterized as positive for virulence genes *eae* and *vtx2*. The identification of the strains belonging to clade 8 was performed by detecting a SNP located in the *rhsA* gene by PCR-RFLP. The restriction digestion of DNA was carried out with HaeII and Sau961 enzimes. In relation to the *nle* genes, *nleA*, *nleH1-2*, *nleF*, *nleG*, *nleG2-1* and *nleG9*, encoded in genomic island O-I 71, *nleB*, *nleE* and *ent/esp L2*, O-I 36, *nleB2*, *nleC*, *nleH1-1* and *nleD*, O-I 122, *nleG2-3*, *nleG5-2* and *nleG6-2* O-I 57, were amplified. PCR products were visualized in agarose gels stained with SYBR Safe. All isolates belonged to clade 8. Regarding the presence of *nle* genes, isolates were grouped into six profiles. Twenty isolates (61%) were positive for all *nle* genes analyzed, while the remaining isolates, except two, showed incomplete O-I 71, particularly lacked the *nleF* gene. No source-specific profiles were observed. According to these studies, O157:H7 strains circulating in the pampean region are a homogeneous group in relation to clades assignment. The belonging of the isolates studied to hypervirulent clade 8, and the high prevalence of *nle* genes, genetic determinants that enable the pathogen to persist in the host and cause disease, would allow to assign most O157:H7 strains of this region a high risk to public health.

Código de Resumen: MM-016

Sección: Microbiología Molecular

Modalidad: Poster

CARACTERIZACIÓN DEL MEGAPLÁSMIDO EN CEPAS *Escherichia coli* PRODUCTORAS DE TOXINA-SHIGA (STEC) O157 Y NO-O157, AISLADAS DE GANADO BOVINO

CHARACTERIZATION OF A LARGE PLASMID IN SHIGA TOXIN-PRODUCING *Escherichia coli* O157 AND NON-O157 STRAINS ISOLATED FROM CATTLE

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Shiga toxin-producing *Escherichia coli* (STEC) is an important cause of disease in humans such as haemorrhagic colitis (HC) and haemolytic-uremic syndrome (HUS), the leading cause of acute renal failure in children (Siegler, 2003). The most common STEC serotype associated with sporadic cases and large outbreaks of diseases is *E. coli* O157:H7. However, other STEC serotypes, especially O26:H11/NM, O91:H21, O103:H2, O111:H8/NM, O113:H21, O104:H4, O145:NM, and O157:NM have been isolated from human disease (Paton, et al. 1999; Center for Disease Control and Prevention, 2007; Karama, et al. 2008; Frank, et al. 2011). Cattle are the main reservoir and can transmit STEC to humans through contaminated food and direct contact with the animals and with the environment farm (Fernández, et al. 2011; Polifroni, et al. 2012).

E. coli O157:H7 possess a putative virulence plasmid carrying genetic information for the enterohemorragic haemolysin -*ehxA*-(Schmidt, et al. 1995b, 1996) and other virulence factors such as a periplasmic catalase-peroxidase -*katP*- (Brunder, et al. 1996), an extracellular serine protease -*espP*- that is able to cleave pepsin and coagulation factor V in humans (Brunder et al, 1997); a zinc-metalloprotease -*stcE*-, (Grys, T.E. et al. 2005), and a subtilase-cytotoxin -*subA*- which can produce an extensive microvascular damage, thrombosis, and necrosis in multiple organs (Paton & Paton, 2005).

In this study, 255 enterohaemolysin-positive STEC strains (O157 and non-O157), isolated from different categories of cattle (newborn, weaning and rearing calves, and cows) in Buenos Aires province, were analyzed by PCR for subtyping the megaplasmid genes.

The most prevalent detected gene was *espP* in 250/255 isolates (98.04%) followed by *subA* 48.63% (124/255), *katP* 19.22% (49/255) and *stcE* in 5.49% (14/255).

We found differences in the distribution of genes between the different categories of cattle, except for *espP* that was kept at a high prevalence in all of the categories studied. *katP* was found in similar percentages in newborn, weaning and rearing calves (38.89%, 31.37% and 33.33% respectively), but decreased in cows (6.06%). Moreover, the presence of *subA* increased with the age of the animals, being 11.11% in newborn, 15.69 % in weaning calves, 37.04 % in rearing calves and 71.21 % in cows. *stcE* was the least prevalent gene of all, with an overall average of 5,49%.

These results show the variability of STEC megaplasmid. It is important to note the high percentage of *espP*-positive strains and the high levels found of *subA*. Knowledge about the putative virulence genes megaplasmid can provide data to assess the potential risk of bovine STEC isolates to cause human infection.

Código de Resumen: MM-017

Sección: Microbiología Molecular

Modalidad: Poster

EL INHIBIDOR DE PROTEASAS TIPO GERMINA DE TRIGO EXPRESADO HETERÓLOGAMENTE EN *Escherichia coli* TIENE ACTIVIDAD ANTIFÚNGICA SOBRE *Fusarium solani*

A WHEAT GERMIN LIKE PROTEIN EXPRESSED IN *Escherichia coli* HAS ANTIFUNGAL ACTIVITY AGAINST *Fusarium solani*

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A Germin-like Protein with trypsin inhibitor activity has been purified from intercellular fluid of wheat leaves and it was named Germin-like Protein Inhibitor (GLPI). Since GLPI is a heat-resistant protein, a heating step (30 min at 70ºC) is included in the protocol of purification. In addition to its inhibitor activity, GLPI has at least two more enzimatic activities: superoxide dismutase (SOD) and adenosine glucose pyrophosphatase/phosphodiesterase (AGPPase). Moreover, its antifungal activity against plant fungal pathogens was confirmed recently. Also, the expression of GLPI (rGLPI) in *E. coli* was achieved. The original protocol includes proteins resolubilization from inclusion bodies using 6M urea. Although this compound is removed by followings steps of the protocol, a modification was applied. After induction, *E. coli* cells were pelleted and the soluble fraction was heated at 70 ºC for 30 minutes. The heat-resistant proteins were loaded in a nickel matrix and the recombinant protein obtained by the new protocol was called rGLPI70. To confirm the homogeneity of the eluted fraction and to confirm its identity, the purified protein was analyzed by SDS-PAGE and protein gel blotting followed by inmunodetection with antiserum against GLPI isolated from wheat. The purified protein showed one clear band of the expected molecular mass (21 kDa). With the objective to study whether rGLPI and rGLPI70 maintain the antifungal activity observed with the native protein, we analyzed the effect of both on *F. solani* spores germination. *In vitro* quantitative assays showed that 240 ug/ml of rGLPI and rGLPI70 inhibited a 70 % of *F. solani* spores germination. These results were similar to those obtained for native GLPI. In conclusion, the modified protocol consists in an easier, faster and less astringent alternative to obtained rGLPI. The rGLPI antifungal activity against *F. solani* suggests that this protein could be used to develop new natural pesticides.

Código de Resumen: MM-018

Sección: Microbiología Molecular

Modalidad:Poster

AISLAMIENTO Y SELECCIÓN DE BACTERIAS OXIDANTES DE MANGANESO

ISOLATION AND SELECTION OF MANGANESE OXIDIZING BACTERIA

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Manganese represents the 12th most abundant element in the Earth's crust. It can exist in 11 oxidation states ranging from –3 to +7 with two major forms in aquatic environments: Mn (II) and Mn (IV). Changes between the two major oxidation forms occur via oxidation and reduction reactions that may be abiotic or microbially mediated. Although bacterial Mn (II) oxidation is widespread, little is known about why bacteria oxidize Mn (II) and if this process gives any

advantages to the bacteria that can perform it. Aside from a requirement for oxygen and iron, as well as the observation that oxidation occurs in stationary phase, very little is known about this regulation. Identifying signals or conditions that regulate oxidation could provide some insight into the role of Mn (II) oxidation in bacterial cells.
The primary objective of this study was to isolate manganese-oxidizing bacteria, and study, in a future, the physiological function of the oxidation process in these isolates.
Mn-oxidizing bacteria are ubiquitous, but several works demonstrated that habitats containing high levels of Mn tend to have high numbers of these bacteria. Therefore, in order to isolate Mn-oxidizing bacteria we collected samples from a biofiltration system that enables the removal of the Mn present in underground waters by the biological oxidation of this metal. Sediments were dissolved in PBS and were plated on different solid selective media containing high MnSO4 concentrations. The isolated strains were found to oxidize MnSO4 (present in the agar) as deduced from color changes. In the absence of Mn, the colonies on the agar were whitish, in contrast, in Mn-containing cultures a shift from whitish to brownish was observed. From a total of 2080 isolates evaluated we could select 82 Mn-oxidizing strains. Oligonucleotides were designed in order to amplify specific regions of the 16S RNAs by PCR. These sequences may allow the molecular identification of each strain by phylogenetic analysis. Genome data bases will be analyzed in order to screen the presence of genes with a role in Mn-oxidation processes and a strategy to obtain mutant strains in these genes will be designed.
With the aim of know the environmental grown conditions of the isolated strains, we determined the general physicochemical parameters of the waters and sediments from which the bacteria were obtained. Mn and iron concentrations were measured using Flame Atomic Absorption Spectroscopy. Total sediments, pH, electrical conductivity and oxygen concentration, were measured using procedures acceptable by Standard Methods for the Examination of Water and Wastewater (2005). This may allow us to introduce modification in the culture media in order to improve the *in vitro* culture conditions.
This work thus will contribute to the understanding of the role of Mn (II) oxidation in the evolution of bacteria.

Código de Resumen: MM-019

Sección: Microbiología Molecular

Modalidad: Poster

MECANISMOS CONCENTRADORES DE CARBONO: ESTUDIOS FILOGENÉTICOS DE PROTEÍNAS DE CARBOXISOMAS EN CIANOBACTERIAS

CARBON-CONCENTRATING MECHANISMS: PHYLOGENETIC STUDIES OF CARBOXYSOME PROTEINS IN CYANOBACTERIA

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Cyanobacteria live in a diverse range of ecological habitats, including both freshwater and marine ecosystems and play a key role in the biogeochemical carbon and nitrogen cycle. The ability of this diverse group of photoautotrophs to assimilate carbon dioxide (CO_2) from the environment comes from the Carbon Concentrating Mechanisms (CCM). The CCM comprises inorganic carbon (Ci, as CO_2 and HCO_3^-) transporters for Ci uptake and protein microbodies called carboxysomes for CO_2 concentration and fixation by Rubisco.
There are two main phylogenetic groups within the cyanobacteria based on Rubisco and carboxysome phylogenies. Alpha-cyanobacteria have alpha-carboxysomes with form-IA Rubisco, whereas beta-cyanobacteria have beta-carboxysomes with form-IB Rubisco. The two types of carboxysomes are morphologically similar but differ in that shell proteins are encoded by the *cso* (csoS123AB) operon, in the case of alpha-carboxysomes, or by *ccm* (ccmKLMN) operon, in the case of beta-carboxysomes. HCO_3^- dehydration is catalyzed by carbonic anhydrase (CA) specifically localized in the center of the carboxysomes. Its function results in the accumulation of CO_2 in high concentrations in the vicinity of Rubisco to promote Ci fixation.

Recently, the number of sequenced cyanobacterial genomes has been duplicated and more than 190 genomes are available. Thus, new phylogenetic and genetic studies are needed. In this work, phylogenetic analyses indicate that beta-cyanobacteria evolved first than alpha-cyanobacteria. Beta-cyanobacteria are widely distributed and occupy a more diverse range of habitats than alpha-cyanobacteria, including freshwater, estuarine, and hot springs. Furthermore, alpha-cyanobacteria, which inhabit in marine environments, have evolutionary relationship to the Proteobacteria. Alpha-cyanobacteria include only marine genera *Prochlorococcus* and *Synechococcus.*
Besides, our genetic analyses, summarized the genetic diversity in the presence/absence of the CCM genes of the sequenced genomes. Most beta-cyanobacteria have beta-CAs inside its carboxysomes, but those that live in hot springs have a gamma-CA, potentially active, because they have the appropriate amino acids for a correct structure and a functional enzyme. Alpha cyanobacteria have only epsilon-CAs in their carboxysomes.
Our results are the first step to a more comprehensive study of these proteins to shed some light on the evolution and function of cyanobacterial carboxysomes. Supported by CONICET, UNMdP and FIBA.

Código de Resumen: MM-020

Sección: Microbiología Molecular

Modalidad: Poster

CARACTERIZACIÓN MORFÓLOGICA Y MOLECULAR DE DOS CEPAS TOXÍGENAS DE CIANOBACTERIAS NATIVAS, FORMADORAS DE FLORACIONES

MORPHOLOGIC AND MOLECULAR CHARACTERIZATION OF TWO NATIVE TOXIGENIC BLOOM-FORMING CYANOBACTERIA

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Under certain environmental conditions, some cyanobacteria (Cyanophytes, Cyanoprokaryotes, also known as blue-green algae) are capable of growing rapidly and form harmful blooms (CHABs), phenomena that consists in a rapid increase in the density of one or two species that dominate the phytoplankton. The major problem associated with CHABs is that many species are capable of producing toxic compounds called cyanotoxins that constitute a health-risk for human beings worldwide via recreational and drinking water. In Argentina, the potential public health risk due to exposure to CHABs has grown in the last decades.
In this study, we report the presence of *Raphidiopsis mediterranea* (Nostocales) and *Planktothrix agardhii* (Oscillatoriales) in Los Patos shallow lake, a small eutrophic reservoir located on Ensenada city, 60 Km South-West of Buenos Aires city (34º 50´ 44´´S, 57º 57´ 26´´W). Both species are known to co-occur in eutrophic shallow lakes and have been reported in other Argentinean freshwater bodies.
One strain of each species was isolated and grown in MLA medium. Morphological traits of the two strains were analyzed by light microscopy and transmission electron microscope. Molecular analyses were performed using the sequences of the *16S rRNA* gene and the *cpcBA-IGS* region, which includes the highly variable intergenic spacer region (*IGS*) between two phycobilisome (bilin) sub-units genes (*cpcB* and *cpcA*).
R. mediterranea has been associated with the production of several cyanotoxins, such as homoanatoxin-a, anatoxin-a and cylindrospermopsin, while *P. agardhii* is known to synthesize microcystins. Thus, we investigated the presence of genes related with microcystin (MYC) and cylindrospermopsin (CYN) production by using primers specially designed for *Planktothrix* and *Raphidiopsis*, respectively.
Isolated strains were successfully cultured on MLA medium and are now part of the culture collection of the FIBA-INBIOTEC laboratory. The identification of the strains as *P. agardhii* and *R. mediterranea* by morphological characterization was also supported by phylogenetic analyses.
PCR analyses of *P. agardhii* were positive for the microcystin synthetase gene E (*mcyE*), whereas the *cyr*C gen, involved in CYN production, was absent in *R. mediterranea.*

This it is the first report on the molecular characterization of filamentous bloom forming cyanobacteria native from Argentina.
Supported by CONICET, UNMdP and FIBA.

Código de Resumen: MM-021

Sección: Microbiología Molecular

Modalidad: Poster

CAMINOS CATABÓLICOS DE LA SACAROSA EN CIANOBACTERIAS

SUCROSE CATABOLISM PATHWAYS IN CYANOBACTERIA

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Sucrose metabolism has been studied extensively in plants and in a lesser extent in cyanobacteria. The increasing number of complete sequenced genomes of oxygenic photosynthetic microorganisms led us to continue our study on sucrose metabolism proteins. Sucrose metabolism occurs in cyanobacteria in a similar way as described previously in plants. Sucrose synthesis through a two-step pathway has been described in both unicellular and filamentous nitrogen-fixing strains. On the other hand, sucrose catabolism can take place through its irreversible hydrolysis to hexoses by alkaline/neutral invertases (A/N-Inv) or its cleavage by sucrose synthase (SuS, UDP–glucose:D-fructose D-glucosyltransferase), supplying sugar nucleotides, precursors in the formation of structural and storage polysaccharides. Recently, a new hydrolysis pathway has been described involving an amylosucrase (AMS) that belongs to glycoside hydrolase family 13 that not only hydrolyze Suc to monosaccharides supplying carbon and energy, but also to synthesize amylose-like polymers using sucrose as sole substrate.
First, based on the presence of SuS gene homologs in the most recently radiated cyanobacterial species, whose genomes have been fully sequenced (198 in total), and after phylogenetic analysis, we concluded that SuS may play a key role in heterocyst-forming strains, while in unicellular strains is likely to be dispensable in some cases or related to environmental adverse conditions as it has been demonstrated in *Microcystis aeruginosa*. Second, the presence of A/N-Inv gene (*inv*) homologs in the 45% of the available cyanobacterial genomes and in strains related to the base of the radiation, indicate the early origin of A/N-Inv. Additionally, in nearly 60% of the cases, the *inv* homologs are present in nitrogen-fixing cyanobacterial genomes and Inv-A/N are present in the majority of all heterocyst-forming cyanobacteria, as occurs with SuS, where they may play a relevant role in the heterotrophic metabolism within the nitrogen-fixing specialized cells named heterocysts. Third, occurrence of AMS gene homologs is limited to its presence in 2 filamentous and in 8 unicellular cyanobacterial strains, where this was the only sucrose catabolic pathway.
Phylogenetic analyses of the three Suc catabolic pathways in cyanobacteria showed that the origin of the A/N-Inv might have occurred early in the evolution, while the appearance of SuS might have happened later, being fundamental in the development of more evolved morphological forms. In contrast, cyanobacterial AMS seems to have a bacterial origin and might have been acquired in separate events.
Taken together, the results presented here show that Suc catabolic pathways play different roles in cyanobacteria. Supported by PIP 134, UNMdP (EXA645/13), and FIBA.

Código de Resumen: MM-022

Sección: Microbiología Molecular

Modalidad: Poster

EVALUACIÓN DEL EFECTO MICOBACTERICIDA DEL NITROXILO (HNO)

EVALUATION OF THE MYCOBACTERICIDAL EFFECT OF NITROXYL (HNO)

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Tuberculosis, caused by *Mycobacterium tuberculosis* (Mt), is one of the most important infectious diseases in the world, with nearly 2 million deaths annually, mostly in developing countries. Reactive oxygen and nitrogen species (RNOS) are key compounds used by the immune system to fight the intracellular infection, particularly showing concentration and time-dependent mycobactericidal activity even in the bacillus stationary phase. RNOS are generated as the result of nitro-oxidative stress generating compounds such as NO and HNO (nitroxyl). These compounds are very unstable at physiological pH, so for laboratory manipulation NO (e.g. sodium nitroprusside, SNP) or HNO releasing agents (e.g. Angeli's Salt) are used. The aim of this work is to evaluate the viability and biological response of mycobacteria towards a new set of pro-drugs with the potential of generating RNOS inside the bacilli through the release of nitroxyl.

In the first part of the project, we used *E. coli* as a model to evaluate the effect of different RNOS generating compounds. We have constructed, a RNOS hypersensitive mutant strain Δ*hmp* (*hmp* codes for flavohemoglobin, a protein that is known to be important for the organism's RNOS scavenging and detoxification process) using "*recombineering*". We determined the MIC for SNP and Angeli's Salt in the mutant and *wt* strains. As previously reported, Δ*hmp* hadincreased sensitivity to SNP, corroborating the phenotype of the constructed mutant. Interestingly, we have also observed a higher sensitivity of the mutant to Angeli's Salt. When we evaluated the mode of action of these compounds, we observed that while SNP acts as a bacteriostatic, Angeli's Salt has a bactericidal effect in *wt* and mutant *E. coli*. The Δ*hmp* strain constructed in this work, will be used in further studies for evaluation of an ample set of RNOS compounds.

In the second part of the project, we tested the effect of the mentioned compounds in *Mycobacterium smegmatis* mc2155 (a nonpathogenic strain used as a model for the study of Mycobacteria). For both compounds an inhibitory effect on bacterial growth was observed but at very distinctive concentrations. A MIC of 100 mM for SNP and 7.5 mM for Angeli's Salt was obtained. The mode of action of these NO and HNO releasing compounds in this microorganism was bacteriostatic.

Taking this as a starting point we project to study in detail the mechanism of action of these compounds by including RNOS sequestering proteins, such as myoglobin, as well as extend the study the effect of these compounds in *Mycobacterium tuberculosis* mc2 6230 viability.

Código de Resumen: MM-023

Sección: Microbiología Molecular

Modalidad: Poster

¡TRABAJO EN EQUIPO! PRESENCIA DE COMPLEJOS DE CITOCROMOS EXTRACELULARES EN BIOFILMS ELECTRO-ACTIVOS DE *Geobacter sulfurreducens*

TEAM WORK! PRESENCE OF EXTRACELLULAR CYTOCHROME COMPLEXES IN ELECTRO-ACTIVE BIOFILMS FROM *Geobacter sulfurreducens*

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The discovery of electro-active microorganisms in the past decade fastly became one of the top areas of study mainly due to the great number of technological applications these organisms present. *Geobacter sulfurreducens* is one of many anaerobic gram (–) bacteria capable of using extracellular insoluble electron acceptor like Fe (III) (hydro)oxides during respiration. These bacteria can also use a polarized electrode for this function, thus producing an electric current. The redox molecules involved in this process have been identified as type c cytochromes named Omc (Outter membrane cytochromes). Molecular aspects of this process as well as the mode of action of these redox molecules remain unclear. Aiming to gain more information we studied external soluble cytochromes Omc extracted from the extracellular matrix of electro-active *G. sulfurreducens* biofilms developed over polarized electrodes. We first attempted to separate these proteins by hydroxyapatite chromatography and observed that, unexpectedly, each elution contained more than one cytochrome. It has been previously described that respiratory proteins can and will, in many cases, associate forming multi-protein complexes necessary for the electron transport process to occur. Then, it may also be possible for *G. sulfurreducens´*s soluble external cytochromes to associate forming multi-protein redox complexes. Therefore we performed two dimensional blue native (2D-BN) gel electrophoresis stained with specific cytochrome staining to further corroborate the presence of Omc cytochrome complexes. Results indicated the presence of two high molecular weight extracellular redox complexes described here as COmc200 (MW≈200 kDa) and COmc150 (MW≈150 kDa). Also, a rather low molecular weight band, denominated COmc80 (MW≈80 kDa), was observed. Second dimension non-reducing SDS-PAGE of each complex was performed to determine the components of each complex. Results showed that while COmc150 and COmc80 are composed by two cytochromes, COmc200 is composed of four different cytochromes. Interestely, all three complexes shared at least one component with the other two. This result may indicate a dynamic and possibly reversible protein interaction between these proteins. In this sense, we believe these redox complexes and their dynamic interaction are necessary for the redox process occurring outside the cell of electro-active bacteria.

Código de Resumen: MM-024

Sección: Microbiología Molecular

Modalidad: Poster

EL CIRCUITO REGULATORIO Rcs-CASCADA FLAGELAR MODULA LA EXPRESIÓN DE LA HEMOLISINA ShlA de *Serratia marcescens*

THE REGULATORY Rcs-FLAGELLAR CIRCUIT MODULATES *Serratia marcescens* ShlA HEMOLYSIN EXPRESSION

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Serratia marcescens produces numerous exoproteins with toxic effects, including ShlA pore-forming toxin which has been catalogued as its most potent cytotoxin. ShlA belongs to the two-partner secretion family and is coded by the *shlBA* operon. Previously, we have determined that the expression of ShlA is responsible for the autophagic response that is promoted before bacterial internalization in epithelial cells. However, the regulatory mechanisms that govern ShlA expression have remained unclear. A predictive *in silico* analysis of *shlBA* promoter region, showed consensus sequence for the recognition of the FliA and RcsB regulators. FliA is an alternative sigma factor in the regulatory cascade of flagellar biogenesis, and RcsB is the response regulator of the RcsCDB phosphorelay system. Hemolytic activity and transcriptional assays in flagellar and mutant strains in the genes encoding Rcs system components were performed. An inducing action of the flagellar regulatory cascade over *shlBA* expression was observed**.** On the other hand, RcsB showed a negative modulation over *shlBA* expression. Our previous work has shown that flagellar biogenesis is under negative regulation of RcsB, therefore we hypothesized that the control of *shlBA* expression was modulated by a transcriptional cascade involving the Rcs-flagellar circuit. Direct binding of RcsB response regulator to a specific promoter motif upstream *shlBA* operon was confirmed by *in vitro* assays, demonstrating that RcsB is also able to exert a direct inhibition on *shlBA* transcriptional expression. In sum, we show that the expression of *shlBA* is subjected to a transcriptional feed-forward regulatory circuit dependent on RcsB that can involve or bypass the flagellar regulatory pathway. This feed-forward regulatory path may have evolved to provide multiple access points for the fine-tuning of ShlA expression, highlighting the functional importance of this cytotoxin throughout *Serratia* life cycle.

Código de Resumen: MM-025

Sección: Microbiología Molecular

Modalidad: Poster

ACTIVIDAD *IN VITRO* ANTI-*Helicobacter pylori* DE PROPÓLEOS COLECTADOS DE DISTINTAS ÁREAS GEOMORFOLÓGICAS DE LA REGIÓN DEL BIOBÍO, CHILE

ANTI-*Helicobacter pylori* ACTIVITY OF PROPOLIS FROM VARIOUS GEOMORPHOLOGICAL AREAS AT THE BIO-BÍO REGION, CHILE

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Helicobacter pylori is a gram-negative bacteria found in the human stomach of roughly 50% of the world's population. Pathologies such as gastritis, duodenal and gastric ulcer, gastric carcinoma and MALT lymphoma have been etiologically associated with this pathogen. Thus, prevention of *H. pylori* infection and eradication of the bacteria are priority in public and private health care system. Currently, the failure of eradication therapies reach 20% to 40% depending on the clarithromycin

resistance observed among clinical isolates. Therefore, the search for new biological molecules active upon *H. pylori* is a crucial key. The aims of this study were to evaluate the anti-*H. pylori* activity of propolis collected from five different geomorphological areas of the Bio Bío region and to establish the main active compound of propolis. *H. pylori* J99 was used as reporter strain in this study. The propolis was dissolved in DMSO 20% and assayed for their antimicrobial activity by both agar diffusion and agar dilution assays. In addition, the active fractions of the propolis samples showing the highest antimicrobial activity were analyzed by centrifugal partition chromatography (CPC) and Thin-layer chromatography (TLC). All propolis samples showed anti-*H. pylori* activities, with inhibition zones showing diameters between 17 to 25 mm (propolis concentration 10000 μg/mL). On the other hand, the most active extract shows a MIC of 8 μg/mL. CPC results showed the presence of nine fractions, all of them with anti-*H. pylori* activity with inhibition zones of 14 to 27,5 mm diameters. These fractions also showed antibacterial activity against *S. aureus* ATCC 6538P and *E. coli* SI. TLC results revealed the presence of galangin, pinocembrin, chrysin, caffeic acid phenethyl ester (CAPE), kaempferol and quercetin. To the best of our knowledge, this is the first study done in Chile describing the anti-*H. pylori* activity of propolis. This activity seems to be related to the area were honeybees collect the pollen to make propolis.
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Código de Resumen: MM-026

Sección: Microbiología Molecular

Modalidad: Poster

Streptococcus uberis: CARACTERIZACIÓN DE BACTERIOCINAS CON ACTIVIDAD ANTIMICROBIANA PARA CONTROLAR LA MASTITIS BOVINA

Streptococcus uberis: CHARACTERIZATION OF BACTERIOCINS WITH ANTIMICROBIAL ACTIVITY IN ORDER TO CONTROL BOVINE MASTITIS

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Bovine mastitis is an important disease that causes severe economic losses in dairy herds. Although antibiotic therapy is effective, it can be detrimental too, because of the emergence of antibiotic resistant human pathogens. An effective treatment by other substances than antibiotics becomes an urgent need. Probably one of the biggest challenges of the modern dairy industry is to reduce the use of antibiotics in food-producing animals. Bacteriocins provide an alternative to antibiotics in the treatment of dairy cows or for sealing nipple during the dry period. *Streptococcus uberis* is commonly found in the natural environment of dairy cows and therefore competes with other bacteria in this niche. *S. uberis* is known to produce inhibitory substances type bacteriocin such as nisin U, a circular peptide, called uberolisina and ubericina A. The aim of this work was to select *Streptococcus uberis* strains isolated from the central dairy region of our country based on the production of active bacteriocins against the most prevalent mastitis pathogens. Fifty nine *S. uberis* strains isolated from subclinical mastitis were studied. The inhibitory capacity detection was performed by cross-streaking method and the production of bacteriocins-like inhibitory substances (BLIS) by the well diffusion assay. Despite the disadvantages and limitations, this last technique, is still the most used. Seven strains showed ability to inhibit the growth of the main mastitis pathogens as: *E. coli*, *S. aureus, S. agalactiae, S coagulase* negative (SCN), *L. plantarum* and *Enterococcus* by cross-streaking method. The strains were grown in broth medium and cell-free supernatant of each strain was obtained. The first stage consisted of an ammonium sulphate precipitation 95%, allowing the concentration of the active fraction. Then, it was tested for antagonism assay by agar well diffusion. The concentrated supernatant of *S. uberis* strain 150 was active against *S. aureus* and SCN. After neutralization with NaOH, the antagonistic activity of the strain remained active and the treatment with catalase did not affect the antimicrobial activity. The results of this work allow the conclusion that the *S. uberis* strains produce bacteriocins with capacity to inhibit the main pathogens causing bovine mastitis, so these microbial products would serve as potential candidates for the treatment of this pathology.

Código de Resumen: MM-027

Sección: Microbiología Molecular

Modalidad: Poster

DISEÑO Y EVALUACIÓN DE NUEVOS PÉPTIDOS ANTIMICROBIANOS CON ACTIVIDAD EN *Staphylococcus aureus* Y *Pseudomonas aeruginosa*

DESIGN AND EVALUATION OF NEW ANTIMICROBIAL PEPTIDES WITH ACTIVITY AGAINST *Staphylococcus aureus* AND *Pseudomonas aeruginosa*

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Antimicrobial cationic peptides are a crucial component of the innate immune system as a first line of defense against infectious agents [1] . The properties of these peptides make them very attractive for the development of new therapeutic drugs, as they not only have action against bacteria, but also have overcome the problem of resistance that other antibiotics have [2,3] . The research topic includes the design, modification and evaluation of new antimicrobial peptides with activity against strains resistant to commonly used antibiotics. In a first step we proceeded to the analysis of antimicrobial and cytotoxic activity of a group of de novo designed peptides. Subsequently we performed specific modifications in the amino acid sequence in order to increase the amphipathicity, net charge and content of alpha helix; and the therapeutic index of these modified versions was compared to the original versions. The antimicrobial activity was analyzed using the microdilution minimum inhibitory concentration (MIC) technique in broth. As a test of cytotoxicity, hemolysis of murine and human erythrocytes was performed. Afterwards the inhibitory activity of bacterial biofilm formation in strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was quantified. With these tests it was determined that two peptides, in addition to having strong antimicrobial activity, have low cytotoxicity and the ability to inhibit the production of bacterial biofilm thus are possible candidates for future development of antimicrobial drugs.

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Código de Resumen: MM-028

Sección: Microbiología Molecular

Modalidad: Poster

UNA MIRADA MÁS PROFUNDA DENTRO DE LOS EXTREMOFILOS: ANÁLISIS DEL GENOMA DE UNA NUEVA ESPECIE DE ARQUEA TERMOACIDÓFILA

A DEEPER LOOK INTO EXTREMOPHILES: ANALYSIS OF THE DRAFT GENOME OF A NOVEL THERMOACIDOPHILIC ARCHAEON

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Copahue geothermal area is located on the Northwest corner of Neuquén, crowned by Copahue volcano (30000 m o.s.l. in Cordillera de los Andes). The constant volcanic activity has determined the extreme environments found in the area, specially the existence of many high-temperature, acidic, sulphur-reach ponds and hot springs. Such habitats are populated by extremophilic microorganisms well adapted to develop under hard physicochemical conditions and the lack of organic carbon sources. In acidic high temperature environments archaea are important members of the communities, however many of the species are yet unknown and uncharacterized.

Acidianus copahuensis is a thermoacidophilic archaeon (phylum *Crenarchaeota*, order *Sulfolobales*) that has beenisolated from various hot springs in Copahue. *A. copahuensis* is a facultative chemolithoautotroph that grows optimally at 75°C and pH 2.5. It is capable of autotrophic growth; aerobically using iron and different sulfur compounds as energy sources, and anaerobically using H2 and S as electron donors and Fe(III) or S as final electron acceptors. It can also grow heterotrophically using yeast extract or glucose.

In this work we present the first analysis of the genome sequence of *A. copahuensis,* obtained using a whole-genome shotgun (WGS) strategy with a 454-FLX Titanium pyrosequencer. The draft genome is 2,454,023 bases in length and the G+C content is 35.63 mol%. According to RAST annotation a total of 2,548 coding sequences (CDSs) and 52 structural RNAs (49 tRNAs, 3 rRNA) were predicted. A 47% of the CDSs were classified as coding for hypothetical proteins and 20% for known enzymes.

The genome of *A. copahuensis* revealed specific genes that could be associated with the metabolic activities of this organism. The key enzymes for sulphur compounds oxidation described for other acidophilic archea, such as sulfur oxigenase-reductase (SOR) and thiosulfate-quinone oxidoreductase (TQO), were detected in *A. copahuensis*. Iron oxidation as an energy source was also represented in the genome by some of the fox cluster enzymes. *A. copahuensis* presents genes coding for proteins of the five major terminal oxidase complexes of *Sulfolobales*, so far reported in only two species. As regards autotrophic metabolism, carbon fixation through the 3-hydroxypropionate–4-hydroxybutyrate cycle could be inferred by the presence of the key enzymes of this pathway. An interesting discovery is the presence of genes encoding for arsenite oxidase (aioAB), not common in *Sulfolobales*. This could be related with the use of arsenite as an energy source or the arsenic bioremediation potential of *A. copahuensis*.

The present analysis confirms the presence of key enzymes that allow *A. copahuensis* survival in the environmental conditions of Copahue and make it a good candidate for biomining of sulphide minerals. Further analysis would be required to for a better understanding of this archaeon extreme metabolic features.

Código de Resumen: MM-029

Sección: Microbiología Molecular

Modalidad: Poster

DESARROLLO DEL SISTEMA *IN VITRO* DE RIBOZIMA P PARA *Acinetobacter baumannii*

DEVELOPMENT OF RIBOZYME P *IN VITRO* SYSTEM FOR *Acinetobacter baumannii*

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Multidrug resistant (MDR) *Acinetobacter baumannii* is emerging as the causative agent of numerous nosocomial outbreaks worldwide. The frail health of hospitalized patients that are usually infected together with its multiresistant nature make *A. baumannii* a highly problematic pathogen to treat. Since available antibiotics effective against MDR *A. baumannii* are running out, newer treatment strategies must be devised. EGS (External Guide Sequence) technology is a type of antisense technology that is utilized to silence essential or resistance genes. It consists of the use of antisense oligonucleotides, known as EGSs, to elicit Ribozyme P (RNase P)-mediated cleavage of a target RNA. To design EGSs that can silence essential or resistance *A. baumannii* genes, we first identified and characterized its RNase P enzyme.

Bacterial RNase P is a ribonucleoprotein composed of a catalytic RNA subunit (M1) and a cofactor protein (C5). Its main natural substrates are pre-tRNAs but it can be induced to digest other RNA molecules if an appropriate double stranded three dimensional structure is formed.

Genes with high homology to the *E. coli* M1 and C5, called M1Ab and C5Ab, were identified within the genome of *A. baumannii.* Genomic comparisons showed that the M1Ab sequence can be classified as type A, and has high homology with those of other gram-negative opportunistic pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*. However, while the M1 sequences of these two enterobacteria were nearly identical, M1Ab presents two 9- and 11-nucleotides gaps located upstream Universally Conserved Region I (CRI) and between CRI and CRII regions. On the other hand, C5Ab is predicted to be a 102 amino acid peptide that includes a 30-amino acids conserved region. The amino acid sequences flanking this conserved region have low homology with the *E. coli* C5 sequence. However, three-dimensional modeling comparing C5Ab to all known C5 proteins showed that highest structural homology occurs with the *E. coli* C5 protein. Likewise, C5Ab shows a high pI (10.8) typical of nucleic acid binding proteins, although not as high as that of the *E. coli* C5, which is 11.8. Following, the M1Ab and C5Ab genes were cloned under the control of the T7 promoter. Using these recombinant clones M1Ab was synthesized in vitro and C5Ab was overexpressed and purified. Both components were used to reconstitute the *A. baumannii* holoenzyme.

In conclusion, the *A. baumannii* RNase P was identified, the M1Ab and C5Ab components were characterized in silico, cloned, purified, and used to reconstitute the ribozyme in vitro. Future directions include the utilization of RNase P to test synthetic EGSs that elicit cleavage of mRNA corresponding to essential genes as a first step towards designing new pharmacological tools to control the growing threat of MDR *A. baumannii* infections.

Código de Resumen: MM-030

Sección: Microbiología Molecular

Modalidad: Poster

DETECCIÓN DEL MARCADOR EPIDÉMICO DE CEPA *Burkholderia cepacia* (BCESM) Y GENE DEL CABLE PILI (*cblA*) EN AISLADOS LOCALES DE *Burkholderia contaminans*

DETECTION OF *Burkholderia cepacia* EPIDEMIC STRAIN MARKER (BCESM) AND THE CABLE PILIN SUBUNIT GENE (*cblA*) IN *Burkholderia contaminans* LOCAL ISOLATES

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Pulmonary infections with bacteria belonging to *Burkholderia cepacia* complex species (Bcc) in cystic fibrosis (CF) patients are usually chronic, resistant to antibiotic therapy and associated with increased morbidity and mortality. Previous studies identified transmissibility markers in Bcc bacteria, which were associated with certain epidemic strains, having an increased ability to spread. These markers included the cable pili (*cblA*) gene (Sajjan et al., 2008) and the *B. cepacia* epidemic strain marker (BCESM) which is part of a genomic island encoding genes linked to metabolism and virulence such as quorum sensing signals (AHLs).

The prevalence of infection in CF population worldwide has shown that *B. multivorans, B. cenocepacia,* and *B. cepacia* are the most predominant Bcc pathogens, causing on average 80% of the Bcc infections. However, in a previous study we demonstrate that in Argentina 8 out of the 18 Bcc species could be recovered from CF patients, with a remarkably high representation of *B. contaminans,* detected in 60% of the CF patients analyzed (Martina et al., 2013). Thus, despite the implementation of strict infection control protocols, *B. contaminans* species have been recovered from both CF and non-CF patients, industrial products, and environmental samples.

In order to analyze some virulence factors and get insights into the identification of possible problematic strains and assist in the diagnosis and management of *B. contaminans* infections in CF patients, in the current study we investigated the presence and distribution of transmissibility markers as well as the association with the presence of QS signals in *B. contaminans* isolates recovered from CF patients, non-CF patients and environmental samples.

A total of 93 *B. contaminans* isolates recovered in the period 2003-2013 from different regions of Argentina were analyzed. This collection consisted in 72 clinical isolates belonging to CF patients (63 children and 9 adults), 12 clinical non-CF isolates and 9 environmental samples. Isolates were characterized by *recA*-PCR-RFLP and identified by sequencing of the *recA* gene. BCESM and *cblA* gene were detected by PCR using specific primers. QS signals were identified by means of AHLs biosensors.

Our results showed that 82.6% of the isolates recovered from CF patients carried the BCESM while, in only 10.9% and 6,5% of the isolates recovered from non-CF patients and environmental samples respectively the BCESM was detected. On the other hand, in none of the *B. contaminans* isolates the *cblA* gene could be detected. In order to study the distribution of the BCESM along the 10 years of our survey, we analyzed the presence of this marker in CF children patients. It is important to note that the presence of the QS signals was positively correlated with the incidence of the BCESM in the population analyzed in this work.

Sajjan et al, 2008. Infect Immun76,12:5447-55; Martina et al 2013, J Clin. Microbiol. 51: 339-344

Código de Resumen: MM-031

Sección: Microbiología Molecular

Modalidad: Poster

DISEÑO Y CONSTRUCCIÓN DE HERRAMIENTAS PARA EL ANÁLISIS POR CHIP-SEQ DEL REGULÓN DE *vjbR* EN *Brucella*

DESIGN AND CONSTRUCTION OF TOOLS FOR CHIP-SEQ ANALYSIS OF THE *vjbR* REGULON IN *Brucella*

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The genus *Brucella* comprises several species of facultative intracellular bacteria that are the causative agent of brucellosis, a widespread zoonotic disease that affects animals and humans. VjbR is a LuxR-familiy regulator that plays a central role in the virulence of *Brucella* and affects transcription of a vast number of genes including virulence determinants. Despite extensive efforts to identify the VjbR regulon by microarray and proteomic analyses, the only VjbR-binding site characterized so far corresponds to the *virB* promoter. The aim of this work is to identify all VjbR binding sites in the genome of *Brucella.* To achieve this, we took the challenge to apply an innovative technique called ChIP-seq. This technique comprises several steps including crosslinking of bacteria, chromatin fragmentation, and immunoprecipitation of specific protein-DNA complexes.

In order to mimic the intraphagosomal environment found by *Brucella* within the host, bacteria were incubated under a restricted set of conditions that trigger the expression of the VjbR protein, thus allowing the regulator to bind to its target sequences. Following incubation under low nutrients and low pH (5.5), bacteria were crosslinked and disrupted. Sonication of chromatin was optimized and a fragment size of 250 bp suitable for further analysis was obtained. Proper folding, expression, and integrity of the protein after sonication and decrosslinking was analyzed by Western blot.

In order to prepare a VjbR derivative that can be used for chromatin immunoprecipitation through the use of a high affinity and high specificity monoclonal antibody, we constructed a mutant strain containing a 3xFLAG epitope fused to the N-terminus of the protein VjbR. To check that the 3xFLAG-VjbR protein retains its biological activity, we constructed transcriptional fusions between a target promoter of VjbR and the lacZ reporter gene, in order to perform b-Galactosidase assays.

The developed tool will allow to carry out the subsequent steps, that consist of immunoprecipitation of VjbR-DNA complexes, confirmation by real-time PCR by measuring the amount of immunoprecipitated DNA corresponding to the known binding site for VjbR, and de-crosslinking of DNA. Finally, co-immunoprecipitated DNA fragments will be analyzed by deep sequencing on a platform Illumina.

FISIOLOGÍA MICROBIANA

2 al 4 de Julio de 2014
Hotel 13 de Julio
Mar del Plata, Argentina

Código de Resumen: FM-001

Sección: Fisiología Microbiana

Modalidad: Oral

PRESERVACIÓN DE ALIMENTOS: EFECTOS BACTERICIDAS Y ANTI-COLONIZATION DE POLIFENOLES DEL VINO SOBRE BACTERIAS PATÓGENAS

FOOD PRESERVATION: BACTERICIDE AND ANTI-COLONIZATION EFFECTS OF WINE POLYPHENOLS ON PATHOGENIC BACTERIA

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Synthetic preservatives (i.e. organic acids) play an important role in food quality protection although the economical cost of these chemicals and their intrinsic potential toxicity drives the food industry for the development of alternative preservatives. To this respect, the grape marc, the residue consisting of seeds, stalks and peel after pressing in the production of wines, constitutes a cheap and natural source of a plethora of polyphenolic compounds that would be used as natural food preservatives. In this work, we studied the antioxidant and potential antimicrobial properties of polyphenolic extracts obtained from the marc (orujos) of a varietal wine grape from Argentina (Malbec wine). We show that the marc polyphenolic extracts have a potent antioxidant capacity (evaluated by the % of free radical scavenger effect, ECRL) that correlated well with the total flavonoid content (TFC) of these extracts. Also, we investigated the antibacterial properties of the grape-extracts against the growth of several human-pathogenic bacteria (*Salmonella enteritidis, Salmonella infantis, enterohemorrhagic Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes*) under planctonic- and biofilm-growing conditions as well as the inhibition of bacterial social surface-associated motility (swarming, gliding and sliding). Finally, we demonstrate that the incorporation of low amounts of grape-extracts in fresh foods (i.e. cookies) protect them from the spoilage provoke by aging (antioxidant effect) and contamination with Gram-positive and Gram-negative bacterial pathogens (antimicrobial effect). Overall, these results suggest the potential use of marc grape extracts as natural and cheap preservatives to maintain food quality and avoid its microbial spoilage.

Código de Resumen: FM-002

Sección: Fisiología Microbiana

Modalidad: Oral

IMPACTO DE LAS SEÑALES DE QUORUM SENSING Y HIERRO EN LA ARQUITECTURA DEL BIOFILM Y LA VIRULENCIA DE *Stenotrophomonas maltophilia*

IMPACT OF QUORUM SENSING AND IRON SIGNALS ON BIOFILM ARCHITECTURE AND VIRULENCE OF *Stenotrophomonas maltophilia*

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Stenotrophomonas maltophilia (Sm) is an opportunistic nosocomial pathogen. A key virulence factor is its capacity to form biofilms. In many species quorum-sensing (QS) and iron control biofilm formation and virulence. In Sm the QS signal is the diffusible signal factor (DSF), which synthesis depends on *rpfF.* DSF has been implicated in biofilm dispersal and formation and virulence in a nematode model. We reported the isolation of Sm Fur mutants which produced higher biomass and EPS than wt strains. The Fur mutant F60 presented a higher SOD activity

than K279a. Interestingly K279a*rpf*F mutant (DSF-) showed poor adherence and resistance to oxidative stress. The aim of this work was to study the role of QS and iron on biofilm architecture and virulence of Sm. Studies were done on K279a, K279a*rpf*F and F60 cultured in TSB or in TSB with dipyridyl (Dip) or DSF. Biofilms used for CSLM were stained with SYTO9. 3D images were reconstructed using the ZEN 2009 light edition and ImageJ. For estimation of DSF the bioassay based on its ability to restore endoglucanase production to *rpfF* mutants was used. *Galleria mellonella* was used as an infection model. Ten larvae were used for group, and 5 µl aliquots containing from 10 4 to 106 CFU were injected into the prolegs. Larvae were incubated at 30°C and assessed over 4 days. Survival curves were plotted using the Kaplan-Meier method and differences in survival were calculated by using the log-rank test. Confocal images acquired from K279a biofilms grown in TSB showed a confluent growth with microcolonies scattered on the surface. The presence of Dip produced a more compact biofilm with enhanced thickness, as revealed by the *z*-projection of the *x–y* stacks. In 3D reconstructions peaks of 30 µm in height were observed, while iron restriction resulted in the formation of taller peaks up to 50 µm. F60 showed enhanced extent and complexity of biofilms with several big aggregates and taller peaks up to 60 µm. The QS mutant showed a patchy coverage that failed to form a monolayer and few peaks of 10 µm. Addition of DSF produced an almost confluent growth with numerous microcolonies and peaks of 20 µm. The role of iron in DSF production was assessed by a bioassay. The restriction of iron increased 15% the production of DSF for K279a, a result similar to that obtained with F60. In *G. mellonella* model optimal killing was observed using 105 CFU/larva. K279a*rpf*F was not virulent. A statistically significant difference in *G. mellonella* killing was observed between K279a and F60, being the Fur mutant more virulent. The enhanced virulence could be related to the formation of biofilms with greater biomass and complexity and the production of higher amounts of EPS and SOD than K279a, which could protect F60 from the innate immune system of larvae. In conclusion, DSF and iron restriction are positive signals for Sm biofilm formation and virulence. Results suggest a role of iron in the regulation of QS and virulence factor‘s genes.

Código de Resumen: FM-003

Sección: Fisiología Microbiana

Modalidad: Oral

PROPIEDADES PROBIÓTICAS Y ANTI-*Helicobacter pylori* DE LA CEPA ÁCIDO-TOLERANTE *Lactobacillus salivarius* UCO_979C

ANTI-*Helicobacter pylori* AND PROBIOTIC PROPERTIES OF ACID-TOLERANT *Lactobacillus salivarius* UCO_979C STRAINS

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Helicobacter pylori is a gram-negative microaerophilic human gastric pathogen, which causes several gastric pathologies. Current treatment of *H. pylori* infection reports serious side effects and falling efficacy because of antibiotic resistance. Therefore, the trend is to look for alternative therapies, such as natural food substances and probiotics. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Most probiotics are strains of *Bifidobacterium* or *Lactobacillus* species.The acidic environment and the bile salts in the gastrointestinal tract are major impediments to the survival of ingested bacteria. Hence, the ability to survive passage through the gastrointestinal tract is vitally important physiological attribute for a bacterium to function as a probiotic. The aim of this work was evaluate the effect of acid-stress acclimatization of *Lactobacillus salivarius* UCO_979C on its exopolysaccharide (EPS) production, cell morphology, protein profile and anti-*H. pylori* activity. First, we acclimated the strain *L. salivarius* UCO_979C at pH 2.6. EPS were isolated using ethanol precipitation method and quantified by phenol-sulphuric acid method. For discriminating external variations between wild *L. salivarius* UCO_979C and

acclimated strain, we used Fourier Transform Infrared Photoacoustic Spectrocopy (FTIR-PAS). To measure total protein concentration, Bradford Method was done. Membrane proteins were analyzed by SDS-PAGE. Anti-*H. pylori* activity was evaluated by agar diffusion assay. The results show EPS overproduction in acid stress conditions until the first 24 hours. Acid stress modified important components at macromolecular level, such as amides in proteins, glycosidic linkages and branches in sugars and methyl groups and ester linkages in fatty acids. Proteins profiles showed changes between bands patterns of native and acclimated strain. Finally, anti-*H. pylori* activity increases at acid stress conditions. Our results provide new insight into the changes involved in the acid tolerance of *L. salivarius* UCO_979C and shows that tolerance to acid stress is an important factor in the evaluation of strains with potential probiotic activity.
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Código de Resumen: FM-004

Sección: Fisiología Microbiana

Modalidad: Oral

PERFILES REDOX EN BIOFILMS DE *G. sulfurreducens* DETERMINADOS MEDIANTE MICROSCOPÍA RAMAN CONFOCAL

REDOX PROFILING OF *G. sulfurreducens* BIOFILMS BY CONFOCAL RAMAN MICROSCOPY

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Exocellular electron conduction is one of the most fascinating discoveries of microbiology in the last decade. In natural and man-made environments it is thought to play a fundamental role, allowing the exchange of electrons between bacterial cells and between these and external electronic acceptors. Moreover, it is one of the fundamental processes behind the production of electricity by electroactive biofilms grown on electrodes, which are of paramount importance in emerging technologies as microbial fuel cells, microbial electrolysis cells, the microbial electrosynthesis process, and whole cell biosensors. Aiming at gaining information on structural and physiological features of electricity-producing biofilms, we have constructed an electrochemical cell that can be mounted on the stage of a microscope. The cell is designed to use thin film transparent electrodes, thus allowing the observation of biofilms *in situ* and *in vivo* through the electrode. By this cell in conjunction with a confocal Raman microscope (CRM), the redox state of molecules at different focal planes of *Geobacter sulfurreducens* biofilms was explored. Obtained results showed a redox gradient across electricity-producing biofilms which was dependent on the potential applied to the electron acceptor, with the fraction of reduced species being higher in upper layers of the biofilm. Therefore, respiration in these layers is expected to be limited. The approach provided new information about internal biofilm physiology, relevant for the understanding energy production constrains and electron conduction mechanisms in these systems.

Código de Resumen: FM-005

Sección: Fisiología Microbiana

Modalidad: Poster

OMPQ, UN FACTOR DE VIRULENCIA INVOLUCRADO EN LA FORMACIÓN DE BIOFILM POR *Bordetella bronchiseptica*

OMPQ, A VIRULENCE FACTOR INVOLVED IN *Bordetella bronchiseptica* BIOFILM FORMATION

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Bordetella species are small aerobic, Gram-negative bacteria that colonize the respiratory tract of humans and animals. *Bordetella pertussis*, a strictly obligate human pathogen, is the etiologic agent of whooping cough, while *Bordetella bronchiseptica* mainly infects animals causing a variety of respiratory diseases. We and other groups have recently demonstrated that these bacteria are capable of living as sessile communities known as biofilms on different abiotic surfaces and in the respiratory tract of mice. OmpQ is a general outer membrane porin of *Bordetella*, whose expression is positively regulated by the two-component signal transduction system BvgAS, which is also involved in the expression of the most important virulence factors of these bacteria. Using proteomic approaches we had found higher expression levels of the general porins OmpP and OmpQ in *B. pertussis* biofilm cells than in planktonic counterparts, either for reference and clinical strains. The purpose of this work was to study the contribution of OmpQ to biofilm formation by *B. bronchiseptica*. To this aim an in-frame, non-polar mutation was obtained through allelic exchange on *B. bronchiseptica* RB50 strain. No differences were observed under planktonic growth conditions in Stainer-Scholte (SS) medium between the wild-type and Δ*ompQ* strains, nevertheless, quantification of the biomass adhered to the wells of microtiter plates after 48 h of biofilm development showed significant differences between both strains, observing a reduction of the biofilm biomass in the OmpQ defective strain. Next, we studied the *ompQ* gene expression profile during the biofilm formation process, detecting an increment of its expression at 48 h of biofilm culture, indicating a specific role of OmpQ at later stages of biofilm development. In addition, we evaluated the potential use of OmpQ as an antigen that could prevent biofilm formation. To this aim, OmpQ was recombinantly expressed in *E. coli*, purified and inoculated into mice to obtain afterwards polyclonal serum. The anti-OmpQr serum showed recognition against OmpQ and recombinant OmpQ (OmpQr). When the anti-OmpQr serum was added to the growth medium at the beginning of experiments, a reduction of the biofilm biomass level of *B. bronchiseptica* was observed after 48 h of incubation in a dose-dependent manner. Normal serum was added to SS medium as negative control. In conclusion, our results contribute to the identification of new key factors for *Bordetella* biofilm development that could act as protective antigens by inhibiting biofilm formation and, in addition, lead to a better understanding of the mechanisms involved in the process of *B. bronchiseptica* biofilm formation.

Código de Resumen: FM-006

Sección: Fisiología Microbiana

Modalidad: Poster

AUSENCIA DE COMPETENCIA NATURAL EN *Lactobacillus casei BL23* BAJO CONDICIONES DE ESTRÉS EN LAS QUE SE SIMULA EL AMBIENTE DEL TRACTO GASTROINTESTINAL

ABSCENCE OF NATURAL COMPETENCE IN *Lactobacillus casei BL23* UNDER STRESS CONDITIONS RESEMBLING THE GUT ENVIRONMENT

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Most of the probiotic bacteria currently available in the dairy industry belong to the genera *Lactobacillus.* Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotics are used as starter cultures in dairy and non-dairy products. They are considered GRAS (generally recognized as safe) and QPS (Qualified presumption of safety status) for their safe history of usage. Probiotic *Lactobacillus* strains exceptionally show transferable antibiotic resistance. However, transferability studies have not been assayed systematically. Transferable antibiotic resistance is the only relevant cause for caution since they might potentially serve as hosts of antibiotic-resistance genes, with the risk of transferring these genes to other bacteria. This in fact is the reason why genetically modified microorganisms are not approve to be used in food as they might experience horizontal gene transfer with the autochthonous microbiota, however this fact has never been clearly established.

Lactobacilli are able to survive the gastrointestinal transit and transiently colonize our gut. In that environment they encounter multiple stress conditions. To address transferability we decided to evaluated if those gut conditions are able to modify the gene expression of for *comX*, annotated as alternative sigma factor of the RNA polimerase, YP_001986877.1, Gene ID: 6404650, a putative regulatory element of competence cascade coded in the genome of *Lactobacillus casei* BL23. For this purpose we checked conditions that resembled those found in the gastrointestinal tract like salt stress or acid pH with bile salts and compared them to those known to promote competence gene expression like starvation, UV and heating. Messenger RNA of *comX* gene, was analyzed by Dot Blot and qPCR and related to their expression in early stationary phase growth condition and the housekeeping gene 16S rRNA. We also verified the transfer of an antibiotic resistance marker (CmR) evaluating the naturally transformations in any of these conditions by evaluating the most probable number (MPN).

The results showed that an increase in the expression of *comX* was observed in the condition of UV and acid pH with bile salts.

However the numbers of naturally transformants obtained in those conditions were not significantly different from non-induced condition as verified by MPN. Although an induction of expression was observed it was not enough to supply competence for plasmid transformation. These results argue in favor of the fact that Lactobacilli might not be able to be naturally transformable, although it is not sufficient to ensure the absence of transfer mechanisms.

Código de Resumen: FM-007

Sección: Fisiología Microbiana

Modalidad: Poster

EVALUACIÓN DE ACTIVIDAD INHIBITORIA DE *Lactobacillus* spp. AISLADOS DE CERDO SOBRE *Escherichia coli* PRODUCTOR DE TOXINA SHIGA

EVALUATION OF THE INHIBITORY ACTIVITY OF SHIGA-LIKE TOXIN PRODUCING *Escherichia coli* BY *Lactobacillus spp.* ISOLATED FROM PIGS

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Foodborne diseases represent health and economic problems of relevance in the world. Symptoms vary depending on the causal agent, the dose of intake and the age of the group affected. Bacteria most implicated are Shiga-like toxin producing *Escherichia coli* (STEC). To reduce the risk of infection by pathogen is necessary a strict microbiological monitoring throughout the food production chain. One strategy is the use of biopreservation systems as lactic acid bacteria or their metabolites. The genus *Lactobacillus* is of interest as new biotechnological applications and by their probiotic properties. Usually, these strains are isolated from humans because of their greater ability to adhere to and colonize the intestinal epithelium. However studies have shown that strains of *Lactobacillus* isolated from animals also have beneficial effects on human. Through this study, it was determinate the inhibitory activity of *Lactobacillus* spp. isolated from pigs. This was evaluated on *EDL933* STEC reference strain isolated from a case of hemolytic uremic syndrome. *Lactobacillus* spp. was inoculated by puncture on MRS agar plates. After incubation, the plates were exposed to chloroform vapors for inactivating bacteria. Later, the plates were covered with LB agar containing STEC. Finally, the inhibitory activity was evaluated by observing the translucent area around the colony. Of the three strains tested, two inhibited growth of STEC, with the halo of inhibition greater than 1 mm. This suggests that *Lactobacillus* have characteristics of antagonism against pathogens. It could be concluded that the biopreservation ability of *Lactobacillus* spp. generates alternatives for producing foods naturally protected and improve the microbiological quality in the food industry.

Código de Resumen: FM-008

Sección: Fisiología Microbiana

Modalidad: Poster

EL SISTEMA INMUNE DEL COMPLEMENTO Y SU EFECTO SOBRE LA ARQUITECTURA DEL BIOFILM BACTERIANO

THE IMMUNE COMPLEMENT SYSTEM AND ITS EFFECT ON THE BACTERIAL BIOFILM ARCHITECTURE

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Spores of the human-friendly bacterium Bacillus subtilis represent a new generation of probiotics due to their high stability and additional utilization as non-refrigerated antigen-delivery vaccines. An interesting premise for probiotics is their capacity to modulate the immune system. The Complement System (C) is a major effector of innate response and is under the control of three different pathways of activation (Classic, CP; Alternative, AP; and Lectin pathway, LP). However, the understanding of the probiotic effect on C is waiting. Using a battery of bacterial genetic techniques, in combination with specific methods to evaluate C activation, we demonstrate that B. subtilis is capable of activating the three C pathways without MAC formation. This activation is under the control of the master transcriptional regulator of biofilm development SinR. Using different genetically-constructed *B. subtilis* mutants, we uncovered each component of the biofilm involved in C regulation. *B. subtilis* mutants deficient in the

synthesis of exopolysaccharide (EPS) and the antimicrobial and antibiotic-repellent proteins TasA and BslA, respectively, were severely affected in C activation. Furthermore, we demonstrate a specific role for each biofilm component on each C activation-pathway. TasA was essential and specific for CP-activation whereas BslA and EPS were essential and specific for the activation of the AP and LP, respectively. Overall, our results show for the first time how different components of the bacterial biofilm (which colonize the human mucosa) modulates the activity of C and contribute to a better understanding of the beneficial effects of probiotics on innate immunity at molecular level.

Código de Resumen: FM-009

Sección: Fisiología Microbiana

Modalidad: Poster

EFECTO DE NANOPARTÍCULAS DE PLATA SOBRE EL DESARROLLO DEL BIOFILM

EFFECT OF SILVER NANOPARTICLES ON BIOFILM DEVELOPMENT

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In recent years, the resistance of bacteria to antimicrobials has increased due to the slapdash use of antibiotics in animal feed as growth promoters as well as their abusive utilization in medicine and veterinary. As a consequence of the emergency in antibiotic-resistance, there is a failure in antibiotic-treatment that results in a significant increase of mortality, morbility, cost of medical treatments and spreading of infectious bacteria between the human population.The bactericidal effects of ionic silver are known and applied since antiquity. Silver is used in several medical devices and surgical equipments such as burn dressings, scaffolds, water purification systems and medical devices because, and specially, silver nanoparticles (SNPs) may damage the activity of bacterial enzymes and cell structures, which cause bacterial cells to die. Biofilms are surface associated bacterial communities, in which bacteria are enveloped by polymeric substances known as the biofilm matrix. *Bacillus subtilis* biofilms display persistent resistance to liquid wetting and gas penetration, which probably explains the broad-spectrum of resistance and tolerance of bacterial biofilms to antimicrobial agents. In this work we analyze the ability of *B. subtilis*, and isogenic mutants affected in the synthesis of the extracellular matrix (ECM), to form biofilms in the presence of AgNPs. Our results show that silver nanoparticles have a greater inhibitory effect on biofilm development that the inhibition of biofilm formation produced by the germicide compound silver nitrate. In addition, it was observed that strains defective in the formation of particular ECM components (lipids, exopolysaccharide, etc.) differentially responded to the presence of AgNPs, suggesting a selective and exclusive effect of this novel nanomaterial on biofilm architecture.

Código de Resumen: FM-010

Sección: Fisiología Microbiana

Modalidad: Poster

El REGULADOR GLOBAL CREC DE *Escherichia coli* AFECTA EL BALANCE REDOX INTRACELULAR Y LA RESISTENCIA A ESTRÉS OXIDATIVO

THE GLOBAL REGULATOR CREC OF *Escherichia coli* AFFECTS THE INTRACELLULAR REDOX BALANCE AND RESISTANCE TO OXIDATIVE STRESS

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The survival of an organism depends, at least in part, on its ability to sense and respond to changes in the environment. In bacteria, changes in the physical and nutritional characteristics of the environment generate immediate responses, controlled through the regulation of sets of genes in response to specific environmental stimuli and metabolic signals.
In this work we studied the effect of CreC, a global regulator, in different aspects of bacterial fisiology, such as redox balance, oxygen consumption, and oxidative stress, in conditions in which CreC is active (M9 mineral media suplemented with glucouse and low oxygen availability).
In the firsts attemps to caracterize the metabolic profile of strains with mutations in *creC* , a clear decrease of the intracellular redox state could be observed in the creC strain (DC1060) when compared to the wild type strain (K1060), evidenced through the [ethanol]/[acetate] rate. This results led us to test the growth of these strains in M9 glucouse with toluidine blue. In this media, cells with a lower respiratory activity tend to produce more reactive oxygen species (ROS) due to the dye present in the media, giving smaller colonies. Surprisingly, the mutant strain, which was supposed to have a more oxidative intracellular environment, gave rise to smaller colonies than the wild type. To see if the absence of CreC was the cause of the higher ROS suscenpitibility, strains were grown in the presence of oxidative chemicals, such as hydrogen peroxide and paraquat, to determine the minimum inhibitory concentration (CIM). In these experiments, we could corroborate that the absence of CreC augmented the susceptibility of the cells to oxidative stress. Finally we measured the rate of reduced/oxidized cofactors [NAD(P)H/NAD(P)$^+$] to confirm the oxidized intracelluar state of *creC* mutants. Strain DC1060 was observed to have a lower NADH/NAD$^+$ rate than the K1060 strain in oxygen limiting conditions.
These results suggest that CreC affects the redox balance, most probably by affecting carbon flux in the fermentative routes. As the molecular mechanisms involved in the response of ROS production require cofactors, the lower NADH/NAD$^+$ rate observed in strain DC1060 could be the reason for the increased susceptibility to oxidative chemicals.

Código de Resumen: FM-011

Sección: Fisiología Microbiana

Modalidad: Poster

ANÁLISIS GENÓMICO COMPARATIVO DE CEPAS DE *Enterococcus mundtii* AISLADAS DE DIFERENTES FUENTES

COMPARATIVE GENOMIC ANALYSIS AMONG *Enterococcus mundtii* STRAINS ISOLATED FROM DIFFERENT SOURCES

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Enterococci are a diverse and versatile group of gram positive lactic acid bacteria. They live as commensal microorganisms in the gastrointestinal tract of humans and animals, but they are also found in soil, water, plants and are used in the production of fermented foods and probiotics. Due to their intrinsic characteristics and a remarkable metabolic adaptability, they are capable of surviving and growing under different conditions and to tolerate many forms of stress.

The study of enterococci´s genomics has grown considerably in recent years and has allowed us to obtain new insights into the physiology of this genus. Using different approaches it has been concluded that many metabolic genes and pathways vary, even within single species of this genus. Particularly, genome comparisons are really important for determining the genotypic differences between closely related bacteria and for understanding genomic and molecular evolution while considering the phenotypic evolution and population genetics.

Enterococcus mundtii CRL35 is a bacteriocinogenic non starter lactic acid bacteria strain whose genome was recently sequenced. Based on this information, the goal of the present work was to perform a comparison of the metabolic reconstruction of this strain against those belonging to *E. mundtii* CRL1656 (cow milk isolate), *E. mundtii* QU 25 (ovine faecal origin) and *E. mundtii* ATCC 882 (fermented product origin), through the RAST tool. The results obtained revealed that QU 25 has, at the chromosome level, 67 coding DNA sequences (CDS) assigned to subsystems that are not present in CRL35. Some of these genes are involved in gram positive competence, CRISPR-Cas system, citrate metabolism and toxin-antitoxin replicon stabilization systems. On the other hand, CRL35 has CDS related to bacterial restriction-modification system, sialic acid metabolism and biotin biosynthesis, that are not present in QU 25. Regarding CRL1656, 42 functional roles have genes assigned to subsystems that are not found in CRL35, some of which are related to the citrate metabolism and the com system. Also CRL35 has 46 roles with genes not localized in CRL1656. Finally, the strain ATCC 882 has 60 functional roles with genes involved in citrate metabolism, com system and phages production, that are not present in CRL35. Only 13 roles with genes assigned are found in CRL35 and not in ATCC 882.

Furthermore, an orthology analysis evidenced that *E. mundtii* CRL35, CRL1656 and ATCC 882 have 1772 orthologous proteins while the last two strains have 2141 suggesting, therefore, a less evolutionary distance among them. These analyses help us to understand how enterococci adjust to different environments. Transcriptomic studies of CRL35, now under way, will provide an overview of the physiological responses of this microorganism in fermented foods.

Código de Resumen: FM-012

Sección: Fisiología Microbiana

Modalidad: Poster

CARACTERIZACIÓN DE PLÁSMIDOS CONTENIENDO *bla*OXA-58 EN CEPAS MULTIRRESISTENTES CLONALMENTE RELACIONADAS DE *Acinetobacter baumannii*

CHARACTERIZATION OF PLASMIDS HARBORING *bla*OXA-58 IN CLONALLY RELATED MULTIRESISTANT *Acinetobacter baumannii* CLINICAL STRAINS

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Acinetobacter baumannii is an important opportunistic pathogen responsible for a variety of nosocomial infections and outbreaks that can rapidly evolve multidrug resistance (MDR) when confronted with antibiotic therapy. In this context, the acquisition of resistance determinants mediated by horizontal gene transfer (HGT) is considered a major determinant of MDR particularly in environments under strong antibiotic pressure such as the clinical setting. In particular, the emerging resistance to carbapenems represents a major concern worldwide. One of the mechanisms playing a significant role in *A. baumannii* carbapenem resistance is the production of OXA-type carbapenemases, whose genes are generally carried by plasmids. Thus, the analysis and understanding of the dissemination mechanisms of genetic platforms carrying the *bla*OXA-58 genes is fundamental for the prevention of outbreaks. In this context, we have recently described the presence of a plasmid overexpressing *bla*OXA-58 as a result of an ISAba825-generated hybrid promoter which largely enhances the carbapenem resistance to harboring bacteria. We additionally found that this arrangement can be carried in an adaptability module.

Here we present the characterization of two different plasmids (pAb242 and pAb825), both carrying the ISAba825-*bla*OXA-58 arrangement, which were isolated from clonally related carbapenem-resistant clinical *A. baumannii* strains designated Ab242 and Ab825, respectively. Both plasmids were isolated from the corresponding strains, used to transform the *A. baumannii* ATCC 17978 multisensitive strain, and further selecting for imipenem (IPM) resistance. We found that both plasmids could replicate and could direct expression of *bla*OXA-58 gene in this new host, as shown by the 8-fold increases in MIC values towards IPM (from 0.5 μg/ml to 4 μg/ml for both ATCC 17978 transformants). Production of OXA-58 was evident as judged by SDS-PAGE analysis of bacterial extracts. Restriction analysis of pAb242 and pAb825 recovered from recombinant bacteria showed however relevant differences in sizes indicating that both plasmids are similar but not identical.

Overall, the presence of ISAba825-*bla*OXA-58 in plasmids from clonally related *A. baumannii* clinical strains suggest dissemination of this arrangement through HGT and further selection by carbapenem pressure in the nosocomial environment. The possibility of transference of the adaptability module between *A. baumannii* plasmids is discussed.

Código de Resumen: FM-013

Sección: Fisiología Microbiana

Modalidad: Poster

ACEPTABILIDAD SENSORIAL Y ACTIVIDAD ANTIMICROBIANA DE ACEITES ESENCIALES SOBRE BACTERIAS CONTAMINANTES Y PATOGENAS DE PURE DE TOMATE.

SENSORIAL ACCEPTABILITY AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS ON SPOILAGE AND PATHOGENIC BACTERIA FROM TOMATO PURÉE.

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The new trend in food industry, preferred by consumers, is the use of natural preservatives to replace traditional methods. Investigations are being oriented on the application of new methods of making food safe which have a natural or 'green' image. Application of essential oils (EOs) is a very attractive method for controlling postharvest diseases and spoilage in various fruits and vegetables. In a previous work we isolated and identified *Klebsiella ornitolytica* and *Candida tropicalis* from tomatoes surface. In addition we demonstrated that *Escherchia coli* was able to growth in tomato puree during storage at 30ºC. So, the aim of this work was to test and compare the antimicrobial activities of four EOs against undesirable bacteria and yeasts isolated tomatoes and obtained from collection cultures. Inhibition of growth by different concentration (50-300 ppm) of EO from lemon(*Citrus limon*), oregano(*Origanum vulgare* L.), onion (*Allium cepa*) or garlic(*Allium sativum*) was tested by the paper disc agar diffusion method. First, the sensory acceptability of the EOs was evaluated in tomato purée (TP) by an untrained panel using a hedonic scale of four points. Lemon essential oil was the most acceptable for all the different concentrations tested except 300 ppm. Contrary, onion essential oil was not acceptable under any condition. Garlic essential oil did not show antibacterial activity, being only active against the *Candida tropicalis.* The growth of all tested microorganisms, *E. coli* ATCC 25922, *Klebsiella ornitholytica*, *Listeria monocytogenes* and *C. tropicalis* were inhibited by lemon and oregano essential oils at 300 ppm, which was considered unacceptable from view point sensorial. In this condition, oregano essential oil was the most effective, producing inhibition zones between 12 and 27 mm. Lemon essential oil was the only one capable of inhibit the growth of *E .coli* ATCC 25922, *K.ornitolytica* and *L. monocytogenes* at lower concentrations (150-200 ppm). At these concentrations the organoleptic contribution of lemon EO to the flavor was satisfactory. In conclusion, lemon, oregano and garlic Eos showed promising results, especially lemon EO for the utilization of as suitable preservative agent for use in minimallyprocessed fruit products.

Código de Resumen: FM-014

Sección: Fisiología Microbiana

Modalidad: Poster

CARACTERIZACIÓN BIO-ELECTROQUÍMICA DE CULTIVOS LÍQUIDOS AEROBIOS DE LA MICROALGA *Scenedesmus dimorphus*

BIO-ELECTROCHEMICAL CHARACTERIZATION OF AEROBIC LIQUID CULTURES OF THE MICROALGA *Scenedesmus dimorphus*

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The use of microbial metabolism for the conversion of chemical energy into electricity has given raise to a series of bio-electrochemical devices. In the most promising approach, bacteria that transfer electrons directly to electrodes, have been utilized in hybrid anodes of microbial fuel cells (MFCs). Current production in MFCs depends largely on the kinetics of the reduction that takes place at the cathode. Power output has been shown to be proportional to the concentration of dissolved O2 in the catholyte, but increasing the dissolved O2 concentration is limited by its solubility in water. The addition of photosynthetic organisms to the cathodic compartment of MFCs has been proposed as a way to reduce cathodic limitation, due to their ability to increase the concentration of O2 to much higher concentrations than its solubility, process driven by the photo-oxidation of water during photosynthesis. In addition, this strategy has the environmental added value of enabling electricity generation from sunlight and carbon dioxide. The bio-electrochemical properties of photosynthetic microorganisms have not been characterized to detail. Specifically, although electron transfer between cathodes and microalgae has been proposed, the mechanisms have not been described. In this work, we initiate the characterization of bio-electrochemical properties of the green microalga *Scenedesmus dimorphus* in aerobic liquid cultures, in relation to three different electrode materials: stainless steel, graphite and platinum. We measured the open circuit potential (OCP) of cell cultures at different cell densities, either during light or dark periods. We also measured current generation when potentials from 0 to -0.9 V were applied. OCPs showed characteristic values for each material, with slight variations with culture conditions. Current generation on the other side, depended on the light:dark cycle, but this was strictly related to the concentration of O2 in the culture. In fact, current production using the three electrode materials showed a linear relationship with O2 over a wide range of concentrations, from 2 to above 20 mg/L of dissolved O2 . Platinum proved to be the best of the three electrode materials. Current production using platinum was 3-fold higher than using graphite and 2- to 6-fold higher than that provided by stainless steel at the most negative potentials that were applied (-0.4 to -0.9 V). Furthermore, at potentials more compatible with the use as a bio-cathode in *Geobacter* sp. MFCs (e.g. -0.15 V), platinum was 8-fold better than graphite and 60-fold better than stainless steel for current generation. However, considering the high cost of platinum, graphite electrodes constitute, overall, an appropriate option for use in photosynthetic MFCs.

Código de Resumen: FM-015

Sección: Fisiología Microbiana

Modalidad: Poster

ANÁLISIS DE SECUENCIA DE UN NUEVO PLÁSMIDO PORTANDO *bla*OXA-58 AISLADO DE UNA CEPA CLÍNICA LOCAL DE *Acinetobacter baumannii*

SEQUENCE ANALYSIS OF A NOVEL PLASMID CARRYING *bla*OXA-58 ISOLATED FROM A LOCAL *Acinetobacter baumannii* CLINICAL STRAIN

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The emergence of carbapenem resistance in *Acinetobacter baumannii* has been reported worldwide and correlated with the acquisition of carbapenem-hydrolyzing oxacillinases. Dissemination of *bla*OXA-58 genes by plasmids may explain the wide propagation of these genes among nosocomial *A. baumannii* strains. We reported previously the presence of *bla*OXA-58 -expressing plasmids in local clinical strains of this opportunistic pathogen. Still, little is known about the replicon types of *bla*OXA-58 -carrying plasmids in *A. baumannii* strains from Argentina. We present here the sequence analysis and characterization of a plasmid isolated from a multiresistant (MDR) *A. baumannii* clinical strain (Ab242) from a Rosario hospital and carrying the ISAba825-*bla*OXA-58 arrangement that we described previously.

The complete Ab242 sequence was done by pyrosequencing (INDEAR-Rosario). Exhaustive analysis of its whole genome allowed the identification of four plasmids, one of them harboring the ISAba825-*bla*OXA-58 arrangement and designed as pAb242. pAb242 contigs were manually gathered in a 22.8 kbp circular construct, and this assembly validated by PCR. The plasmid was further classified on the basis of its replication systems. Phylogenetic analysis employing the plasmid-borne replicase genes including representative Rep groups could classify pAb242 as a multireplicon plasmid showing homology with both p11921 Rep Aci8 and pACICU1 Rep AciX, both isolated from clinical *A. baumannii* strains in Italy. Remarkably, pAb242 represents a chimera of these two replicons. Further sequence analysis shows that pAb242 is composed of different modules responsible of replication, maintenance, adaptability, and mobility. The replication and maintenance modules are closely related to those of pACICU1, which also carries *bla*OXA-58 but lacks ISAba825. The adaptability module comprises a backbone region containing a ISAba3 located immediately upstream *bla*OXA-58 and a *araC1* and *lysE* downstream of this gene. Comparative sequence analysis showed that this adaptability module has unique features: i) the presence of ISAba825 inserted within ISAba3 generating a hybrid promoter overexpressing *bla*OXA-58 ; ii) an ISAba125 interrupting the *lysE* gene. In turn, the pAb242 mobility module showed no detectable homology to other plasmids harboring *bla*OXA-58 genes.

The pAb242 adaptability module showed similar backbone regions than other plasmids isolated from other *A. baumannii* strains as well as *A. pittii* and *A. nosocomialis*, thus suggesting the dissemination of this region among the *A. calcoaceticus-baumannii* (ACB) complex. Still, pAb242 represents a novel plasmid exhibiting distinctive characteristics, including its adaptability module, an unusual multireplicon, and a mobility module. Genetic platforms such as pAb242 may be responsible for the wide dissemination of *bla*OXA-58 genes among strains of the ACB complex.

EDUCACIÓN EN MICROBIOLOGÍA

2 al 4 de Julio de 2014
Hotel 13 de Julio
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Modalidad: Oral

BIOTECNOLOGÍA INDUSTRIAL Y MICROBIOLOGÍA APLICADA (BIMA): UN ENFOQUE DIFERENTE PARA EL DICTADO DE UN CURSO DE GRADO

INDUSTRIAL BIOTECHNOLOGY AND APPLIED MICROBIOLOGY (IBAM): AN INNOVATING COURSE FOR UNDERGRADUATE LEVEL

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In our country, emergent biotechnological processes are focused on agriculture, health and environment. Biotechnology courses are chosen by Chemistry and Biology students of the Faculty of Sciences (FCEN-UBA) eager to acquire tools for industrial activities. Until 2009 two courses were offered, but mainly dedicated to animal or agrobiotechnology. To cover a curricular deficiency, IBAM was implemented ever since as a 128 h-course, optional, in first instance, either for undergraduate or graduate students. The learning objectives of IBAM are to introduce the basis of the industrial application of prokaryotes, to update students about daily problems with biotechnological processes and train them in finding solutions, to improve both intellectual and manual skills to be applied in fermentation and bioremediation technologies and to stimulate the critical criterion, both in technical aspects and in ethic implications. Based on the hypothesis that students are not trained in solving problems that integrate topics, IBAM was planned as an innovative approach. IBAM is organized in theoretical-practical classes, with a simultaneous combination of lectures and 8 lab exercises. The challenge is the evaluation of comprehending experimental methodology and the training in result analysis instead of appealing to memory to reproduce taught topics. The average of two written exams with the contribution of lab performance, reports and oral expositions should surpass 8 over 10 points; if it does not, an integrated final exam is required. In spite of the declared extra effort and the difficulty of the exams, the number of students has been increasing from 12 to 40, with 6-10 graduated students. Proof of IBAM effectiveness is the students´ performance and opinions. Surveys revealed that for students some of the topics are new (23%) and others are more deeply discussed and taught in an integrated way than in other courses (68%). IBAM helped with the understanding of industrial application of bacteria (94% students), in bacteria and environment (45%) and metabolism and its regulation (45%). As a 97% of students would strongly recommend IBAM to peers, an increase of their number is expected for 2014.

Código de Resumen: EM-002

Sección: Educación en microbiología

Modalidad: Oral

CLASE EXPERIMENTAL PARA ILUSTRAR DAÑO MUTAGÉNICO LETAL POR RADIACIÓN UV, FOTOREPARACIÓN Y REPARACIÓN MUTAGÉNICA EN UNA CEPA DOBLE AUXOTRÓFICA DE *Pseudomonas aeruginosa*

A SIMPLE LABORATORY CLASS USING A *Pseudomonas aeruginosa* AUXOTROPH TO ILLUSTRATE UV-MUTAGENIC KILLING, DNA PHOTOREPAIR AND MUTAGENIC DNA REPAIR

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A simple and cheap laboratory class is proposed to illustrate the lethal effect of UV radiation on bacteria and the operation of different DNA repair mechanisms. The experimental session involves two separate experiments: one dedicated to illustrating the lethal effect of UV radiation and the protective effect of DNA photorepair; the second to explore the operation of DNA repair mechanisms that prioritise survival but introduce mutations. The procedure makes use of a *Pseudomonas aeruginosa* double auxotroph (Lys- , Arg-) carrying base replacements in the biosynthetic genes *lysA* and *argH*, which serves to detect UV-induced back-mutations to prototrophy. The experimental procedure can be carried out in a single lab session of about 3 hours, with the simultaneous participation of no more than twenty-five students divided into groups of two or three (depending on the availability of laminar flow chambers). A second session of about 2 hours is required for colony count and analysis of the results.

The proposed scheme is carried out by undergraduate students of the Bacterial Physiology and Genetics course, as part of our Biotechnology curriculum, after approving previous related courses like General Microbiology and Introduction to Molecular and Cellular Biology. The design of the practical lab session follows that of scientific experiments, in which students collect, analyse and interpret their own data, and utilize them to challenge the hypotheses discussed during the theoretical part of the course in which concepts on bacterial DNA replication, mutagenesis and repair have been already discussed. We think that it will be a valuable tool for microbiology students to increase their understanding of basic genetic concepts.

BIOREMEDIACIÓN Y BIOCONTROL

2 al 4 de Julio de 2014
Hotel 13 de Julio
Mar del Plata, Argentina

Código de Resumen: BB-001

Sección: Bioremediación y Biocontrol

Modalidad: Oral

CONTRIBUCIÓN DE LA PROTEÍNA S-LAYER EN LA ACTIVIDAD MOSQUITOCIDA DE *Lysinibacillus sphaericus*

CONTRIBUTION OF S-LAYER PROTEINS TO THE MOSQUITOCIDAL ACTIVITY OF *Lysinibacillus sphaericus*

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Lysinibacillus sphaericus strains belonging the antigenic group H5a5b, produce spores with larvicidal activity against *Culex* mosquito's larvae. C7, a new isolated strain, that presents similar biochemical characteristics and Bin toxins in their spores as the reference strain 2362, was however up to 20-fold more active against *Culex* mosquitos' larvae. The contribution of the surface layer protein (S-layer) to this behaviour was envisaged since this envelope protein has been implicated in the pathogenicity of several bacilli and we have previously reported its association to spores. Microscopic observation by immunofluorescence detection with anti S-layer antibody in the spores confirms their attachment. S-layers and BinA BinB toxins formed high molecular weight multimers in spores as shown by SDS-PAGE and western blot detection. Purified S-layer from both *L. sphaericus* C7 and 2362 strains were by itself toxic against *Culex sp* larvae, however that from C7 strain was also toxic against *Aedes aegypti*. Synergistic effect between purified S-layer and spore-crystal preparations was observed against *Culex* sp. and *Aedes aegypti* larvae in particular with the C7 strain. *In silico* analysis of the S-layer sequence suggest the presence of hemolytic and chitin-binding domains. Both biochemical characteristics were observed for both S-layers´ strains that must justify their contribution to pathogenicity.

Código de Resumen: BB-002

Sección: Bioremediación y Biocontrol

Modalidad: Oral

BACILLUS FORMADORES DE ESPORAS COMO AGENTES NATURALES DE BIOCONTROL DE ENFERMEDADES DE PLANTAS

SPORE-FORMING *BACILLI* AS NATURAL AGENTS OF BIOCONTROL OF PLANT-DISEASES

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Pests and plant diseases constitute a problem that began with agriculture itself. The massive use of synthetic (chemical) agents of biocontrol (i.e. funguicides and insecticides) with potential toxicity to the users and the environment pushes for the development of alternative (ecological and natural) compounds. To this respect, the use of natural and human-friendly bacteria with insecticide and funguicide activities (biocontrol agents) represents an interesting alternative. In this research we focused our attention in the isolation and characterization of natural isolates of spore-forming bacteria (*Bacilli*) from different agricultural regions of Argentina seeded with soybeans (spring-summer) and maize-wheat (autumn-winter). The ability of spore formation in *Bacilli* makes this type of bio-controlling bacteria very attractive for industrial purposes and scaling-up due to the high stability and longevity of the spores which, after germination, would

produce the biocontrol agents. Hundreds of *Bacillus* strains (n=743) were intensively isolated from agricultural soils during a one year period and assayed against fifteen different and relevant fungal phytopathogens (i.e. *Fusarium*, *Cercospora*, *Alternaria*, *Phomopsis*, *Mucor, Phomopsis, Penicillium*, *Rhizoctonia* and *Aspergillus* strains). The phylogenic analysis indicated that most of the biocontrol isolates possessed great and broad antifungal activities and belonged to the *Bacillus subtilis* / *Bacillus amyloliquefaciens* eco-group. Because of their importance for the colonization of the plant rhizosphere we studied and characterized their ability to form biofilms and surface translocation.
Similarly, we studied the solar and UV-B tolerance of the novel isolates during foliar treatments of crops of agricultural importance under laboratory and field conditions. The role of global transcriptional gene-regulators (i.e. Spo0A, AbrB, SinR) and quorum sensing molecules (autoinducer Phr-peptides and furanone AI-2) were evaluated for their participation in the control and prevention of plant-diseases and are discussed in this work.

Código de Resumen: BB-003

Sección: Bioremediación y Biocontrol

Modalidad: Oral

***Rosmarinus officinalis*: UNA FUENTE POTENCIAL DE DROGAS ANTI-INFECCIOSAS**

***Rosmarinus officinalis*: A POTENTIAL SOURCE OF ANTI-INFECTIOUS DRUGS**

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With the emergence of new bacterial strains resistant to antibiotics, the efficacy of antibiotics has dropped and antibiotic resistance has become a global public health issue. Research into novel antibiotics has decreased due to a change that occurred in the 1970s, where the focus shifted from research and development of new antibiotics to modifying existing classes[1-2]. One possibility to resolve the antimicrobial resistance crisis is to combine antibiotics with adjuvants or antimicrobials selected from the reservoir of bioactive compounds in nature[3]. In the last years, special attention has been paid towards secondary metabolites (essential oils, polyphenols) of edible plants many exhibiting antimicrobial activity and/or resistance-modifying activity[4]. Several modes of action have been put forward by which antibiotics and plant components may act synergistically, such as by affecting multiple targets, by physicochemical interactions and inhibiting antibacterial-resistance mechanisms[5-7].
This work aims to provide the antibacterial modes of action of *Rosmarinus officinalis* constituents. This plant growing worldwide and has been cultivated since long ago for its use in pharmaceuticals and food industries to eliminate pathogenic bacteria[8]. We demonstrated the active efflux pump inhibition activity of the main diterpene constituent, carnosic acid, as well its capacity to modify the cell membrane potential gradient in *E. faecalis* and *S. aureus.* In addition, this compound at sub-inhibitory dose in combination with traditional antibiotics inhibited the growth of methicillin-resistant *S. aureus* (MRSA), an important nosocomial and community-acquired pathogen that has also developed resistance to various antibiotics. Carnosic acid may be used in combinational therapy with fluoroquinolone, aminoglycosides and tetracycline to treat infections caused by Gram-positive bacteria. Our finding also showed that the 1,8-cineole, constituent of the *R. officinalis* essential oil, in combination with β-lactam-type antibiotics was active against Gram-negative *K. pneumoniae* multiresistant strains. We suggest possible mechanism of action for the active molecules under investigation and their potential targets. These phytochemicals may be good candidates to be employed as a novel therapeutic agent in combination therapies against drug-resistant bacteria.
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Código de Resumen: BB-004

Sección: Bioremediación y Biocontrol

Modalidad: Oral

EFECTO DEL SURFACTANTE NO-IONICO TRITON X-100 SOBRE LA BIODEGRADACION DE PAH Y LA COMUNIDAD MICROBIANA DE UN SUELO CRONICAMENTE CONTAMINADO

EFFECT OF THE NON-IONIC SURFACTANT TRITON X-100 ON PAH BIODEGRADATION AND SOIL MICROBIAL COMMUNITY IN A CHRONICALLY CONTAMINATED SOIL

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Polycyclic aromatic hydrocarbons (PAH) are poorly soluble, hydrophobic compounds which have been released into the environment on large scale. These facts, along with its high bioconcentration factor, toxicity and mutagenicity, make PAH priority pollutants. Bioremediation can be limited by the bioavailability of soil-bound PAH and strong sorption to soil, which is exacerbated by the long aging of contaminants in soils. A possible way of enhancing the bioavailability of PAH is the application of surfactants, molecules which consist of hydrophilic and hydrophobic parts. Because of this property these molecules decrease levels of surface and interfacial tension. PAH-degradation processes involving surfactant utilization need to be optimized for each of the factors influencing biodegradation, including surfactants type and concentration and the microorganisms present in the process.

The aim of this study was to examine the effect of adding the non-ionic surfactant Triton X-100 to a soil chronically contaminated with a petrochemical sludge, on PAH biodegradation and bioavailability and its impact on soil microbial community.

A critical micelle concentration was determined for Triton X-100 inthe aged soil (26 mg/gDRY SOIL). Microcosms consisting in 60 g of soil (20% moisture content) were assembled in two different conditions, without surfactant (C) and with Triton X-100 at 26 mg/$g_{DRY\ SOIL}$ (T). The microcosms were incubated at 24 ºC, for 30 days. Analysis of the hydrocarbons soil concentration and quantification of its bioavailable fraction (Amberlite XAD-2) were carried out by GC-FID. The number of cultivable heterotrophic bacteria (R2A), aliphatic-degrading bacteria (NMP) and PAH-degrading bacteria (NMP), and the genetic diversity of the bacteria soil community (PCR-DGGE of 16S rDNA) were also determined.

The initial soil hydrocarbons concentration was 573 ± 38 mg/g DRY SOIL .The extraction with Amberlite XAD-2 showed that only 5% of hydrocarbons were bioavailable. After 30 days of treatment, while the C microcosm showed 17% of hydrocarbons elimination, the T microcosm reached a 45%, along with a significant increase in the bioavailable hydrocarbons fraction (25%).

The analysis of the chromatograms showed that the surfactant enhanced the removal of both alkanes and PAH. However, the T microcosm showed a stimulatory effect on the number of alkanes hydrocarbon degrading bacteria, but not in PAH-degrading bacteria counts. The DGGE profiles showed that the Triton X-100 produced drastic changes in the structure of soil microbial community, but important changes in the DGGE profiles were also observed when the surfactant was added to an uncontaminated soil.

Our results show that the addition of surfactant could be an efficient strategy to enhance PAH biodegradation in aged contaminated soils. While the increase of hydrocarbons bioavailability was demonstrated, we cannot exclude a direct biostimulatory effect of surfactant on the soil microbial community.

Código de Resumen: BB-005

Sección: Bioremediación y Biocontrol

Modalidad: Poster

DESARROLLO DE BIOPELÍCULAS DE *Pseudomonas veronii* 2E SOBRE GRAFITO PARA CELDAS DE COMBUSTIÓN MICROBIANAS Y BIOSENSORES

Pseudomonas veronii 2E BIOFILM DEVELOPMENT ON GRAPHITE FOR MICROBIAL FUEL CELLS AND BIOSENSORS

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The use of fossil fuels has accelerated in recent years triggering a global energy crisis. New electricity production from renewable resources without a net carbon dioxide emission is much desired. Recently, a technology using microbial fuel cells (MFCs) has generated considerable interests among academic researchers. In these cells the energy stored in chemical bonds is converted to electrical energy through the catalytic reactions by microorganisms: for this, biofilm establishment on a conducting surface is required. Also, biofilms should have appropriate conducting properties. These characteristics are also required for the development of electrochemical biosensors, which combine at a molecular level the specificity and selectivity of biological processes with the sensitivity of the detection technique. In order to develop either a biosensor or a MFC, a strain able to form a biofilm on a conducting material and a reasonably good electrical response are necessary.

The aim of this work is to study the ability of *Pseudomonas veronii* 2E for biofilm development on graphite and to explore the electrochemical behaviour.

For that purpose, different graphite surface were used (Faber Castell® 9B; CRETACOLOR® 4B; Conarco C, ESAB®; HARDTMUTH 2B, KOH-I-NOOR®). The ability to produce biofilms was tested in different media and growth conditions (temperature, carbon source). Cyclic voltammetry was used for the electrochemical evaluation.

P. veronii 2E is able to produce biofilms on Faber Castell® and CRETACOLOR® surfaces, but both have not a good voltammetry response. On the other hand, KOH-I-NOOR® surface shows a good voltammogram, but *P. veronii* 2E is not able to develop a biofilm. ESAB® graphite has a reasonably good electrochemical behaviour and *P. veronii* 2E produce biofilms. ESAB surface and *P. veronii* 2E resulted a promising candidates for future studies in MFC and biosensors development, considering the abilities of *P. veronii* 2E to biosorb Cd(II), Zn(II) and Cu(II), to biotransform Cr(VI) and to produce siderophores.

Código de Resumen: BB-006

Sección: Bioremediación y Biocontrol

Modalidad: Poster

CONTROL DE *Paenibacillus larvae*, AGENTE CAUSAL DE LA LOQUE AMERICANA, USANDO EXTRACTO HEXANO DE *Minthostachys verticillata*

CONTROL OF *Paenibacillus larvae*, CAUSAL AGENT OF AMERICAN FOUL BROOD BY USING HEXANE EXTRACT FROM *Minthostachys verticillata*

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American Foulbrood (AFB) is a common bacterial disease of honey bees (*Apis mellifera*) brood. AFB is produced by the Gram positive, spore-forming bacterium *Paenibacillus larvae*. AFB is still among the most deleterious bee diseases, highly contagious, presents a serious problem in apiculture causing considerable economic looses to beekeepers all over the world.
Different antibiotics have been used to control AFB, but concerns still remain regarding the emergence of resistant strains or the residues that they may leave in hive products. Thus, the development of new strategies to treat AFB infected honey bee colonies is necessary. Essential oils and extracts of plants are known to retard or inhibit the growth of *P. larvae*. Previous studies in our research group showed that extracts from different vegetal species have antibacterial activity against *P. larvae*.
Among these plants, *Minthostachys verticillata*, a native specie widely used in natural and popular medicine, was one of the most effective.
The aim of this research is to identify compounds with antimicrobial activity against *P. larvae* in different extracts of *M. verticillata*.
We used strains of *P. larvae* isolated from apiaries in the Río Cuarto department in Córdoba, exhibiting clinical symptoms of the disease, and strains of *P. larvae* provided by INTA Balcarce. *M. verticillata* was collected in Santa María, a town in Cordoba province. From the dried plants were obtained hexane extract (HE), benzene extract (BE), ethyl ether extract (EE) by liquid-liquid extraction. Bioactivity was evaluated by broth microdilution method. EH had the best antibacterial activity, with Minimum Inhibitory Concentration (MIC) values between 0,032 and 0,125 mg/ml, and Minimum Bactericidal Concentration (MBC) values between 0,125 and 0,5 mg/ml. MBC / MIC ratio showed that HE has bacteriostatic activity against *P. larvae*.
Separation by Thin Layer Chromatography (TLC) of the components of HE showed the presence of 6 bands with different Rf. (revealed under UV light at 254 nm), and 5 bands (revealed with iodine). Two of the last bands (Rf 0,83 and Rf 0,58) showed antibacterial activity when studied by bioautography technique.
These results provide evidence of the potential use of *M. verticillata* extracts as antibacterial agents against *P. larvae*, and also shows that these extracts could be used as a natural alternative treatment to control AFB, increasing production and quality of the products. Further studies are needed not only to elucidate the compound/s with biological activity but also to test their toxicity.

Código de Resumen: BB-007

Sección: Bioremediación y Biocontrol

Modalidad: Poster

COMPOSTAJE Y BIOESTIMULACION COMO ESTRATEGIAS DE RECUPERACION DE UN SUELO CRONICAMENTE CONTAMINADO CON HIDROCARBUROS

COMPOSTING AND BIOSTIMULATION AS STRATEGIES FOR CHRONICALLY HYDROCARBON CONTAMINATED SOIL RECOVERY

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The concept of treating PAH-contaminated soil by means of co-composting with organic materials or by mixing soil with mature compost has proved to be effective in the degradation of PAHs at the laboratory and/or field-scales.
A contaminated soil was collected from a petrochemical area, near La Plata. It was characterized by a very low biological activity, probably due to the hydrocarbon content (4000ppm of aliphatic and 300ppm of PAH). The microbial populations were similar to those determined in pristine soils near of the area.
The goals of our study were to investigate the potential of composting related practices in the biological recovery of the contaminated soil.
Composting treatment (CT). A sample of soil was treated with amendment in ratio 0,7: 0,3 (w/w) and the resulting material was mixed with bulking agent. It was incubated at room temperature in reactors of 34L during 4 months. The moisture was adjusted to 45%.
Biostimulation treatment (BT). A sample of soil was mixed with compost (prepared 30 days before use) in ratio 0,7: 0,3 (w/w) and it was incubated during 1 month at 25°C. This experiment was carried out in triplicate trays. The moisture was adjusted to 45%. Control reactors (S). Contaminated soil microcosm with any additive was used as control system.
Microbial population densities. Viable heterotrophic bacterial count was performed using R2-Agar. The value for CT was higher than for S whereas the BT did not show differences with S. Fungi were enumerated on Rose Bengal Agar. For both treatments the counts were higher than for S. Phosphorus solubilising bacteria was enumerated on PIM media: the count in CT was higher than in S while BT did not show any difference. The most probable number of aromatic hydrocarbon-degrading bacteria was determined using mineral salts medium with the addition of a mix of PAHs. No differences were detected after both treatments in the hydrocarbon degrading bacteria counts.
Biological Activity. Dehydrogenase assays were performed using soluble tetrazolium salt as an artificial acceptor. Both treatments produced significant increase in the dehydrogenase activity that was higher than those determined in S, during all the incubation time.
Toxicity assays. Seed germination test using *Lactuca sativa* was performed on water extracts. Only the CT increased significantly the germination.
Hydrocarbons concentrations. The extracts were analyzed by GC-FID. No significant decrease was detected after 1 and 4 months respectively.
Molecular analysis. PCR-DGGE analysis was performed. Both treatments produced a significant diversity increase of the populations.
A successful composting treatment was evidenced by the visible changes in the matrix aspect in the CT treatment, in agreement with the higher bacterial counts, biological activity and percentage of seed germination. Although this treatment did not reduce significantly the hydrocarbon concentration, it was able to improve the soil quality in the experiment time.

Código de Resumen: BB-008

Sección: Bioremediación y Biocontrol

Modalidad: Poster

LA RESISTENCIA A COBRE EN *Pseudomonas extremaustralis* Y SU RELACION CON LA ACUMULACION DE POLIHIDROXIALCANOATOS

COPPER RESISTANCE IN *Pseudomonas extremaustralis* AND ITS RELATIONSHIP WITH POLYHYDROXYALKANOATES ACCUMULATION.

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Copper is an essential micronutrient, critical for cell function. However at high concentrations copper tends to accumulate in soils, plants and animals, increasing its harmful effects within superior levels of food chains.
There are different process for removal of Cu(II) from wastewater and soils, including precipitation, ion-exchange, evaporation, oxidation, electroplating and membrane filtration. But application of such technologies has technical or economic constraints.
Bioremediation, an environmental friendly and cheap approach, was proposed to access the cleanup these sites. One of bioremediation mechanisms involve the seeding of microorganisms with known ability to transform or to adsorb the contaminant (bioaugmentation), but success also depends on the adaptability of the microorganism to the stressing environment.
Pseudomonas extremaustralis was isolate from a temporary pond in Antarctica and shows high resistance to several stresses in association with the production of polyhydroxyalkanoates (PHAs).
The genome analysis of *P. extremaustralis* exhibited the presence of several sequences that could be involved in copper resistance like: several cytochrome oxidases, 5 copies of a Type P Cu-translocation system (homologous to CopAB) and an efflux system CusAB.
Copper minimum inhibitory concentration (Cu(II) CIM) was assayed using LB broth 0.5X supplemented with glucose 2% (PHA accumulation conditions) and different concentrations of CuSO4 (1mM-5mM). As PHAs enhance stress resistance, a *phaC1ZC2* mutant was also assayed. Cu (II) CIM was the same for both strains (2mM), so PHA seems to have no effect on copper resistance.
Biosorption is an strategy used by microorganisms to cope with high Cu(II) concentration. *P. extremaustralis* 14-3 and its *pha* mutant's sorption capacity were examined. In order to describe Cu (II) biosorption characteristics, Langmuir and Freundlich models were evaluated. Wild type strain´s Cu biosorption was higher than the mutant, although both strains adjust to Freundlich model.

Código de Resumen: BB-009

Sección: Bioremediación y Biocontrol

Modalidad: Poster

LA SINTESIS DE POLIHIDROXIALCANOATOS AFECTA A LA ADHESION MICROBIANA A HIDROCARBUROS Y LA BIOSORCION DE COBRE EN *Pseudomonas* sp KA-08

POLYHYDROXYALKANOATE SYNTHESIS AFFECTS CELL ATTACHMENT TO HYDROCARBONS AND COPPER BIOSORPTION IN *Pseudomonas* sp. KA-08

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Biological treatment is an innovative technology available for the cleanup of contaminated sites. In some cases, bioremediation includes the seeding of selected bacteria to enhance contaminant's removal. In this case, strains that show high stress resistance and increased fitness are desirable. *Pseudomonas* sp. KA-08 was selected by its ability to grow in high amount of kerosene, diesel and xylene as sole carbon source, to tolerate high copper concentration, and to accumulate polyhydroxyalkanoates (PHA). We analyzed the relevance of PHA accumulation capabilities on cell membrane hydrophobicity and copper biosorption as a strategy to improve bioremediation. The PHA- mutant, *Pseudomonas* KA-mut, was obtained by CrossOver PCR deletion of *phaC1ZC2* genes. As *Pseudomonas* sp. KA-08 is highly tolerant to xylene and is able to degrade this compound, microbial attach to hydrocarbons (MATH) assays were performed using xylene as hydrocarbon to test bacterial adherence. Briefly, xylene was added to a cell suspension, the mixture was vortexed and left to stand to allow phases separation. Aqueous phase was taken, and OD600nm was measured. Under no PHA-accumulating conditions (LB Medium), both strains presented no significant differences in their affinity to xylene but under PHA accumulatiing conditions (ME + sodium octanoate), the wild type strain showed a significant lower affinity to xylene than the mutant strain ($P< 0.0001$).
On the other hand, copper's MIC was determined in LB broth 0.5X. Both strains showed a copper's MIC close to 4mM. Due to this high resistance, copper biosorption assays were performed. The wild type strain showed a significant higher biosorption capability than the mutant strain and both of them adjusted to the Freundlich´s model.
In conclusion, our results suggest that PHA accumulation capabilities affect bacterial outer membrane characteristics of *Pseudomonas* sp. KA-08 that are relevant for its fitness in hydrocarbon and heavy metal-contaminated environments.

Código de Resumen: BB-010

Sección: Bioremediación y Biocontrol

Modalidad: Poster

BÚSQUEDA DE BACTERIAS CON POTENCIAL PROBIÓTICO A PARTIR DE AMBIENTES MARINOS SOBRE LA COSTA PATAGÓNICA PARA SU APLICACIÓN EN SISTEMAS ACUÍCOLAS

SCREENING OF POTENTIAL PROBIOTIC BACTERIA FROM MARINE ENVIROMENTS ON THE COAST OF PATAGONIA FOR THEIR APPLICATION IN AQUACULTURE SYSTEMS

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Recently, aquaculture has become the fastest growing food-producing sector; therefore it has turned in an intensive activity where animals are exposed to high stress conditions. The abuse of antibiotics to control and/or prevent bacterial diseases has led to the apparition of antibiotic-resistant pathogens. The use of probiotics as sanitary prevention method improves the farmed

fish quality and safety in aquaculture systems. The aim of this study was to assess *in vitro* probiotic characteristics of bacteria isolated from the Patagonian coast for their potential application in aquaculture. The strains isolated from sediments, algae and marine organisms were grown on TS and MRS whith NaCl (2% w/v) at 25ºC for 24-72h. To detect antimicrobial activity against fish pathogens the double layer method was performed. The culture supernatant of the strains showing antimicrobial activity was assessed by the agar-well diffusion assay. The following fish pathogens were used as indicator strains: *Aeromona salmonicida, Yersinia ruckeri, Lactococcus garvieae, Carnobacterium piscicola, Listonella anguillarum,* and *Vibrio alginolyticus.*
Agar-cellophane assay was performed to remove the extracellular agent released by the strain M26 on solid medium. The cell adhesion ability was tested using the Crystal Violet assay. Siderophore production in the culture cell-free supernatants was evaluated by the agar CAS diffusion assay. Hemolysin and gelatinase production was determined as an indication of the microbiological safety of the selected strains. Eighty-two percent of the isolated strains showed inhibition against at least one of the indicator strains. Several strains isolated from gut of sea bass (*Acanthistius patachonicus*) and brazilian sand perch
(Pinguipes brasilianus) showed a wide inhibition halo indicating that the antimicrobial agent diffused through the agar. None of the strains showed extracellular antimicrobial activity. The antimicrobial agent could not be removed by agar–cellophane assay.
Strain 73 showed the highest adhesion ability to a hydrophilic surface. The strains evaluated by agar-CAS diffusion assay did not show siderophore production under the tested conditions. Two strains (T29 and T35) presented a slight haemolytic activity and only one (T39) showed gelatinase activity. Efforts to isolate and assess probiotic properties in native strains, such as those performed in this study, may lead to probiotic strains that could be used as biological control agents, reducing the antibiotic use and its negative environmental impact.

Código de Resumen: BB-011

Sección: Bioremediación y Biocontrol

Modalidad: Poster

INTERACCIÓN ENTRE *Azospirillum brasilense* CON *Fusarium graminearum* EN TRIGO

INTERACTION BETWEEN *Azospirillum brasilense* WITH *Fusarium graminearum* IN WHEAT

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In the last decade the literature cites promising results in vitro and in vivo with the genus *Azospirillum brasilense* as biocontrol of *Fusarium sp* and other fungal pathogens that attack crops of economic interest. Based on this background and in order to expand on these possibilities in order to evaluate in vitro and in vivo in wheat antagonistic effect of *A. brasilense (Az)* of *F. graminearum (Fg)* was formulated .In vitro both organisms have been tested on a dual culture system in Petri dish.
Firstly the commercial inoculant AZ was planted in triplicate and by extension with spatula Drigalsky superficie dilutions 10^{-3} ,10^{-4} ,10^{-5} y 10^{-6} Petri dishes containing medium RC (15-20 ml/plate). Plates were incubated at 30 ° C in the dark for 4 days. Later, sow in the center of the disc plate of 6 mm in diameter with the fungus (FG) and a control without Az. To quantify the radius measured in mm of the control colony (R1) and the colony in interaction with Az (R2), at 4, 7 and 11 days. In vivo: Tests were conducted on disinfected seeds of wheat BIOINTA cultivar planted in containers with soil / perlite sterile seeds, which were subjected to four treatments (T); T1 (Wheat untreated) and T2 (Wheat + *Fusarium*) T3 (Wheat + *Azospirillum*) and T4 (Wheat+*Azospirillum*+*Fusarium*)The assay was performed with four replicates and an experimental design was applied in completely randomized blocks. To evaluate the antagonistic/synergistic effect of microorganisms on wheat the following growth parameters were measured: germination, dry weight of shoot and root. The results were analyzed by

ANOVA using the LSD test with P < 0.05 to determine the differences between means. In the in vitro results, inhibition of 66 to 69% was determined in presence of 3 CFU from 10^2 to 3.10^3 and Az and a growth rate of 33mm/ds versus 100 mm / ds in the untreated Az was determined. The antagonistic effect of Az- Fg manifested morphologically by the presence of aerial mycelium called " aerial mycelium fluffy " with conidia truss .In the in vivo results, the stimulatory effect on germination rate from 5th day was observed in T3 (Wheat + *Azospirillum*) 58 % and T4 (Wheat *Fusarium* + *Azospirillum*) 68 % of T1 (Wheat untreated) 47 % and T2 (Wheat +*Fusarium*) 42%. Observations on the 9th day seedlings were matched resulting in T1: 90 % , T2: 90 % T3: 95% and T4:88 % .There were no significant differences in fresh weights of aboveground biomass between treatments in contrast to the fresh weight of root that gave highly significant differences for T1 (control) less developed compared T2 , T3 and T4 . Contrary to expectations the presence together with the biocontrol fungus has had a synergistic effect in stimulating the growth of the roots of the host, probably due to a metabolic establishing association between two microorganisms.

Código de Resumen: BB-012

Sección: Bioremediación y Biocontrol

Modalidad: Poster

LA CEPA MARINA *LACTOBACILLUS PENTOSUS* H16 PROTEGE A *ARTEMIA FRANCISCANA DE LA INFECCIÓN POR VIBRIO ALGINOLYTICUS*

MARINE STRAIN *LACTOBACILLUS PENTOSUS* H16 PROTECTS *ARTEMIA FRANCISCANA* FROM *VIBRIO ALGINOLYTICUS* PATHOGENIC EFFECTS

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Diseases caused by *Vibrio* spp. are commonly implicated in episodes of mortality in mariculture, particularly at the larval phases.
In aquaculture, bacterial diseases have habitually been controlled using antibiotics. This practice may lead to the emergence of multiresistant strains, causing serious sanitary concerns. Currently, probiotics have been considered to prevent bacterial infections. Probiotics are microorganisms or their products that when administered via the feed or to the rearing water improve the host condition by providing both a nutritional benefit and protection against pathogens. In a previous work, we demonstrated *in vitro* probiotic properties of *Lactobacillus pentosus* H16 which included production of antimicrobial compounds against the fish pathogen *Vibrio alginolyticus*, high adhesion to fish mucus and the capability to inhibit *V. alginolyticus* adhesion to fish mucus. Furthermore, H16 successfully bioencapsulated in *Artemia franciscana* nauplii. The aim of this study was to assess *in vivo* H16 probiotic capability. Two challenge experiments using *A. franciscana* nauplii infected with *V. alginolyticus* were performed.
Bacteria-free nauplii were distributed at a density of 100 nauplii per vessel containing 100 ml of sterile seawater. In both experiments, 3 uninfected units were used as controls. In the first experiment, each treatment included 3 vessels containing bacteria-free nauplii and 1) H16 (4.6 E+05 CFU/ml), 2) *V. alginolyticus* (5.3 E+05 CFU/ml), and 3) H16 and *V. alginolyticus* simultaneously co-inoculated at those concentrations. After 48h of incubation, the nauplii survival rate was recorded. The second experiment was similar to the first except that in 3) H16 was inoculated at the beginning of the experiment and *V. alginolyticus* after 24h of incubation. After 72h of incubation, the nauplii survival rate was recorded. Our results showed that H16 protects the nauplii against *Vibrio* infection only when it was administrated previously to *V. alginolyticus* inoculation. Probably, an early addition of H16 allowed the colonization of *Artemia* nauplii, and consequently the inhibition of *V. alginolyticus*. The probiotic properties of *L. pentosus* H16, its capability of outcompete with *V. alginolyticus in vivo* and its successful bioencapsulation in a live carrier such as *Artemia* for administration to marine fish and crustacean larvaes, make this strain an interesting alternative for the prevention of vibriosis in mariculture.

Código de Resumen: BB-013

Sección: Bioremediación y Biocontrol

Modalidad: Poster

EFECTO DE EXTRACTOS DE PROPOLEOS SOBRE EL CRECIMIENTO *IN VITRO* E *IN VIVO* DE *Penicillium sp.*

EFFECT OF PROPOLIS ALCOHOLIC EXTRACTS ON *IN VITRO* AND *IN VIVO* GROWTH OF *Penicillium sp.*

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There is concern among produce consumers regarding approved chemical fungicides and their residue levels in crops. In this sense, the manufacture and application of natural products for control of plant pathogenic fungi, such as propolis is emerging as an interesting alternative. Propolis is a bee-collected resinous substance with antimicrobial properties. Previous studies in our lab showed the *in vitro* effect of propolis on the growth of the plant pathogenic fungus *Botrytis cinerea*. The objective of this new research was to evaluate the effect of poplar-type propolis on *in vitro* and *in vivo* growth of *Penicillium* sp., fungus collected from garlic heads (*Allium sativa* L.). Two trials were run using propolis extracts from the province of Mendoza. For the *in vitro* trial four treatments with four repetitions were carried out: a control (T); a commercial fungicide (F); a solution of diluted hidroalcohol propolis extract (water:ethanol 30:70) (PHA), and its respective control (CHA). For treatments F, PHA, and CHA, three mL of the corresponding solutions were each poured into sterile Petri dishes, they were allowed to evaporate at 45 ºC for 48 h, and then 20 mL of Hongos y Levaduras (BritaniaÒ) culture medium were added. For treatment T, only 20mL of culture medium were poured. Ten mm diameter fungus discs obtained from 72 h-incubated cultures using a sterile hole punch were placed in the dishes. Petri dishes were incubated at 22ºC and the mean diameter of the mycelium (average of the measurements of diameter in two directions) in cm was recorded at 24 h intervals for 14 days. The results of the assay showed that the mean diameters of the fungi in the propolis treatment (PHA) were significantly lower ($p \leq 0.01$) than the control (CHA). It is important to point out that the PHA treatment was significantly more effective as a mycelial growth inhibitor than the synthetic fungicide (F). For the *in vivo* assay, garlic cloves were artificially infected with *Penicillium* sp. Five treatments were carried out with five repetitions that consisted of non-infected cloves (T1); fungus-infected cloves dipped in the propolis solution (T2); fungus-infected cloves dipped in hidroalcohol (water:alcohol 30:70) (T3); non-infected cloves dipped in hidroalcohol (T4); non-infected cloves dipped in propolis solution (T5). Plastic trays were layered with moistened paper towels and covered with Saran wrap-type film to provide a moist environment. Colonization of the garlic clove by *Penicillium* sp. was evaluated. After 14 days of incubation at 24ºC fewer colonies of the fungus were observed in the propolis treatments T2 and T5 with respect to other treatments. The results of this trial lead us to conclude that propolis has an *in vitro* growth inhibition effect on *Penicillium* sp. In spite of the non-conclusive results of the *in vivo* assay, they still allow to establish new work conditions to improve the protocol for this kind of work.

Código de Resumen: BB-014

Sección: Bioremediación y Biocontrol

Modalidad: Poster

BACTERIAS TERMOFÍLICAS FORMADORAS DE ESPORAS CON ALTOS NIVELES DE RESISTENCIA A LA RADIACIÓN UV Y AL ARSÉNICO

THERMOPHILIC SPORE-FORMING BACTERIA WITH HIGH LEVELS OF RESISTANCE TO UV-RADIATION AND ARSENIC

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The North-Western part of Argentina is particularly rich in wetlands located in the Puna in an altitude between 3,600 and 4,600 m above sea level. Most of these high-altitude Andean lakes are inhospitable areas due to extreme habitat conditions such as high contents of toxic elements, particularly arsenic (As). In this work, we have characterized wild strains of three spore forming bacteria of the *Bacillus* genus that were initially isolated from the Puna based on their ability to form biofilms at high temperature.
We examined their ability to grow, colonize solid substrates (surface-associated translocation) and biofilm formation in the presence of different concentrations of toxic As (III) (up to 10mM) and As (V) (up to 100 mM). These analyses were conducted at different growth temperatures (up to 50°C). Our results indicate that the wild Andean isolates were able to grow and cooperatively colonize surfaces at concentrations of arsenic above the maximum concentrations of other *Bacillus* strains (i.e. the Marburg-related reference strain NCIB3610) isolated from more moderate environments. The Andean *Bacillus* isolates were further characterized by having a greater tolerance to high temperatures, salinity and UV-B and UV-C radiations lethals to NCIB3610 cells. These results allow the potential utilization of these strains in bioremediation practices of pristine environments contaminated with metals.

Código de Resumen: BB-015

Sección: Bioremediación y Biocontrol

Modalidad: Poster

ESTUDIO DE LA INTERACCIÓN ENTRE METALES Y EXOPOLÍMEROS O SIDERÓFOROS PRODUCIDOS POR Pseudomonas veronii 2E

INTERACTIONS BETWEEN METALS AND EXOPOLYMERS OR SIDEROPHORES PRODUCED BY *Pseudomonas veronii 2E*

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P. veronii 2E secretes exopolymers (ES) and siderophores (SP) with potential metal-binding ability. ES is a complex mixture of proteins, polysaccharides, nucleic acids, lipids and other polymeric compounds. SP are iron-chelating ligands synthesized under iron-limited conditions. These ligands show high affinity for Fe (III) and can also chelate Ni, Zn, Co, Cu, Pb and Cd. Both ES and SP can protect the cell from the harsh external environment.
The objectives of this study were: a) to characterize the bacterial exopolysaccharide, major component of the ES, and study its Cd(II) complexation and b) to evaluate the production of SP in different Zn(II) and Cd(II) concentrations.

ES produced in a minimal medium M9-2%(v/v) glycerol was precipitated with ethanol and purified by dialysis and enzymatic treatments. The biochemical composition was determined with Bradford method for protein content; Anthrone assay for neutral sugar; Molybdenum blue assay for phosphate and sulfamate/m-hydroxydiphenyl assay for uronic acid. ES total acid hydrolysis with Trifluoroacetic; HPAEC-PAD analysis of hydrolyzated products showed the presence of fucose, galactosamine, glucosamine, galactose, glucose, mannose and glucuronic acid. Oxalic acid treatment after acid hydrolysis revealed the presence of a pyruvilated sugar. Deoxy-cholate-polyacrylamide gel electrophoresis and silver/alcian blue staining of the purified ES showed the presence of both extracellular polymeric substances EPS and LPS. Monosaccharide analysis of the isolated EPS showed no difference with the whole ES fraction, suggesting that the oligosaccharide linked to the Lipid A moiety of the LPS would have a similar structure.
The ability of ES to complex Cd (II) was studied at 25°Cand pH range 5.5-8.1 using an electrochemical monitored titration (ASV).One family of binding sites of moderate strength was detected: [L] ≈15µmol of sites per g of dry weigh of EPS and conditional complexation constant K´≈105 , for the highest pH values.
On the other hand, supernatants of M9-succinate cultures (25˚C) obtained with 0.01; 0.1 and 0.5 mMof Zn (II) or Cd (II) were tested for SP production and their interactions were studied based on the spectral characteristics of these metabolites and their metal complexes. SP production were stimulated by Zn(II).
Electrochemically monitored titrations of EPS and LPS combined with structure characterization (*Mass spectrometry*) will help to understand the ES structure and complexing capacity responsible for the interaction with metals. Further steps involve characterization of metal - siderophore interactions for future applications in environmental biotechnologies.

Código de Resumen: BB-016

Sección: Bioremediación y Biocontrol

Modalidad: Poster

SELECCIÓN DE MUTANTES NO PRODUCTORAS DE BIOSURFACTANTES PARA EL ESTUDIO DEL COMPORTAMIENTO BACTERIANO EN AMBIENTES NATURALES O DISEÑADOS ARTIFICIALMENTE

SELECTION OF LOW BIOSURFACTANT PRODUCING MUTANTS FOR THE UNDERSTANDING OF BACTERIAL BEHAVIOUR IN NATURAL OR ENGINEERED ENVIRONMENTS

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Biosurfactants (BS) are surface-active molecules synthesized by a variety of microorganisms. In bioremediation processes, they can promote the biodegradation of hydrophobic pollutants. The exact physiological role of BS is unknown but in *Pseudomonas aeruginosa* PA01, for example, they are fundamental in processes like swarming and biofilms development. Swarming motility could be boost by the addition of exogenous BS, enhancing biofilm stablishment, useful in wastewater biotreatments. The goal of this work is to produce mutants for further studies on biosurfactant roles in microbial behaviour. Using *Escherichia coli* S171λpir/put tc we produced mutants from the natural isolates *Pseudomonas veronii* 2E and *Ralstonia taiwanensis* M2. These strains were previously studied for motility and biosurfactant production. We tested 1500 clones of each strain using methylene blue agar plates (g/L: $(NH_4)_2HPO_4$ 1.5, KH_2PO_4 4, yeast extract 0.4, CTAB 0.2, glucose 20, methylene blue 0.015, MgSO4.7H_2O 1.97, agar-agar 15) evaluating their capability for biosurfactant production. Non-producing surfactant strains were identified by the lack of coloration and absence of blue precipitation halo, isolated and inoculated in Plate Count Agar (PCA).

From *P. veronii* 2E, 11 white mutants were obtained but only 6 were viable after storing them at 4ºC. The mutants named as 2E02, 2E03, 2E05 and 2E06 showed a negative response in blood agar, so they were discarded. From *R. taiwanensis* M2, 4 mutants were isolated but only M201 was culturable after the storage at 4o C. Both selected mutants presented the growth in
PYG broth (g/L: casein peptone 2.5, yeast extract 1.25, glucose 0.5) and the swimming motility in soft agar (PYG broth supplemented with agar 3 g/L) similar to the wild type. In contrast, none of them presented swarming motility in swarming agar plates. The secretion of biosurfactants performed by 2E01 and M201 was compared to *P. aeruginosa* PA01 and the respective wild type strains in Kay´s medium (g/L: $(NH_4)_2HPO_4$ 1.5, KH_2PO_4 4, yeast extract 0.4, $MgSO4.7H_2O$ 1.97) supplemented with glucose 5%(w/v). The results indicated that 2E01 produced only a 34 % of the surfactants of PA01. M201 produce the 46 % of surfactants when glucose was the carbon source and only a 21 % when vegetable oil (5 %v/v) was used as carbon source. As vegetable oil is the optimal carbon source for surfactant production by *R. taiwanensis* M2, the observed decrease of anionic tensoactive compounds was acceptable to consider this strain as a low-producing mutant. These mutants are an important tool for the study of BS role on the acquisition and utilization of hydrophobic compounds as carbon source by *P. veronii* 2E and *R. taiwanensis* M2. In addition, the selection of mutants will contribute to study the significance of BS on swarming motility and any other related process such as biofilm development and surface colonization relevant in natural or engineered environments.

Código de Resumen: BB-017

Sección: Bioremediación y Biocontrol

Modalidad: Poster

ESTUDIO DE UN CONSORCIO BACTERIANO DEFINIDO Y ADAPTADO A BAJA BIODISPONIBILIDAD DE FENANTRENO

STUDY OF A BACTERIAL DEFINED CONSORTIUM ADAPTED TO LOW PHENANTHRENE BIOAVAILABILITY

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A general problem of bioremediation of polycyclic aromatic hydrocarbons (PAH) contaminated soil is that the degradation appears rather slow, due to the propensity of these nonpolar contaminants to adsorb strongly to organic matter.
The establishment of microniches of PAH degrading bacteria in an aged PAH contaminated soil represents a process strongly influenced by the manner in which the PAH are exposure in the soil and bacterial capability to develop physiological strategies to adapt to the PAH bioavailability. Due to the reduced bioavailability, the bioaugmentation strategy with cultures obtained by enrichment in solid phase systems, could be a promising strategy to apply in aged contaminated soils.
Applying an enrichment method with the phenanthrene provided in a sorbed state, a degrading consortia was previously obtained. The predominant strains from the consortia were isolated and identified as belonging to *Sphingobium, Acidovorax*, *Rhodococcus* and *Arthrobacter* genera (strains C1, C2, C3 and C4, respectively) suggesting that the strains could develop different strategies to integrate the degrader consortium.
Because the successful of survival and establishment of a consortium in soil is probably higher than those obtained with the inoculation of only one strain, we focussed our study in understanding how a defined consortium built by these four strains could be more efficient in reducing sorbed phenanthene than pure cultures.
The Amberlite® XAD-2 preloaded with phenanthrene (10.8 mg of phenanthrene g^{-1} XAD2) was used as solid model in batch system with mineral medium (10% p/v). Each strain and the defined consortium were inoculated in the solid model in batch system with phenanthrene as only carbon source. Systems without phenanthere were inoculated to evaluate the inespecific

adsortion to the resin beads.
The biomass developed on the beads was monitoring by R2 agar count and FTIR spectroscopy (evaluating the increase in Amide I band at 1650cm^{-1}) after 10 and 30 days of incubation at 28C.
Only the *Arthrobacter sp.* C4 strain growing as pure culture exceeded the 10^6 CFU/g of XAD2-Phen after 10d. Both by plate count and FTIR we could determine that, C1 and C4 biomass obtained in pure cultures were more abundant on XAD2 beads than XAD2-Phen, suggesting the strains could degrade the resin.
When the four strains were inoculated together in XAD2-Phen, each strain rose to 10^7 CFU/g and remained this value after 30d.
Both the high amount of carbohydrates observed for *Acidovorax* sp. C2 strain (1200-900cm^{-1} and 3500cm^{-1}) and the significant production of lipids for *Rhodococcus sp.* C3 strain (3000-2800 cm^{-1}) could be detected in the defined consortium. These results demonstrated that at least these two strains could establish and express their phenotypes during the consortium development.
Both FTIR spectroscopy and HPLC techniques allow the detection of phenanthrene by products accumulation along the pure cultures, which were reduced in the consortium.

Código de Resumen: BB-018

Sección: Bioremediación y Biocontrol

Modalidad: Poster

EVALUACIÓN DE *Bacillus thurigiensis var israelensis* COMO LARVICIDA PARA *Musca domestica*, FORMULADO COMO ALIMENTO PARA AVES DE CORRAL

EVALUATION OF *Bacillus thurigiensis var israelensis* AS A LARVICIDE FOR *Musca domestica*, FORMULATED AS POULTRY FOOD ADDITIVE

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The health pest infestations caused by domestic fly (*Musca domestica*) is a serious problem around poultry facilities. In these facilities, laying hens are kept in cages while feces accumulate for a long period of time and fly larvae reproduced in this substrate. Chemical control methods employed in these facilities are expensive and involve several drawbacks. The aim of the present work is the development of an effective larvicide against domestic fly larvae, based on *Bacillus thuringiensis varisraelensis*(B ti). During sporulation this bacteria produces a parasporal inclusion formed with Cry 4 and Cry 11 proteins with toxic activity against dipterans. These proteins are encoded in a 127.9 Kbp plasmid (pBtoxis). This biological larvicide will be incorporated into a specific formulation as a poultry food additive, in order that the spores or vegetative cells of Bti are consumed and excreted, preventing the fly reproduction. Molecular identification of the bacillus was performed with five Bti strains available in our laboratory by PCR amplification with specific primers for cry4 and cry11 genes. Only one isolate was positive for the presence of cry genes and the extracted plasmid matched the pattern of Bti. The extraction and purification of the toxic crystals of Bti were obtained by the induction of sporulation in liquid media and verified with comassie blue and acetic acid special staining techniques. Preliminary toxicity tests were carried out in mosquito larvae (*Culex pippens*) and the previously extracted crystals were tested. The maximum dilution that retains toxic activity was found to be 1:100,000 of the purified crystals. A formulation made of bentonite and zeolite alumninosilicates with Bti was incorporated into hen's food and it was found to be capable of adsorbing viable spores of Bti. However, it showed no effective toxic activity in aqueous solution against mosquito larvae. For the domestic fly larvae toxicity assay, ten larvae at stage 3 were tested with Bti crystal formulation, commercial Bti formulation and water as negative control. At this growth stages larvae were not affected either in its survival nor its development to adult flies. However when larvae at stages 1 and 2 where used, Bti showed a high toxic activity (mortality > 90 %) These results encourage us to continue the optimization and development of an effective larvicide that effectively replace the use of chemical insecticides.

Código de Resumen: BB-019

Sección: Bioremediación y Biocontrol

Modalidad: Poster

AISLAMIENTO DE CEPAS NATIVAS DE HONGOS ENTOMATOGENOS PARA EL CONTROL DE *Varroa destructor*

ISOLATE OF STRAINS OF NATIVE ENTOMOPATHOGENIC FUNGI FOR THE CONTROL OF *Varroa destructor*

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Varroa destructor Anderson and Trueman (Acari: Varroidae) is an obligate ectoparasite of the western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae). This mites feed on honeybee haemolymph, and transmit diseases that reduce bee longevity, lower reproductive capacity, and induce deformities. At present, beekeepers attempt to control varroa with chemical pesticides, but resistance to these chemicals is developing. The biological control of varroa warrants attention as a partial or total alternative to chemicals, and it could form part of an integrated pest management system. Fungal species that infect mites and insects are known as entomopathogens. Because the temperature within the honey bee hive is determined by ambient conditions, it would be logical to screen local isolates of fungi that fall within the ambient environmental conditions. This study report natural occurrence of entomopathogenic fungi pathogenic towards local Varroa destructor. Varroa mites were collected from experimental apiary of the National University of San Luis located in Villa Mercedes, San Luis, Argentina. Apiary hives were provided with technical floors to collect mites. After disinfection, specimens were incubated in a humids chambers at 100% of relative humidity for 48 hours and fungal growth was observed. In the colonies where fungal growth were plated on Potato dextrose agar (PDA) medium and incubated at 30 °C for 3-4 days. At the end of the growth period, a single colony was transferred onto another PDA agar medium and incubated at 30 °C, and the resultant mycelium was used to identification.
Microscopic features observed are consistent with Entomomophthorales (Zygomycetes).

INTERACCIONES PROCARIOTA-EUCARIOTA

2 al 4 de Julio de 2014
Hotel 13 de Julio
Mar del Plata, Argentina

Código de Resumen: IN-001

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

ESTUDIO DE ASOCIACIÓN A CÉLULAS THP-1 DE CEPAS POTENCIALMENTE PROBIÓTICAS DE *Bifidobacterium*

STUDY OF ASSOCIATION TO THP-1 CELLS OF POTENTIALLY PROBIOTIC STRAINS OF *Bifidobacterium*

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Bifidobacterium are potentially probiotic microorganisms that colonized the new born intestine and they are also present in children and adult intestine where they lead to immunomodulatory effects. Since macrophages are key players in the immune response, the aim of the present study was to gain insight on the interaction between *Bifidobacterium* and human monocytic THP-1 cells.

According to the surface properties and their ability to adhere onto cultured enterocytes, two strains were selected: *B. bifidum* CIDCA 5310 (high surface hydrophobicity and adhesion) and *B. adolescentis* CIDCA 5317 (low surface hydrophobicity and adhesion).

Bifidobacteria were cultured in anaerobic conditions and the planktonic or whole (planktonic + sessile) population were used in experiments. Monocytic THP1 cells were differentiated with phorbol myristate acetate (PMA) 200 nm for 3 days. Co-incubations (THP1 cells-bacteria) were performed for 1h or 2h at 37°C at different bacteria/monocyte ratios (multiplicity of infection, MOI). Cells associated to FITC-labeled bacteria were determined by flow cytometry (FL1+ cells) and plate counts on MRS agar (Samples were treated with gentamicin to assess bacteria internalization). Flow cytometry was also used to evaluate necrosis (propidium iodide staining; FL2+ cells).

Microscopy analysis revealed different interaction patterns for the two strains. Whereas strain CIDCA 5310 lead to large clusters around the phagocytic cells, strain CIDCA 5317 lead to a more diffuse interaction. Flow cytometry analysis of THP1 cells incubated 1 h with the planktonic population of bacteria (MOI=20) showed 90.10 ± 1.56 % of cells associated to strain CIDCA 5310 and 57.15 ± 0.35 % associated to strain CIDCA 5317. Results for the whole population (sessile + planktonic) microorganisms were 92.30 ± 4.80 and 12.60 ± 3.80 of cells associated (FL1 + events) to strain CIDCA 5310 and strain CIDCA 5317, respectively. For strain CIDCA 5310, MOI of 50 or higher lead to around 70 % necrosis whereas values remains similar to controls (20 %) for strain CIDCA 5317.

The ratio of invading/associated bacteria was: 0.16 ± 0.10 (1 h of incubation) and 0.20 ± 0.02 (2 h incubation) for strain CIDCA 5310, whereas for strain CIDCA 5317 values were 0.15 ± 0.03 irrespectively of the timepoints considered.

Código de Resumen: IN-002

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

LEVADURAS ENDOFITICAS DE CAÑA DE AZUCAR PRESENTAN ACTIVIDAD *QUORUM QUENCHING*

ENDOPHYTIC YEASTS FROM SUGARCANE EXHIBIT *QUORUM QUENCHING* ACTIVITY

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Quorum sensing (QS) are signaling mechanisms that govern morphological and physiological changes in responses to cell density increases. QS enables microorganisms to communicate via secreted signaling molecules called autoinducers. *N*-acyl homoserine lactones (AHLs) are autoinducers synthesized by several species of Gram negative bacteria. *Quorum quenching* (QQ) is a process that disrupts QS mechanisms by different ways including the enzymatic inactivation of the signal molecules.
QQ can be exploded as an alternative way for the control of Gram-negative pathogen bacteria whose pathogeny depends on QS mechanisms. QQ properties have been studied in bacteria and filamentous fungi. However, little is known about QQ in yeast. In view of this, the aim of the present work was the isolation and characterization of endophytic yeasts with QQ propertiesfrom sugarcane (*Saccharum officinarum*), a crop of high economic interest.
Isolates were obtained from samples of roots, stems and leaves of sugarcaneafter surface sterilization and plating in YM agar. Endophytic yeasts were also obtained from apoplast fluid after centrifugation of internode sections. Colonies with different morphology were selected for the characterization of QQ properties. Identification of the isolates was performed by amplification with NL1 and NL4 primers and sequencing of the D1-D2 region of the large-subunit rDNA gene. For the characterization of the QQ properties, yeasts were incubated in YM supplemented with the commercial AHLs C6-HSL, 3-oxo-C6-HSL, C8-HSL and 3-oxo-C8-HSL, C10-HSL, 3-oxo-C10-HSL, C12-HSL and 3-oxo-C12-HSL. AHLs degradation was estimated by measuring the residual levels of autoinducers in supernatants with bioassays utilizing the biosensor strains *Chromobacterium violaceum* CV026, *C. violaceum* Vir07 and *Agrobacterium tumefaciens* NT1 (pZLR4).
Nineteen endophytic yeast isolates were obtained. Sequencing of 26S rDNA showed that the strains belonged to the genera
Pichia, *Rhodotorula* and *Sporisorium*. Although under the assayed conditions all yeasts were able to degrade at least one of the comercial standards of AHLs, the strains presented a tendecy to inactivate signal molecules with a long acyl chain. However, two *Rhodotorula* strains exhibited the most outstanding behavior degrading the eight different QS molecules tested. Bioassays with samples acidified with HCl showed that the QS activity could be reestablished suggesting that a lactonase-like activity could be responsible of the AHL inactivation by *Rhodotorula* yeasts
Results presented in this work suggest that endophytic yeasts with QQ activity could modify the physiology of bacteria colonizing the same ecological niche through the inactivation of the AHL signal molecules.

Código de Resumen: IN-003

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

ANÁLISIS DE RASGOS ANTI-PREDATORIOS DE PSEUDOMONAS PROBIÓTICAS VEGETALES EN UN ENSAYO EN MICROPLACA CON EL CILIADO MODELO *Tetrahymena thermophila*

TESTING ANTI-PREDATION TRAITS OF PLANT-PROBIOTIC PSEUDOMONADS IN A MICROPLATE ASSAY WITH THE MODEL CILIATE *Tetrahymena thermophila*

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Protozoa are a major group of bacterial predators. As such, protozoa shape bacterial communities in complex ecosystems like soil and water ecosystems. In turn, bacteria have evolved diverse anti-predatory strategies including biofilm formation, EPS synthesis, swimming motility and toxin production. When considering the possible use of bacterial isolates with plant probiotic traits for inoculant production in agricultural systems, it may be of interest to evaluate their anti-predatory behavior, as predation resistance may promote survival and persistence of the inoculated bacteria in the seed and root environment. In order to test such property, we set up a simple and rapid dual culture test using the model bacterial predatory ciliate *Tetrahymena thermophila*. Axenic cultures of *T. thermophila* were used to prepare suspensions (50.000 individuals/ml) that were aliquoted in 96-well microtiter plates (15 µl/well in Amoeba Saline [AS] solution), and 135 µl of test bacteria (at OD600 = 1.0 in AS) were added to each well. Wells without protozoa were used as reference. Thereafter, bacterial cell density (OD600) was monitored at 25 °C over a period of 15 h. The rationale is that predation-resistant strains will sustain the initial cell density over time, but edible bacteria will be consumed by the protozoa and consequently, cell density will be reduced during incubation. The antibiotic producer and biocontrol strain *Pseudomonas protegens* CHA0 was used as a positive control for anti-predation activity, whereas the isogenic mutant strain CHA19 (unable to produce several antibiotics and extracellular enzymes) was used as a preferred innocuous prey. A collection of 22 pseudomonads strains with antifungal activity isolated from agricultural fields and crop rhizospheres was assayed for their anti-predatory activity in the plate test. Overall, strains could be categorized in 3 groups: those that are resistant to predation (no cell density change during the assay); those are edible by the ciliate (constant cell density decrease during incubation); those that showed mixed responses (delayed onset of resistance or delayed edibility). Such differential behavior may reflect distinct anti-predation strategies and/or ciliate tolerance to bacterial activities. In fact, the cell-free culture supernatant of some predation-resistant strains lysed ciliates in a few minutes (due to extracellular toxic metabolites), whereas those from other predation-resistant strains did not change ciliate shape or motility over a period of 24 h, suggesting that their anti-predatory activity may require induction by the presence of protozoa or that the anti-predatory activity requires bacterial-protozoan contact and/or bacterial ingestion by the ciliate.

Código de Resumen: IN-004

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

EL ÁCIDO INDOL-3-ACETICO ESTARÍA INVOLUCRADO EN LA COMUNICACIÓN ENTRE ALGAS UNICELULARES Y BACTERIAS

INDOL-3-ACETIC ACID MAY BE INVOLVED IN UNICELLULAR ALGAE-BACTERIA COMMUNICATION

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Exhaustion of fossil fuels is one of the major world-wide problems. Thus, the production of biodiesel from alternative feedstocks, such as plants and microalgae, has been attempted as an alternative energy source. It has been demonstrated that oil productivity by several microalgal strains (i.e. *Scenedesmus obliquus*) is 100-fold higher than that of soy. However, massive production of biodiesel from algae is not commercial yet, mostly because production costs are still high. We are exploring the possibility of improving the sustainability of microalge culture in mixed consortia with beneficial bacteria, in a similar way as the current trend in modern agriculture to increase the use of plant growth promoting rhizobacteria.Occurrence of Indole-3-acetic acid (IAA), which promotes the growth of algae and other plant organisms have been reported in many algal species. The role of IAA in plants is linked to cell elongation and division, cell differentiation, among others. *In vitro* studies demonstrated that several algal species respond to synthetic IAA and in *S. obliquus* auxin biosynthesis is linked to stress conditions. However genomic comparison indicated a large divergence of IAA signaling between plants and unicellular algae. Interestingly, microalgae growth promoting bacteria tend to produce high levels of IAA. Even so, the physiological role of IAA in microalgae remains unknown. Since IAA acts as a signal molecule in microorganisms, it has been proposed that algae IAA production could be involved in the interaction with surrounding bacterial strains. *Azospirillum brasilense* is a plant-growth-promoting rhizobacteria (PGPR) that enhances growth of many crop plants. This promoting effect is related to the production of IAA, cytokinin, gibberellin, ethylene and also nitric oxide. Plant growth promotion is IAA dependent, since *A. brasilense* mutant Faj009, with a 90% reduction in IAA production, is impaired in this effect. Incubation of *S. obliquus* with synthetic auxins resulted in growth inhibition at high concentrations. However, co-inoculation with 1:1, 1:5 or 1:10 (*S. obliquus*: *A. brasilense*) cell-ratios produced a slight increase in *S. obliquus* growth. Conversely, the same experiment conducted with the mutant Faj009, resulted in an inhibition of *S. obliquus* growth. Moreover, the bacterial population of the mutant strain remained higher than that of the parental strain. Algae cells appeared to produce IAA under every condition assayed, however it was increased when cells were co-inoculated with the bacterium that produce lower levels of the hormone. None of the treatment led to any changes in neither cell size nor morphology. These results suggest that IAA may participate in unicellular algae-bacteria communication that may result in changes in species relative densities in complex consortia. It is anticipated that advancing in these aspects of microalge-bacteria ecology might have implications for extensive culture of microalgae in the field.

Código de Resumen: IN-005

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

FORMACIÓN DE BIOFILM POR *Burholderia tropica*, UNA BACTERIA PROMOTORA DEL CRECIMIENTO VEGETAL

BIOFILM FORMATION BY THE PLANT GROWTH PROMOTING BACTERIA *Burkholderia tropica*

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Biofilms are recognized as the predominant form of bacterial growth in the environment. Growth in a biofilm provides many advantages for bacteria, including enhanced resistance to environmental stresses, such as desiccation and anti-microbial agents, as well as to host defenses. Biofilms can be defined as structured communities of sessile microbial aggregates, enclosed in a self-produced polymeric matrix, attached to an abiotic or biotic surface. The root surface is an example of biotic surface in which many microorganisms form biofilms for the establishment of an effective plant–microbe interaction. Colonization of plant root surface by Plant Growth Promoting Bacteria (PGPB) has been associated with their capacity of biofilm formation. PGPB are a group of microorganisms able to confer beneficial effects on plant growth and development, applied as an alternative or a supplemental way of reducing the use of chemicals in agriculture. After inoculation process they have to colonize the root. The initial phase of colonization is the bacterial attachment to the root surface followed by a growth forming microcolonies or biofilms, and some of them are able to enter roots and establish endophytic populations. In the present work some common batch systems were carried out in different culture media to evaluate the ability of *Burkholderia tropica* Mto-293 to form biofilm on abiotic surfaces. This bacterium is an endophytic nonpathogenic plant-associated *Burkholderia* specie belonging to the group of PGPB with potential and safe application in an agricultural context. Biofilm studies were carried out using multiwell plates and glass tubes and the biofilm formation was monitored by crystal violet stain. The architecture of biofilm was analyzed on glass slides by fluorescent microscopy (FM) and confocal laser scanning microscopy (CLSM) using a derivative strain of *B. tropica* with the *gfp* marked gene. *B. tropica* was able to attach to both types of surfaces (polypropylene and glass) and developed a biofilm on the air-liquid interphase but not for all culture media tested. The biofilm was observed in the media with ammonia as Nitrogen source in multiwell plates but in glass tubes the best media were those with yeast extract and glycerol, where the polysaccharide production was also observed. Biofilm formation of *B. tropica* was evaluated over time (24, 48, and 72 h) by FM showing sequential microbial aggregates of cells immersed in a dense matrix on the air-liquid interphase. By CLSM it was possible to determine 3D architectural characteristics of the mature biofilms formed by *B. tropica*.

Código de Resumen: IN-006

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

RESPUESTA QUIMIOTÁCTICA DE *Escherichia coli* ACLIMATADA A UN ESTILO DE VIDA ENDOFÍTICO

CHEMOTACTIC RESPONSE OF *Escherichia coli* ACCLIMATED TO AN ENDOPHYTIC LIFESTYLE

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We have previously shown that *Escherichia coli* K12 strain MG1655 acclimates to colonize lettuce plants and shows increased colonization efficiencies after successive cycles of seedlings infection. The aim of this work was to investigate aspects of the acclimated state to have insights into the physiological mechanisms that *E. coli* display when colonizing secondary hosts such as plants. Plant-acclimated *E. coli* cells presented a considerably more active chemotactic response as observed as migration onto TB plates. To confirm this result migration towards glass capillaries filled with leaf-blade or vascular tissue cell-free extracts was scored as a ratio of migration towards sterile PBS buffer (control) from the same bacterial reservoir. Migration towards lettuce extracts was on average 4 to 5 fold more prominent for plant-acclimated bacteria than non-acclimated cells cultivated in LB medium. As a control we show that, conversely to its isogenic line (RP437), a mutant strain impaired in the chemotactic response (*cheA*- Δ*cheA1643* strain RP9535) showed no preferential migration towards leaf-extracts.
Bacterial counts from roots or leaves of lettuce seedlings inoculated with 10 8 non-chemotactic cells x ml^{-1} for 20 days were on average 15-fold lower in comparison with those reached by a similar inoculum of the chemotactic isogenic-strain. It is noteworthy that the minimal infection counts observed from the chemotactic cells were up to 1000-fold higher than those of the non-chemotactic mutant cells. After inoculation of *E. coli* at 10^5 cells x ml^{-1} for 14 days, three out of three pools of three seedlings per pool showed root colonization and one showed leaf colonization when inoculated with chemotactic cells. Conversely, no root or leaf colonization was observed when seedlings were inoculated with non-chemotactic bacteria in a same number of assays.
Thus enhanced chemotactic response might be one of the strategies that *E. coli* display during acclimation to plant colonization.

Código de Resumen: IN-007

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

INCREMENTO DE LA PRODUCTIVIDAD LIPÍDICA EN MICROALGAS OLEAGINOSAS POR MEDIO DE LA INOCULACIÓN CON *Rhizobium sp*.

INOCULATION WITH A *Rhizobium* sp. STRAIN ENHANCES LIPID PRODUCTIVITY OF OLEAGINOUS MICROALGAE

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Increasing demand of food and energy and concerns on environmental decay, represent some of the main current challenges for Humankind. However, what is even more challenging is how to harmonize these three often conflicting interests for sustainable development. The production of biofuels, food, feed and biomaterials from microalgae biomass represent a promising alternative to bring into line all these concerns.

Currently, microalgal biomass is produced commercially mostly for high value products. However, biofuels from microalgae are not commercial yet mostly because production costs are still too high. Some of the drawbacks concern to a very high demand of agrochemicals, which also entails some detrimental effect against the environment.

The use of plant growth-promoting bacteria (PGPB) is widespread in modern agriculture. However, the potential of growth-promotion of aquatic microalgae by bacteria remains mostly unexplored.

This study reports the isolation and identification of cultivable heterotrophic bacteria after 3 years enrichment by serial dilution of monoalgal cultures in mineral medium. We further identified *Rhizobium* strain 10II as a likely general microalgae-growth promoter. An artificial consortium between *Ankistrodesmus* sp. and *Rhizobium* sp. has been characterized in more detail. Upon inoculation with the bacterium, the microalga exhibited increments of up to 50% in chlorophyll, biomass and lipid content of the oleaginous microalgae. The stimulation effect was apparently related to indol-3-acetic acid and/or vitamin B12 produced by the bacterium in exchange for photosynthetic exudates. Inoculated cultures reached a high lipid productivity of up to 112 mg . L^{-1} . d^{-1} after optimization. Lipids from the consortium biomass show no difference in fatty acids composition in comparison to axenically-cultivated microalgae. Interestingly, the consortium oil was highly enriched in Ω3 fatty acids, up to 25 % p/p, with a significant proportion of stearidonic acid, suggesting potential as an alternative land-based source of essential fatty acids.

Código de Resumen: IN-008

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

FAGOCITOSIS DE LACTOBACILOS CON DIFERENTE SUCEPTIBILIDAD A LAS β DEFENSINAS HUMANAS

PHAGOCYTOSIS OF LACTOBACILLI WITH DIFFERENT SUSCEPTIBILITY TO HUMAN β DEFENSINS

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Phagocytic cells play a key role in host´s defenses and constitute a link between innate and adaptive immune responses. The activity of phagocytic cells is crucial to reduce bacterial load and also for antigen processing and presentation. Antimicrobial peptides such as defensins are involved in the inactivation of internalized microorganisms and the differential interaction of microorganisms with those antibacterial molecules could modifies immune response. Human β defensins (HBDs) are cationic antimicrobial peptides secreted by epithelial cells of different mucosa. Positive charged defensins interact with negatively charged bacterial surface structures thus increasing membrane permeability and leading to cell death. Considering that lactic acid bacteria (LAB) have demonstrated an interesting probiotic potential, differences in the interaction of LAB with defensins could modify the fate of internalized microorganisms and cell response.

Since we have demonstrated that related lactic acid bacteria (i. e. *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 and *L. delbrueckii* subsp. *bulgaricus* CIDCA 331) have different susceptibility to human defensins, in the present work we sought to gain insight on the effect of HBD-1 and HBD-2 on the interaction between lactobacilli and cultured macrophages.

Bacteria were incubated with HBD-1 and HBD-2 at different concentrations. Membrane permeabilization was evaluated by flow cytometry (FACS) after propidium iodine (PI) staining. Zeta potential was determined with a Z meter system. Phagocytosis was evaluated by FACS analysis incubating FITC-labeled bacteria at different multiplicities of infection (m.o.i) with THP-1 cells differentiated with phorbol myristate acetate (PMA). Trypan blue was used as quenching solution. Bacterial adhesion was assesed by viable counts.

Strain CIDCA 331 was more susceptible to HBD-2 than strain CIDCA 133. Indeed percentages of PI (+) cells were 2. 6 to 3.5-fold higher for strain CIDCA 331 at 4 and 8 µg/ml respectively. Both lactobacilli were unaffected by HBD-1. The surface potential of both strains were negative, being -5,07 mV for strain CIDCA 133 and -28,9 mV for strain 331. Bacterial adhesion and internalization by THP-1 cells were higher for strain 133 at all the moi tested.

Results indicate that strain CIDCA 331 is more susceptible to HBD-2 than strain CIDCA133. These findings could be ascribed to differences in bacterial surface charges that correlate with the lower glycolipid/phospolipid ratio of the membrane of strain CIDCA 331. This characteristic determines higher negative surface charge. Strain CIDCA 133, which is more resistant to HBD-2, showed higher interaction with THP-1 cells than strain CIDCA 331 and it was endocytosed in high amounts by THP-1 Cells.

We could hypothesize that the internalization of microorganisms with different susceptibility to antimicrobial peptides could modify not only the intracellular traffic of the phagocytosed microorganisms but also the biological response of phagocytic cells.

Código de Resumen: IN-009

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

USO DE MICROORGANISMOS NUTRICIONALES Y SIMBIONTES EN CONTROL BIOLOGICO DE MOSQUITOS DE IMPORTANCIA SANITARIA

NUTRITIONAL AND SYMBIONT MICROORGANISMS TO BE USED IN BIOLOGICAL CONTROL

OF MOSQUITOES OF PUBLIC HEALTH IMPORTANCE

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Some microorganisms are crucial for insect nutrition, as they provide of specific nutrients like vitamins and essential amino acids. The elimination of nutritional and symbionts microorganisms indispensable for the maintenance and development of the insects could be a strategy for biological vector control, and is defined as Symbiotic Control. In a previous work, we evaluated the nutritional quality of different microorganisms (cyanobacteria, algae, yeasts and intestinal bacteria of mosquito) on *Culex pipiens* larvae, among them, only yeasts allowed mosquitoes to complete development until adult stage. Based on these results and the previous evidence in other insects that some yeast species could be inherited to progeny as the first food of neonate larva, we followed the presence of green fluorescence from GFP-labeled *Saccharomyces cerevisiae* as a sole source of food provided to *C. pipiens* first instar larvae. Mosquito‘s four larval instars, pupae, adult as well as offspring eggs (F1) were screened for this yeast by its growth in appropriate culture medium, by fluorescence microscopy of GFP-labeled yeast and PCR amplification of the GFP gene and of the yeast 18S rRNA with generic primers. We only detected fluorescent yeast until fourth instar larvae and GFP gene until adult females. F1 eggs did not indicate the presence of *S. cerevisiae* (not detected by PCR). However, we still obtained some other microorganisms that grew in a suitable culture medium. Based on these results, the aims of this work were to identify those different microorganisms and to analyze if they are inoculated by the mosquito female onto the eggs. The sequences obtained by the amplification of the DNA fragments with specific or degenerate 18S rDNA primers for yeast did not allow the identification of any yeasts. On the other hand amplification of the 16S rDNA gene of these microorganisms led to the identification of bacteria belonging to the genus *Acinetobacter* sp. and *Klebsiella* sp. In addition, we detected both bacteria genera in mosquito guts, by the amplification of 16S rDNA fragments. In order to detect if these bacteria were inoculated onto the eggs by the mosquito female, we reared mosquitoes in sterile and controlled conditions, to rule out the possibility of the contamination of eggs by environmental bacteria. This assay confirms the presence of both bacteria only onto F1 eggs. This suggests that the mosquito female might inoculate the F1 eggs. However, further experiments using other rearing techniques should be performed to confirm this hypothesis, and thus to determine whether these organisms could be used in biological control programs of mosquito populations. This can be carried out by elimination of bacteria that are fundamental for mosquito nutrition, or by expression of mosquitocidal toxins in those microorganisms.

Código de Resumen: IN-010

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

Pseudomonas aeruginosa PROVENIENTE DE AGREGADOS SUPERFICIALES ES ELIMINADA LUEGO DE SU INTERNALIZACION EN CELULAS EPITELIALES

SURFACE-AGGREGATED *Pseudomonas aeruginosa* IS ELIMINATED AFTER BEING INTERNALIZED INTO EPITHELIAL CELLS

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Pseudomonas aeruginosa (PA) is an environmental bacterium of wide distribution. It is also an opportunistic pathogen capable of causing acute as well as chronic infections of high severity. One of the key factors rendering treatment of PA-infections difficult is the formation of biofilms. Biofilms are multicellular communities of bacteria held together by a self-produced extracellular matrix.

In our laboratory we study the formation of PA multicellular structures in the context of host cells, as well as the response of host cells to the formation of such structures.

In previous studies we demonstrated, by infecting cultures of polarized epithelial cells, that when PA contacts the cell surface, it forms biofilm-like aggregates in a sequential and minute-length process. Also, we showed that those recently formed aggregates could be internalized into epithelial cells.

On the basis of these results, we wondered about the fate of PA upon internalization. In order to answer this question we performed standard antibiotic protection assays measuring the number of intracellular bacteria at 3, 6, 9 and 24 hours post-infection (HPI). These assays were carried out using three different antibiotic concentrations, so as to find the suitable antibiotic concentration that eliminates extracellular bacteria but did not cause death of internalized PA by non-specific antibiotic uptake through pinocytosis. An increase in intracellular bacterial number was measured 6 HPI compared to 3 HPI. But at 9 HPI the number of bacteria decreased remarkably, and at 24 HPI no intracellular bacteria were detected. These results suggest that PA is uncapable of surviving within epithelial cells for prolonged periods of time. To assess the mechanisms involved in intracellular PA elimination, subcellular localization studies were performed. 90% of intracellular PA was found in LAMP1 positive-vesicles. It was also found that approximately 50% of PA-containing vesicles were acidified. These findings suggest that PA is transported to lysosomes, where it would be eliminated. Currently, PA metabolic activity within those epithelial subcellular compartments is being studied.

Our results tempt us to speculate that internalization could be part of a mechanism to avoid that surface multicellular structures evolve to form fully mature biofilms.

BIOTECNOLOGÍA Y FERMENTACIONES

2 al 4 de Julio de 2014
Hotel 13 de Julio
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Código de Resumen: BF-001

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

CLONES TRANSFORMANTES DE LA LEVADURA *Pichia pastoris* CON MAYOR NIVEL DE EXPRESION DE QUIMOSINA BOVINA RECOMBINANTE

TRANSFORMANT CLONES OF THE YEAST *Pichia pastoris* WITH HIGHER LEVELS OF EXPRESSION OF RECOMBINANT BOVINE CHYMOSIN

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Bovine chymosin is the most commonly used proteolytic enzyme for the manufacture of cheeses due to its ability to specifically cleave the κ-casein from the milk, thereby causing milk coagulation. Our group has previously cloned and expressed this enzyme in the methylotrophic yeast *Pichia pastoris* under the control of the methanol-inducible promoter AOX1, scaled up chymosin production by fed-batch fermentations using bioreactors and performed its purification by size exclusion chromatography. This expression system has significant advantages such as growth to high cell densities, high expression level of recombinant proteins with post-translational modifications and efficient secretion of extracellular proteins. In the present work, we carried out the selection of clones of *P. pastoris* transformed with the gene of bovine chymosin which showed higher levels of milk-clotting activity. A first selection of transformant clones with higher production of recombinant chymosin was performed by its growth in *Erlenmeyers* containing complex medium with glucose as carbon source and inducing chymosin expression by the addition of methanol. A second selection of clones with higher expression of chymosin was carried out by growing the previous selected clones in flasks with basal salt medium with glycerol as carbon source and the addition of methanol. The enzyme activity was determined by the milk-clotting assay using multi-well plates. The amount of secreted chymosin was determined by gel electrophoresis. Furthermore, we evaluated the growth of one clone in basal salt medium containing crude glycerol derived from biodiesel production. Finally, the stability of the recombinant chymosin was analyzed after storage at different temperatures. Thus, in the complex media we identified 15 over 100 clones that achieved a milk-clotting activity 8 times higher than those with the lowest value. From the analysis of the 15 clones in basal salt medium we determined that the coagulant activity of one clone was 8 fold greater than the lowest value. Furthermore, by gel electrophoresis we established that the profile of the secreted proteins contained mainly recombinant bovine chymosin. Also, we determined that the clone that produced the highest amount of recombinant chymosin could optimally grow in basal salt medium containing biodiesel-derived crude glycerol and subsequent produce chymosin by methanol induction, with similar levels for both types of glycerol. The use of crude glycerol will remarkably reduce the cost of the production of recombinant chymosin in bioreactors by fermentation strategies. The stability study of the recombinant chymosin established that the level of milk-clotting activity does not change when the enzyme is stored at 5° C for at least 3 months. In conclusion, it was determined that one clone of *P. pastoris* reached a higher level of production of recombinant bovine chymosin, which may be industrially used for the elaboration of cheeses.

Código de Resumen: BF-002

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

FÁBRICAS MICROBIANAS MULTIESPECIE FORMADAS POR MICROALGAS OLEAGINOSAS Y BACTERIAS MODIFICADAS GENÉTICAMENTE PARA LA EXCRECIÓN DE AMONIO

MULTISPECIES MICROBIAL CELL-FACTORIES COMPRISING OIL-RICH EUKARYOTIC MICROALGAE AND ENGINEERED AMMONIUM-EXCRETING BACTERIA

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The current and projected increases in human population and general welfare poses a serious concern on the trilemma food, energy and environment, since these interests are often conflicting. A key aspect of this is the additional role imposed to agriculture towards biofuels production that entails dramatic changes in the use of arable land and an increase in the demand of agrochemicals, especially N-fertilizer. The use of non-conventional crops such as aquatic microalgae that may thrive in otherwise non-productive land represents one of the most attractive alternatives. However, large-scale cultivation of microalgae might increase the demand of N-fertilizer up to unsustainable levels. Conversely to current agricultural practices, the use of biological nitrogen fixation to partially substitute for synthetic N-fertilizers in eukaryotic algae culture is just starting to be explored. In this study we modified the capacity of ammonia excretion of the free-living diazotrophic bacterium *Azotobacter vinelandii* by metabolic engineering. For this purpose, regulatory pathways of nitrogen-fixation (ammonium production) and nitrogen assimilation (ammonium consumption) were modified. The first set of mutant strains bear an in-frame deletion of the general *nif*-genes expression anti-activator *nifL*. As expected, these strains were unable to sense intracellular ammonium sufficiency, and as a consequence were impaired in *nif*-genes expression switch-off, produced an excess of ammonium that is released to the medium. In *A. vinelandii* ammonium assimilation occurs only by the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway, making GS mutants lethal. Then, to modify ammonium assimilation, we first made use of the GS inhibitor L-methionine sulfoximine (MSX) to partially inhibit GlnA what allowed us to better understand the relationship between GS activity, cell growth and ammonium excretion. These results encourage us to isolate a set of mutant strains bearing point mutations at the active site of GS. This single mutation, especially D49S, produced strains with slow diazotrophic growth and ammonium excretion properties. Double mutant strains, show a dramatic increase in the initial rate of ammonium release into the medium but failed to sustain the production. We further observed that that was mostly due to a severe impairment of *nif* genes expression in the double mutant strains. D49S strains were more efficient ammonium producers under carbon/energy limiting conditions, as would be expected when growing at the expense of microalgae C-exudates in synthetic microbial consortia. This has been experimentally confirm in co-culturing experiments that resulted in the accumulation of oleaginous biomass using air as the sole source of C and N. Ammonium delivery by the different strains had implications for the cell size distribution of microalga. This study represents a step forward towards sustainable microalgae biotechnology.

Código de Resumen: BF-003

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

PRODUCCION DE ELECTRICIDAD EN LECHOS ACUATICOS CON BACTERIAS ELECTRO-ACTIVAS

ELECTRICITY PRODUCTION IN AQUATIC BEDS WITH ELECTRO-ACTIVE BACTERIA

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Electro-active bacteria can use a polarized electrode as the final electron-acceptor of their respiratory chain. In this way, they can couple degradation of organic compounds and production of electric current. These bacteria are present in most sedimentary environments where they use simple carbon compounds (such as acetate) as carbon and electron source and metallic oxides as electron acceptor. Typically, the activity of these bacteria is limited by the availability of metallic oxides in the media. This limitation can be overcome by introducing polarized electrodes in the sediment that can act as electron acceptor. This leads to the formation of an electro-active biofilm on the electrode surface which produces a stable electric current directly from the bacterial metabolic activity. This energy can be used to operate marine-deployed instruments with lower maintenance requirements and lower cost than the alkaline batteries normally used in those devices. We have studied the production of electricity by electro-active bacteria from natural aquatic environments replicated in the laboratory at small scale. We have tested different electrode materials and configurations and achieved a design that produces almost 50mW per m2 of submerged electrode. This energy would allow charging Li-ion batteries as those typically used in mobile devices or operate a meteorological bouy with a few square meters of electrode.

Código de Resumen: BF-004

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

SCREENING DE POLIGALACTURONASAS BACTERIANAS CON ACTIVIDAD EN MEDIO ALCALINO CON POTENCIAL USO EN LA INDUSTRIA AMBIENTAL

SCREENING OF BACTERIAL POLYGALACTURONASES WITH ALKALOPHILIC ACTIVITY AND ITS POTENTIAL USE IN ENVIRONMENTAL INDUSTRY

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Pectinases present different kind of enzymes that can degrade pectic substances. The classification of these enzymes is based on their way to react with the galacturonan backbone of pectin molecules. The three main types of pectinases are pectin metylesterases (PME), pectin lyases (PL) and polygalacturonases (PG). PME catalyze the deesterification of methyl ester linkages, PL act by ß-elimination on glycosidic bonds and PG act on □-(1,4) glycosidic bonds of pectin by hydrolysis. PG include both endoPG and exoPG which react cleaving internal bonds or terminal galacturonic acid residues, respectively. Pectinases have different applications in food, textile and paper industry, degumming of plant bast fiber and also for the pretreatment of pectic wastewaters. For pretreatment, the pH must be neutral or alkalophilic to remove pectinaceous material and renders it suitable for decomposition by activated sludge treatment. At the present almost all of the PG available commercially are produced by fungi so they present activity at acidic pH. The aim of this research is to obtain PG from bacteria with future applications on wastewaters. So in the present study were screened one hundred bacterial strains for PG activity in a culture medium with citric pectin as inducer. The quantification of the activity was determined in the extracellular medium. The endoPG was

measured by viscosity reduction of 1% polygalacturonic acid solution and exoPG by a colorimetric method to identify the release of reducing sugars. The results showed 64 strains with PG activity. *Bacillus* and *Streptomyces* strains exhibited higher PG activity. The maximum activity was obtained from *Streptomyces halstedii* and the results showed that after 24 hours of fermentation in a culture medium with 10 g/L of citric pectin, 28°C and pH 8 the PG activity was 0.595 U/mL and 94% of viscosity reduction. The enzymatic activity had an increase 1.35 fold using soy peptone as main carbon and nitrogen source and the studies of reaction conditions indicated that the enzyme is highly alkalophilic and stable until 60°C. Finally, the PG activity was 2.95 U/mL and 98% of viscosity reduction at pH 11 and 50°C. *S. halstedii* has a great potential due to its fermentation productivity at 24 hours and the alkalophilic activity is an important advantage to applications in pretreatment of pectic wastewaters. This results show a new development in biocatalysis by means of sustainable and environmentally friendly methods.

Código de Resumen: BF-005

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EXPRESIÓN DE LA PROTEINA S-LAYER DE *Lactobacillus acidophilus* ATCC 4356 EN UN SISTEMA HETERÓLOGO PARA LA EXPOSICIÓN DE ANTÍGENOS SOBRE LA SUPERFICIE CELULAR DE BACTERIAS ÁCIDO LÁCTICAS

EXPRESSION OF S-LAYER OF *Lactobacillus acidophilus* ATCC 4356 IN A HETEROLOGOUS SYSTEM TO DISPLAY ANTIGENS ON THE CELL SURFACE OF LACTIC ACID BACTERIA

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Surface layers (S-layers) have been recognized ubiquitously in both *Eubacteria* and *Archaea.* S-layers proteins normally contain two functional regions: the self-assembly domain and the cell wall-targeting domain. Both regions have been characterized in the S-layer SA protein of *Lactobacillus acidophilus* ATCC 4356. The display of heterologous proteins on the cell surface of lactic acid bacteria (LAB) is an interesting and emerging area that holds great promise in the development of live vaccine delivery system. Various anchoring proteins, including S-layers, have been studied for their efficiency in attaching hybrid proteins to the cell membrane or cell wall of LAB. However, the expressed proteins were anchored to producer cells, thus making the host strain for surface display a genetically modified organism. In this study, we developed an approach for surface display of the heterologous proteins on the LAB cells by mean of S-layer protein fused to MBP (Maltose Binding Protein). For this purpose we cloned the full length S-layer of *Lactobacillus acidophilus* in the pMAL system fused to MBP, this fusion improved the solubility of the recombinant protein. It was shown that a substantial fraction of S-layer commonly extracted with LiCl from *Lactobacillus acidophilus* readily aggregates and forms a precipitate. When the fusion protein was purified, it did not show any precipitate formation. The pLC3 vector was expressed in *Escherichia coli* and the fusion protein was then purified by a one-step affinity purification for MBP using an amylose resin. The confirmation of the correct molecular mass of the fusion protein was checked by SDS-PAGE and Western blotting with anti MBP and anti S-layer antibodies. After overproducing the fusion protein successfully in *E.coli*, the purified protein was used to decorate *Lactobacillus casei* cells in vitro and the binding was viewed by fluorescence microscopy. In order to evaluate different conditions to improve the attachment to the *Lactobacillus* carrier, experiments of flow cytometry are being performed. In conclusion, the S-layer protein fused to a foreign protein like MBP was overproduced in a heterologous organism and shown to maintain its capacity to anchor to the cell surface of LAB. These surface display system offers the possibility of surface display of foreign antigens, suitable for application as an oral delivery vehicle. It is worth highlighting that the lactobacillus decorated is a non- genetically modified organism, therefore its GRAS status is not altered.

Código de Resumen: BF-006

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SCREENING DE RESIDUOS AGRO-INDUSTRIALES PARA LA PRODUCCIÓN DE UNA ACTIVIDAD LIPASA ASOCIADA AL MICELIO DE *Aspergillus niger* MYA 135 POR FERMENTACIÓN SUMERGIDA

SCREENING OF AGRO-INDUSTRIAL WASTES TO PRODUCE A WHOLE-CELL LIPASE ACTIVITY BY *Aspergillus niger* MYA 135 UNDER SUBMERGED FERMENTATION

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Bioconversion of agricultural wastes for enzymes production is turning in an interesting approach in industrial biotechnologies, mainly in developing countries. Lipases (EC 3.1.1.3) are important industrial enzymes due to their versatile applications. Lipases catalyze a variety of reactions, such as partial or complete hydrolysis of triacylglycerols and reactions of esterification, transesterification and interesterification of lipids. Despite the great interests in the application of lipases in various industries, their high costs of production often restrict their use as biocatalysts. One of the research areas involving lipases currently focuses on the use different microorganisms, supplements and substrates to find the best combinations to obtain high-value lipases using operational conditions that facilitate the reduction of the production costs at industrial scale. This can be achieved through the use of low cost culture media, especially residues from agro-industry so that production can become economically viable. Furthermore, several studies have reported the utilization of microorganisms such as bacteria, yeast and fungi as whole-cell biocatalysts in attempts to improve the cost-effectiveness of the bioconversion processes. Among the established whole-cell biocatalyst systems, filamentous fungi have arisen as the most robust whole-cell biocatalyst for industrial applications. The aim of this work was to analyze the production of a lipase activity from *A. niger* MYA 135 including the use of agro-industrial residue wastes as components in a culture medium through a statistical experimental design. These experimental layouts can be adopted at various phases of an optimization process, such as for screening experiments or for finding the optimal conditions for targeted effects. One of the most frequently used choices as screening tool in statistical design is the *Plackett–Burman*. Among the factors analyzed in this design were included agro-industrial wastes such as cane molasses, vinasse, glycerol, waste cooking oil. In these experiments, all analyzed variables had a significant effect on the hydrolytic activity using *p*-nitrophenyl palmitate as substrate. The waste cooking oil showed the most positive incidence (E= 194.82). However, molasses (E= 102.11) and vinasse (E= 131.40) also had an important positive effect on the response studied. Interestingly, in the most of the analyzed media, we found a promising activity increment as compared to the activity previously reported for this strain. On the other hand, some morphological aspects of the biomasses were also analyzed since it is well known that biotechnological production processes performed with filamentous fungi are dependents of morphology control. Finally, the study presented here demonstrates the feasibility of agro-industrial wastes utilization as a sustainable green technology in the lipase activity production.
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Código de Resumen: BF-007

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

CAMBIOS EN LA CALIDAD DE UN QUESO PATEGRÁS ENVUELTO CON PELÍCULAS DE CASINATO DE SODIO INCORPORADAS CON ACEITES ESENCIALES DE ORÉGANO Y LAUREL PARA MEJORAR SU VIDA ÚTIL

QUALITY CHANGES OF PATEGRÁS CHEESE WRAPPED WITH SODIUM CASEINATE FILM INCORPORATED WITH ORÉGANO AND LAUREL ESSENTIAL OILS TO IMPROVE ITS SHELF-LIFE

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Active packaging is intended to change the product conditions in order to extend life or improve safety or sensory properties while the quality of the food is still maintained. Current research, in this topic, has been focused on the strategy of elaboration of an antimicrobial film based on biopolymer matrix incorporated with herbs and spices extract (essential oils) with antimicrobial activity against food borne pathogens, for enhancing the quality and safety of a wide range of food during extended storage. In this work, essential oils of oregano (*Origanum vulgare)* and laurel (*Laurus nobilis*) were obtained by hydrodistillation. Their antimicrobial activities were analyzed by tube dilution method against two food borne pathogens: *Escherichia coli* and *Staphylococcus aureus*. Oregano and laurel proved to have antibacterial effects against both microorganisms. Minimal inhibitory concentration for oregano and laurel essential oils were 1600-1800 and >2000 µg/ml against *E. coli;* and 800-900 and >2000 µg/ml against *S. aureus,* respectively. From these results, sodium caseinate films (3%p/v) with essential oils (1000-10000 µg/ml) were developed by casting. The antimicrobial activities of the films against *E. coli* and *S. aureus* were determined by the inhibition zone technique. For both microorganisms, oregano films showed greater inhibition zone. One selected formulation (10000 µg/ml) was applied on packaging of semi-hard Pategrás cheese. Cheese portions were wrapped with the films by heat sealed and storage (temperature 10±2 °C - 75±5 % relative humidity). Antimicrobial effect of the packaging over the product was evaluated by the petri-dish viable cell count after different time periods (14, 28, 42 and 56 days). The effect against total microorganisms, fungi and yeasts, *E. coli* and *S. aureus* were determined. The results indicated that the obtained films have potential for packaging semi-hard cheeses, increasing the shelf life of the product over time.

Código de Resumen: BF-008

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

UTILIZACIÓN DE HEXOSAS POR CEPAS PATAGÓNICAS DE *Saccharomyces cerevisiae* DE ORIGEN ENOLÓGICO

UTILIZATION OF HEXOSE SUGARS BY PATAGONIAN *Saccharomyces cerevisiae* STRAINS FROM OENOLOGICAL ORIGIN

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During winemaking, *Saccharomyces cerevisiae* yeast is the main responsible for turning grape sugars in alcohol and CO2. Since this species is mostly glucophilic, important amounts of fructose may remain at the end of the alcoholic fermentation (AF) and non desired sweet wines

may finally be obtained. The objective of this work was to select authoctonous *S. cerevisiae* strains with a higher capacity for hexose consumption, particularly fructose. Eight strains of *S. cerevisiae* isolated from red winemaking in the Añelo region (years 2005/2006), Neuquén province, Patagonia Argentina (previously characterized as autochthonous by molecular methods) were evaluated in its ability to grow and consume sugars. Yeasts were grown in YNB broths (Yeast Nitrogen Base, with aminoacids and ammonium sulphate 0,67%) supplemented with 20% (p/v) glucose, 20% (p/v) fructose or a mixture of 10% (p/v) of both sugars. Fermentation progress, carried out at laboratory scale, was evaluated by weighting and growth parameters: maximum specific growth rate (μ), lag phase duration (l) and maximum CO_2 produced (A), were calculated from each treatment by directly fitting CO_2 production versus time to the reparametrized Gompertz equation. Residual sugars were analyzed by enzymatic kits and sugar tolerance was evaluated by drop-test assays. Additionally, hexose transporters expression (Hxt1p to Hxt6p) was detected by RT-PCR. In all cases, assays using *Saccharomyces cerevisiae* EC1118, a commonly used commercial starter, were carried out as comparison. For statistical analysis, ANOVA for multiple data comparison and post hoc tests (α= 0.05) were performed. Three strains were selected from the growth curve profiles obtained, each showing tolerance to must sugar concentration and with statistical differences on growth parameters in the different media: ÑIF8 as glucophilic strain, ÑNM16 as clear fructophilic strain and ÑNM10 as a strain preferring the sugar mixture ($p<0,05$). Residual sugars were analyzed evidencing total sugar consumption by these strains, where ÑIF8 depleted glucose from the media earlier than the commercial strain. Molecular studies showed a significant higher expression of the HXT2 and HXT5 transporters in ÑIF8 and ÑNM16 strains ($p<0,05$), result that could explain the improved performance of these yeasts in sugar utilization. Additional studies will allow to complete autocthonous strain characterization to validate its use in wine fermentation. However, preliminary results suggest that *Saccharomyces cerevisiae* strains from the Comahue region present a fermentative metabolism best adapted to local agroecological conditions, evidenced by an enhanced grape sugar consumption.

Código de Resumen: BF-009

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

NANOBIOTECNOLOGÍA: DESARROLLO DE FILMS FOTOCATALÍTICOS Y BACTERICIDAS CON NANOPARTÍCULAS DE PLATA Y TiO_2 PARA CONTROLAR BACTERIAS MULTIRESISTENTES PATÓGENAS

NANO-BIOTECHNOLOGY: DEVELOPMENT OF PHOTOCATALYTIC AND BACTERICIDE FILMS OF SILVER NANOPARTICLES CONTAINING TiO_2 TO CONTROL MULTIRESISTANT PATHOGENIC BACTERIA

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The bactericidal effects of ionic silver are known and applied since antiquity. Silver is used in several medical devices and surgical equipments such as burn dressings, scaffolds, water purification systems and medical devices because silver nanoparticles (SNPs) may damage the activity of bacterial enzymes and cell structures, which cause bacterial cells to die. In addition, the photo-induced water splitting on TiO2 electrodes when is exposed to UV light (λ=400 nm) generate excited electrons (ecb^-) that are trapped by water (H_2O) or hydroxyl groups (OH^-) adsorbed on the surface to generate hydroxyl radicals (OH•). OH• is a powerful and indiscriminate oxidizing agent and have antibacterial properties. Thus, the combination of noble metals (e.g., silver) and TiO_2 may enhance the photo-catalytic efficiency and so degradation of pathogen microorganisms. In this work we study the antimicrobial activity of Ag nanoparticles in combination with coated TiO_2. The human-pathogenic strains used for the determination of the antimicrobial properties of these materials were multiresistant *Escherichia coli (EHEC), Staphylococcus aureus, Listeria monocytogenes, and Pseudomonas aureaginosa.* In addition,

Bacillus subtilis strains as prototype of spore-forming and biofilm-proficient strains were analyzed. Our results show the efficacy of SNPs coated with TiO_2 to kill dense-cultures of planctonic pathogenic bacteria and the biofilms made by them. In addition, we studied different mutants affected in the formation of the extra cellular matrix of the biofilm to identify the target of SNPs on the biofilm structure. We discuss a scenario of the present strategy to treat multi-resistant infections by biofilm-forming bacteria.

Keywords: Ag-nanoparticles, biofilms, bactericide effects

Código de Resumen: BF-010

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

BIOTRATAMIENTO DE AGUAS RESIDUALES QUE CONTIENEN Cr(VI) UTILIZANDO *Pseudomonas veronii* 2E

Cr(VI)-CONTAINING WASTEWATER BIOTREATMENT BY *Pseudomonas veronii* 2E

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Cr(VI) is a very dangerous pollutant often released into the environment by anthropogenic activities. In contrast, Cr(III) is much less toxic and less mobile in soils. Many industries use Cr(VI) and generate Cr(VI)-containing wastewaters that must be treated prior discharge in water systems in order to fulfill the legal requirements. A treatment commonly used is the chemical reduction of Cr(VI) to the less dangerous Cr(III) using SO_2(g) or $NaHSO_3$(aq) . However, this treatment is very inefficient at low Cr(VI)concentrations. In this work we develop an alternative biological treatment using *Pseudomonas veronii* 2E cells immobilized in calcium alginate. *P. veronii* 2E was grown in 1l nutrient broth (8 g/l nutrient broth and 1 g/l glucose) during 24 hours at 28ºC. The cells were harvested and kept at -20ºC until use. This process was repeated 10 times after which the cells were thawed, washed and resuspended in 500 ml distilled water. This suspension (52 g cell dry weight /l) was mixed with an equal volume of a solution of0.1 ppm sodium alginate. The mixture was then dripped over a 0.05 M $CaCl_2$ solution at 4ºC. The spheres formed were kept at 4ºC for 30 minutes, then washed with distilled water and packed in a 1l glass column. Two industrial wastewaters (wastewaters1 and 2) with 0.26 and 0.69 ppm Cr(VI) respectively supplemented with 200 ppm glucose as electron donor were pumped through the column at 32ºC. The flow rate was adjusted in order to obtain a residence time of the wastewater inside the column of 30 minutes. Every two hours the Cr(VI) concentration in the influent and effluent of the column was determined with a colorimetric method (DPC method). The two wastewaters were treated alternatively to avoid Cr(VI) accumulation in the column, which occurred when the wastewater 2 was treated alone for a long period of time. The concentration of Cr(VI) in the effluent never surpassed 0.1 ppm. In Argentina, any Cr(VI)-containing wastewater must have at least a concentration of 0.2 ppm Cr(VI)in order to be discharged in water bodies. In conclusion, we have proved that the use of immobilized *P. veronii* 2E cells for the treatment of Cr(VI)-containing wastewaters is feasible and more efficient than the conventional treatments used at present.

Código de Resumen: BF-011

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

CAPA S DE *Lactobacillus kefiri* COMO HERRAMIENTA EN NANOBIOTECNOLOGÍA: EN BUSCA DEL "GEN PERDIDO"

S-LAYER FROM *Lactobacillus kefiri* AS NANOBIOTECHNOLOGICAL TOOL: LOOKING FOR THE "MISSING GENE"

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Surface (S) layers are (glyco)-proteinaceous cell envelope structures ubiquitously found in bacterial species and in *Archaea*. The subunits are held together and attached to the underlying cell surface by non-covalent interactions and have an intrinsic tendency to self-assembly. *Lactobacillus kefiri* is a potentially probiotic microorganism carrying a highly glycosylated S-layer protein which showed to have interesting functional properties. Previous MALDI-TOF-MS analysis showed a great structural heterogeneity among S-layer proteins from different *L. kefiri* strains, but no more information about their sequences were obtained until now. The aim of this work is to show the most recent results obtained by MALDI-TOF and LC-MS/MS analysis of the S-layer protein from the autoaggregative strain *L. kefiri* CIDCA 8348, and the way that information was employed to gain insight into the primary sequence of this glycoprotein. A SDS-PAGE band corresponding to the S-layer protein from *L. kefiri* CIDCA 8348 was excised, then it was digested with trypsin, and finally a peptide mass fingerprint (PMF) was carried out in a MALDI-TOF mass spectrometer. Searching was performed against NCBInr/SwissProt database using MASCOT software. As observed few years ago, no significant matching was obtained at global protein sequence level between S-layer protein from CIDCA 8348 and other S-layer proteins available in databases. Interestingly, PMF analysis showed that our protein shares seven different fragments with the S-layer proteins from *Lactobacillus buchneri* CD034 or *Lactobacillus parafarraginis* F0439, two phylogenetically related strains. A 100% homology was observed with fragments from *L. buchneri* CD034 or *L. parafarraginis* F0439, using MS/MS analysis and a *de novo* sequencing method. Each fragment contains from 9 to 25 amino acid residues, showing a 20 % of total homology with proteins of the same family. Based on the genetic code reported for *L. buchneri* CD034, primers were designed and used for PCR reaction. A fragment of 320 bp length was amplified from DNA of *L. kefiri* CIDCA 8348, and the sequence showed a 99% identity with the *L. buchneri* S-layer 320-bp fragment. Since our goal is to sequence the S-layer gene of *L. kefiri*, this information as well as new primers designed in the flanking area of *L. buchneri* S-layer gene, will be used as a starting point for sequencing. Amplification from DNA of other aggregative and non-aggregative *L. kefiri* strains will be also performed. This is the first successful approach in order to obtain the primary sequence of S-layer protein from a potentially probiotic *L. kefiri* strain. The results obtained will be relevant to understand some functional properties of these proteins as surface components of the bacterial cells, and moreover regarding their potentiality in different areas of the nanobiotechnology.

Código de Resumen: BF-012

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

LA FERMENTACIÓN DE EXTRACTOS OBTENIDOS A PARTIR DE LA RAÍZ ANDINA YACON (*Smallanthus sonchifolius*) ES UNA ESTRATEGIA APROPIADA PARA EL DESARROLLO DE PRODUCTOS CON VALOR AGREGADO

FERMENTATION OF EXTRACTS FROM THE ANDEAN ROOT YACON (*Smallanthus sonchifolius*) IS A SUITABLE STRATEGY TO DEVELOP VALUE-ADDED PRODUCTS

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The Andean-native plant called yacon (*Smallanthus sonchifolius*) is valued for its tubers. This ancient culture was recently rescued in mountain areas in the Northwestern part of Argentine and some local food industries have an increasing but early interest. Also, researchers around the world are focusing on its nutraceutical properties, mainly due to its high content of fructooligosaccharides (FOS). In fact, our group determined that the flour and the syrup extracted from this root exhibited positive effects in the health of diabetic patients and in a murine model. As its consumption is limited and the widespread of its cultures may be stimulated, the development of value-added foods based on yacon constitutes a relevant task. Therefore, the aim of this work was to study if yacon extracts (also called yacon juice) could undergo a natural fermentation and to evaluate microbiological quality during this process. In consequence, microbial groups with technological applications and hygienic quality markers were investigated by plating onto general and selective media (Plate Count Agar, MRS, M17, Yeast and Molds, Violet Red Bile Agar and Red Bile Glucose Agar). From the juice before fermentation samples, results showed that Lactic Acid Bacteria (LAB) were the dominant microorganisms with an important contribution of Lactococci (Lc). Yeast and molds were scarcely found, as well as, total and fecal coliforms. During incubation of extracts at 30°C for 72 h, both LAB and Lc grew while pH exhibited a slight reduction and coliforms decreased to a non-detected level. Yeast and mould counts did not present significant changes. Subsequently, LAB and Lc colonies were isolated and partial characterized. In conclusion, fermentation could be a relevant tool to generate a new value-added product from the yacon roots improving the microbiological quality of raw material. It may be noticed that these extracts also emerged as an excellent source of strains with potential usefulness in vegetable fermentations.

Código de Resumen: BF-013

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

DEGRADACIÓN DE PROTEÍNAS CÁRNICAS POR DIFERENTES CULTIVOS INICIADORES AUTÓCTONOS EN UN EMBUTIDO FERMENTADO MODELO CON BAJO CONTENIDO DE SAL

MEAT PROTEIN DEGRADATION BY TWO DIFFERENT AUTOCHTHONOUS STARTER CULTURES IN A LOW SALT DRY FERMENTED SAUSAGE MODEL

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Fermented sausages have a relatively long shelf life; its microbiological quality is guaranteed by the fermentation carried out by lactic acid bacteria (LAB). However it is a product with high levels of sodium so it is not suitable for people with hypertension or cardiovascular diseases. There exist many technological difficulties in reducing the concentration of salt, because sodium

chloride promotes microbiological stability and contributes to flavor formation. The objective of this work was to evaluate the behavior of two different LAB strains to be applied as starter culture in fermented sausage production with reduced sodium contents (1%), focusing on its proteolytic potential towards meat proteins. The presence of LAB with optimal peptidase activities guarantees the production of flavor peptides and amino acids that would balance flavor development in low sodium-fermented sausages. An *in vitro* Beaker Sausage model was used for this purpose. Ground meat with additives was divided into three batches and incubated at 22°C: (T1) *Lactobacillus curvatus* CRL 705/ *Staphylococcus vitulinus* GV 318; (T2) *Lactobacillus sakei* CRL1862/ *Staphylococcus vitulinus* GV 318 and (T3) un-inoculated Control with antibiotics. Analysis of pH and bacterial counts on differential agar media was performed at 0, 3, 6 and 10 days. Total Protein was analyzed by Bradford and the proteolysis was evaluated by Tricine-SDS-PAGE (sodium dodecyl sulfate, polyacrylamide electrophoresis) at 0 and 10 days. Results showed that *Lactobacillus curvatus* (T1) counts remained stable (8 log CFU/ml) and *Staphylococcus vitulinus* decreased 3 logs after 10 days of incubation. The pH decreased rapidly from 5.8 to around 4.7 at the 10th day for both strains (T1 and T2), due to the acidogenic metabolism of LAB. On the other hand, *Lactobacillus sakei* CRL1862 (T2) showed a slight decrease after 10 days whereas the acidic environment resulted in the inhibition of *Staphylococcus vitulinus* in both batches. The proteolytic activity after 10 days of incubation, T2 (*L. sakei* CRL 1862) showed a more intense meat protein degradation profiles than T1 (*L. curvatus* CRL705). A similar tendency was observed in total protein contents, where T2 showed slight higher drop in this parameter (T0: 7.65 mg/ml; T10days: 4.10 mg/ml) than T1 (T0: 6.67 mg/ml; T10 days: 4.70 mg/ml). With respect to amino acids analysis, T1 and T2 beaker sausages, demonstrated high amino acid enrichment after 10 days. In fact, beaker sausages inoculated with *L. sakei* CRL1862 and *L. curvatus* CRL705 increased 7 and 6 times amino acid contents of the meat system respectively. In conclusion, the both assayed starter culture have showed an appropriate growth performance on Beaker Sausage model with reduced NaCl contents, optimal pH drop and ability to hydrolyze meat proteins. *Lactobacillus sakei* CRL1862 + *Staphylococcus vitulinus* GV 318 having the most efficient performance, can be proposed as a candidate for the production of low-sodium fermented sausages.

Código de Resumen: BF-014

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

PRODUCCION DE POLIHIDROXIALCANOATOS POR *Bacillus subtilis* subsp. *spizizenii* EN MEDIOS NITROGENADOS COMPLEJOS

POLYHYDROXYALKANOATES PRODUCTION BY *Bacillus subtilis* subsp. *spizizenii* IN COMPLEX NITROGEN MEDIA

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One of the mayor problems in the society is the environmental contamination with plastics coming from non renewable sources. The use of biodegradable plastics could help to preserve the fossil resources and to reduce the percentage of solids no degradable inside the urban waste. Bioplastics, polyhydroxyalkanoates (PHAs) that are obtained from *Bacillus subtilis* (bacterium considered as generally recognized as safe (GRAS) by the Food and Drug Administration) has the advantage of synthesize a biopolymer free of endotoxins, for this reason the accumulated bioplastic can be used for those products related to medical devices and for food packaging materials such as “fourth gamma product.” The problem of these bacteria are the spore formation when growth conditions are adverse, this mechanism interferes with PHAs production. The present work studies the accumulation of PHAs in *B. subtilis* subsp. *spizizenii* when complex nitrogen and different carbon sources and aeration were used to delay spore formation. One percent (v/v) of 12h at 30°C of *B. subtilis free of plasmids* agar nutritive culture was used as inoculum.The culture media were nutritive broth or peptone with glucose, fructose, mannitol, xylose with different C/N ratios. The sporulation rate in the fermentation media was

determined as the number of heat viable cells present after the treatment at 80°C for 20 min. The PHAs was determined by fluorescence with Nile Blue, by crotonic acid formation and by Gas Chromatography. The aeration was measured with a “DO meter, Hanna Instrument.” The optimization method was based in “a factor per time.” Data were analyzed by ANOVA test. The carbon sources glucose, fructose and mannitol favored PHAs accumulation. When glucose/peptone ratio was 2:1 at 12h culture the percentage of spore formation was 60% with little accumulation of bioplastic (2% of the cells accumulated PHAs). When 1,0% w/v of mannitol was used in the nutritive broth, spore formation was delayed up to 48h, with a PHAs accumulation of 1,1mg/g wet mass. The optimum pH for PHAs synthesis was pH 7,0. When the aeration was 0.1vvm and the shear stress 150rpm with mannitol 1% in a nutritive broth, the PHAs free of endotoxins accumulation in *B. subtilis* was 32,2mg/L at 24h. The presence of a complex organic source in the culture media favored the production of PHAs, this result was also reported in *Azotobacter vinelandii*. In the case of *B. subtilis* subsp*. spizizenii* the combination of a nitrogen organic complex source with mannitol, controlled dissolved oxygen in the culture and shear stress delayed the spore formation and these conditions could maintain the content of PHAs even in the late stationary phase. This differs from other bacteria that form spores under nutritional stress conditions and accumulate PHAs, for which the reserve material accumulated in early stationary phase is quickly degraded towards the formation of the resistance structure.

Código de Resumen: BF-015

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

BIOPROCESOS INTEGRADOS PARA INCREMENTAR LA SUSTENTABILIDAD EN LA PRODUCCION DE BIOMASA DE MICROALGAS

INTEGRATED BIOPROCESSES TO INCREASE THE SUSTAINABILITY OF BIOFUELS PRODUCTION FROM MICROALGAE BIOMASS

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Production of biofuels from microalgae biomass, although very promising, is not commercially available yet. This is mostly because production costs are still too high in comparison with petroleum and primary products of agriculture as feedstocks for first-generation biofuels. Similar to intensive agriculture, one of the drawbacks of implementing massive cultivation of microalgae is the unsustainable requirements of fertilizers, especially N. Microalgae have an average composition of CH1,7 O0,4 N0,15 P0,0094 , with N accounting for 4 to 8% on a dry biomass basis, making the bioprocess considerably more N-intensive than traditional agriculture. It is presumed that the development of breakthrough technologies for integrated bioprocesses comprising a multiplicity of microorganism that allow nutrients recycling and co-production of high value by-product would be necessary to realize the potential of microalgae biomass for bioenergy, food, feed and biomaterials. In this work we show the production of cyanobacterial biomass at the expense of N from the air. Upon rehydration of dry biomass highly concentrated cell-free extracts could be obtained. This extracts were highly enriched in the proteinaceous pigments phycoerytrin and phycocianin that could be purified using simple biochemical techniques. Additionally, this cell-free extracts proved to be a suitable source of nitrogen for the cultivation of many oleaginous microalgae. Moreover, the extracts sustain the algae growth without the need of additional nutrients. Proteins in the cell-free extracts were converted into *Chlorella sorokiniana* strain RP biomass with an efficiency close to 100 %. Control experiments show that while *C. sorokiniana* is able to use short peptides (trypteine) as a sole nitrogen source, it failed to use proteins such as bovine serum albumin or casein, suggesting it does not make use of extracellular proteases to degrade these substrates. Then, by means of an artificial substrate for proteases (azo-casein) we determined that the proteins in the cell-free extracts were degraded by proteases of cyanobacterial origin at a rate that did not impose a nutrient deficit to the microalgae. Interestingly, algae cells fed with cyanobacterial cell-free extracts

presented a 60% increase in the accumulation of neutral lipids up to 35 % (w/w) on a dry biomass basis. The resulted oleaginous biomass had a similar fatty acids profile that microalgae cultivated in mineral medium and would represent a suitable feedstock for biodiesel production.

Código de Resumen: BF-016

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

LA LUCHA CONTRA LOS PATÓGENOS NO HA FINALIZADO: EXPEDICIONES EN LA ANTÁRTIDA ARGENTINA REVELAN UNA NUEVA FUENTE PARA LA BIOPROSPECCIÓN DE ANTIMICROBIANOS ACTIVOS EN FRÍO

THE FIGHT AGAINST PATHOGENS IS NOT OVER: ARGENTINE ANTARCTIC EXPEDITIONS DISCLOSE A NOVEL SOURCE FOR COLD-ACTIVE ANTIMICROBIAL BIOPROSPECTING

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An organism that lives in an extreme environment is an extremophile. Within extremophiles we can find organisms belonging to archaea, prokaryotes and eukaryotes. Key factors, such as frequent and inappropriate use of antibiotic causing increase in bacterial resistance to those commonly used, as well as an irregular emergence generated from a detriment of pharmaceutical companies involved in drugs discovery, makes the development of new antibiotics is a growing worldwide problem and a constant challenge. The need to reduce additives in food industry has triggered the pursuit of natural antimicrobial substances produced by microorganisms from different sources with the aim of preserving processed foods by inhibiting microorganisms associated with food-borne illness. Most antibiotics emerged from screening programs of natural sources, including the isolation of new microorganisms, modification of well-known producers or metabolic engineering of fermentation processes. Extremophiles are considered an important source of metabolites, enzymes and bio-products since they are adapted to their unusual living conditions. This work focused on the isolation of psychrotolerant and psychrophilic microorganisms producing cold-active substances with the ability to inhibit the growth of common food-borne pathogens and phytopatogens for potential use in food preservation, pharmaceutical industry or as agriceutical. Twenty-seven antimicrobial producers were isolated by using microbiological selection techniques from sea water and sediment samples obtained during the 2011 and 2014 summer Antarctic campaigns of the oceanographic survey ship ARA Puerto Deseado. Isolates were grouped in 11 OTUs by ITS-ARDRA techniques; four of them were selected for identification based on rDNA regions sequence analysis together with the biochemical characterization. Isolates 2D, 5D and 6D were closely related to *Halomonas titanicae* (99.8, 98.9, 96.7% respectively), whilst isolate 18SH was related to *Candida sake* (99%). Antimicrobials produced by isolates 2D, 5D and 6D exhibited low molecular weight < 6,000 Da and inhibition spectrum against both, Gram + and Gram - pathogenic bacteria. The antagonist compound produced by *Candida sake* 18SH showed a higher molecular weight > 12,000 Da and a narrower spectrum of bacterial inhibition. However, antagonistic activity against fungi causing rots in fruits was detected. Due to its potential as plant pathogen biocontrol agent was selected for further characterization. Studies indicate antimicrobial stability in 5.0-7.0 pH range and 4-45°C temperature range. Antimicrobial activity was detected during early stationary growth phase retaining antimicrobial activity at low temperatures. To purify the antimicrobial, a protocol involving solid-phase C18 cartridges and HPLC was developed. This work highlights cold environments as a suitable source of microorganisms with the ability to produce cold-active biomolecules of biotechnological interest.

Código de Resumen: BF-017

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

HOJAS Y FRUTOS DE PLANTAS DE *Schinus areira* DE JUJUY, UNA FUENTE POTENCIAL DE COMPUESTOS ANTIBACTERIANOS

LEAVES AND FRUITS OF *Schinus areira* GROWING IN JUJUY, A POTENTIAL SOURCE OF ANTIBACTERIAL COMPOUNDS

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There is an urgent need to replenish our arsenal of anti-infective agents given the dramatic increase of antibiotic resistance around the world. Natural products, as the secondary metabolites ubiquitously distributed in higher plants, are a major source of chemical diversity and have provided important therapeutic agents for many bacterial diseases1-2. These compounds have important roles as defense against plant pathogens and animal herbivore aggression and as response to various abiotic stress conditions. They are gaining increasing interest and can be rationally selected for antibacterial testing based on ethnomedicinal use.3 We investigate the antibacterial activity of the native three *Schinus areira* L. (synonymous: *Schinus molle* L. var. *areira* DC. (aguaribay). The leaves and fruits of this plant widely distributed in the northwestern of Argentina4 have been used as medicines by indigenous peoples historically as antibacterial, antifungal and antirheumatic.5 However, the antimicrobial activity of leaves and fruits of *S. areira* has not been deeply investigated, so limiting the use of their derivatives in the modern medicine. The antibacterial performance of essential oils and ethanol extracts isolated from leaves and fruits of several *S. areira* specimens was performed by the microplate bioassay as described.6 The chemical principal component of essential oils from *S. areira* fruits determined by GC/FID and GC/MS were limonene, α-phelandrene, β-phelandrene, sabinene and myrcene. The strongest antibacterial activity against *Staphylococcus aureus* was exhibited by the fruits oils rich in limonene and sabinene (MIC values <10 µL/mL). When the antibacterial effect of ethanolic leaves and fruits extracts obtained by ultrasound-assisted extraction was determined, a moderate activity against sensitive and meticillin resistant *S. aureus* was observed (MIC50 100-150 µg/mL). The leaves extract was more potent than the fruits extract. To understand the interactions between the compounds of the leaves extracts we use the checkerboard method for detecting interactions between bioactives (non-volatile phenolic compounds), as described previously.7 Results showed that the three ethanolic extracts assayed showed smaller antibacterial activity than that of the sum of the individual substances against *S. aureus*. This antagonistic effect suggested that the bioactive/s in the ethanolic extracts target the same sites in the bacterial cell, at least again the bacteria studied. In conclusion: the present results highlight the presence of some constituents with interesting antibacterial activity in both essential oils and ethanol extracts of leaves and fruits of *S. areira*.

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Código de Resumen: BF-018

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

DISEÑO DE ELECTRODOS BIO-HIBRIDOS PARA EL MEJORAMIENTO EN LA PRODUCCION DE ENERGIA BACTERIANA

DESIGN OF BIOHYBRID ELECTRODES FOR IMPROVING BACTERIAL ENERGY PRODUCTION

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Microbial fuel cells (MFCs) appear as a new and appealing possibility for both the treatment of organic waste and the generation of electric power. They are similar to conventional fuel cells but take advantage of electro-active microorganisms, especially those from the genus *geobacter*, which act as bioelectrocatalysts converting the energy stored in chemical bonds of organic compounds into electric energy. In recent years this concept has triggered considerable interest among academic researchers towards environmentally safe and novel low cost biotechnologies. A MFC consists of an external electric circuit which links anodic and cathodic compartments separated by a proton or cation exchange membrane. Generally used anodes consist of non-porous graphite rods immersed in the bacterial culture medium, upon which the colony of cells can develop. Recent results have indicated that long term development of anodic bacterial biofilms do not correlate with current production, mainly due to a drop in the outside cells redox potential with the distance to the electrode; this potential decay is thought to be due to the imperfect electric conduction of the biofilm matrix and determines that cells composing outer layers of the biofilm structure cannot contribute to the power production process. Applications that make use of electro-active biofilms are not yet economically sustainable, but this situation may change if the electric connection between cells in the biofilms is improved. This work aims at developing hierarchically structured and electrically conducting ceramic supports exhibiting an open structured porosity that allows the proliferation and development of electrogenic bacterial biofilms, in which most of cells are in close contact with the conducting material. These so-called bioHybrid anodes, are expected to produce much more current than traditionally used graphite rods. An ice-templating technique was used to produce macroporous ceramic matrices (based on electrically conducting titanium suboxides) able of being employed as alternative supports (instead of graphite) for the development of electrogenic biofilms of *Geobacter sulfurreducens* in conventional electrochemical reactors. Scanning electron microscopy (SEM) was employed to visualize the formation of biofilms on the surface of the new electrodes. The production of current was followed by chronoamperometry and was found to be three (3) times higher compared to conventional graphite rods. Summarizing, the proposed material appears as an interesting alternative for the replacement of graphite electrodes conventionally used in MFCs by porous ceramic anodes

Código de Resumen: BF-019

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

CONTRIBUCION POSITIVA EN EL AROMA DE VINOS POR LA ADICION CONTROLADA DE *Oenococcus Oeni*

POSITIVE CONTRIBUTION TO WINES AROMA BY THE CONTROLLED ADDITION OF *Oenococcus oeni*

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Lactic acid bacteria (LAB), preferably *Oenococcus oeni*, play an important role in winemaking by undertaking the malolactic fermentation (MLF). As a consequence of this reaction, the total acidity decreases and the organoleptic properties and biological stability of wines are generally improved. Difficulties in triggering MLF and/or the presence of inadequate strains because they either exhibit low fermentative efficient or produce undesirable compounds could be avoided by the inoculation with selected LAB. In a previous research, we selected *O. oeni* MS46 by its high malolactic potential, ability to produce b-glucosidase enzyme and low diacetyl concentration in laboratory media. The aim of this study was to investigate the malolactic potential of *O. oeni* MS46 and its influence on the aromatic profile of wine in microvinification assay. Bottles with 2000 ml of wine sterilized by filtration obtained by alcoholic fermentation of musts from red grapes belonging to the same region as the strain to be tested with a pH 3.7, were inoculated with *O. oeni* MS46 at an average initial cell density of 6.15 ± 0.18 log cfu/ml and incubated at 26±2ºC during 8 days (end of MLF). Wine samples were taken at the beginning and the end of MLF, sterilized as before and analyzed for sugars and organic acids contents by enzymatic tests, volatile compounds by gaseous chromatography coupled to mass spectroscopy (GC/MS) and sensorial profile. In inoculated wine, *O. oeni* MS46 grew by 1.58 log units and efficiently conduced MLF. At this time, the inoculated strain completely consumed L-malic (2 g/l) and citric acids (1.13 g/l) and only 9% of initial residual sugars, even though its concentration was 31 g/l, producing adequate concentrations of D-lactic and acetic acids. Total acidity slightly decreased from 5.63±0.42 to 5.25±0.32 g/l, while the volatile acidity remained almost unchanged. In addition, *O. oeni* MS46 produced significant qualitative and quantitative changes in the composition of volatiles aroma compounds, increasing total concentrations of esters, alcohols and terpenes among 20 and 60% as compared to wine without MLF. For instance, 2-phenylethylacetate and 2-phenyletanol concentrations increased by 38 and 36%, which could contribute to the fruity aromas of wine being 2-phenyletanol one of the most relevant aroma-related alcohols. On the other hand new compounds such as octanol or trans-nerolidol were synthesized. Sensorial analysis of wine samples obtained by microvinification by entreated panel correlated with the modifications in the volatile composition, especially by changes in higher alcohols and fatty acids ethyl esters and acetate. The results obtained in microvinification assay with *O. oeni* MS46, confirmed the importance of inducing the MLF in red wines with selected strains of LAB that can offer a positive contribution to the final aroma in wines.

Código de Resumen: BF-020

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ESTUDIO DE LA FERMENTACIÓN CON GRÁNULOS DE KEFIR DE LECHE FORTALECIDA CON VITAMINAS Y MINERALES, Y SU CAPACIDAD PARA INHIBIR EL EFECTO DE LA TOXINA SHIGA DE *Escherichia coli*

KEFIR MADE FROM MILK FORTIFIED WITH VITAMINS AND MINERALS AND ITS ABILITY TO INHIBIT THE EFFECT OF *Escherichia coli* SHIGA TOXIN

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In Argentina, malnutrition rates are still high; as a result many cases of anemia where iron deficiency anemia is the most common nutritional disorder are frequently recorded. Furthermore, Argentina is the country with the highest rate of cases of hemolytic uremic syndrome caused by enterohemorrhagic *Escherichia coli*. Milk fermented with kefir grains is a natural probiotic known for its beneficial health properties among consumers. Therefore the study of the addition of micronutrients to milk to make kefir with it was proposed. The specific objective of this study was to determine the feasibility of adding vitamins (B9 and B12) and minerals (Fe and Zn) to milk to be used for fermentation with kefir grains. Furthermore, the capacity of kefir produced with fortified milk to exert an inhibitory effect on the shiga toxin enterohemorrhagic *Escherichia coli* was measured. For this, different mixtures of micronutrients in milk were prepared and various tests were performed. On one hand, the acidification kinetics of the milk added with AGK1 CIDCA kefir grains was determined, grain biomass kinetics was performed and counts of lactic acid bacteria and yeast were made. In these trials, no significant differences with control (kefir made from milk without fortification) were observed. Moreover, the evaluation of cytotoxic effect of Shiga toxin from *E. coli* O157H7 in the presence of microorganisms from kefir fermented whey permeate supplemented with vitamins and minerals was tested. For this, a cell proliferation assay was performed using tetrazolium salt assay bromide 3 - (4,5-dimethylthiazol-2-yl) -2,5 - diphenyl tetrazolium bromide (MTT) on Vero cells, it was found that microorganisms of kefir are capable of inhibiting the action of Shiga toxin of *Escherichia coli* O157: H7, exerting a protective effect on Vero cells.

Código de Resumen: BF-021

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

BIOSINTESIS DE FLOXURIDINA MEDIANTE UN NOVEDOSO BIOCATALIZADOR INMOVILIZADO EN SR-ALGINATO

NOVEL SR-ALGINATE BIOCATALYST FOR FLOXURIDINE BIOSYNTHESIS

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Nucleoside analogues play an essential role in the pharmaceutical industry due to their wide use as antiviral and antitumoral agents. In the last decades, the constant progress of industrial microbiology allowed development chemo-, enantio- and stereoselective biocatalytic processes to obtain nucleoside analogues under mild reaction conditions. Biocatalysts (enzymes or microorganisms) can be stabilized by immobilization techniques to achieve a high operational stability, easy downstream separation and scale-up feasibility. Immobilization of microorganisms into gel matrix by entrapment methodologies are the most widely used. In this work, an efficient and green immobilized biocatalyst is reported to obtain 5-fluorouracil-2´-deoxyriboside (5FUradRib), an antimetabolite known as Floxuridine, used in gastrointestinal cancer treatment Alginate is a natural polysaccharide used in the pharmaceutical industry due to its

physicochemical properties, biocompatibility and non-toxicity. Immobilization conditions as multivalent cations (SrCl2), exposure time (2 h) and cross-linking solution concentration (0.2 M) were optimized. Furthermore, compression strength, swelling ratio and fracture frequency were evaluated, improving the mechanical stability of the biocatalyst favoring scale-up. On the other hand, the reaction parameters for 5FUradRib biosynthesis were optimized in order to obtain an immobilized biocatalyst with enhanced activity. *Lactobacillus animalis* ATCC 35046 immobilized in Sr-alginate showed yields of 96% at short reaction times. The obtained biocatalyst was stable for more than 25 days in storage conditions (4°C) and could be reused at least 10 times without loss of its activity. Scale-up bioprocesses in a packed bed column using immobilized *L. animalis* was evaluated preliminarily. Floxuridine productivity obtained was 54 mg/L h. Finally, smooth, cheap and environmentally friendly method to obtain anti-cancer drugs was developed in this study.

Código de Resumen: BF-022

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

INOCUIDAD DE LA APLICACION DE *Lactobacillus plantarum* CIDCA 83114 EN ALIMENTACION DE POLLOS PARRILLEROS

SAFETY USE OF *Lactobacillus plantarum* CIDCA 83114 IN POULTRY FEEDING

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Lactobacillus plantarum CIDCA 83114 has been tested by our working group as probiotic for human consumption. It has been challenged with different pathogens yielding good results. In order to explore its utilization in animal feed and against their pathogens the safety of this strain in poultry feeding was studied. The aim of this study was to evaluate the safety of *L. plantarum* CIDCA 83114 in the drinking water of broaster chickens. Whey permeate was fermented with strain CIDCA 83114 and dosed in the drinking water. Roasted chickens (336) were employed in a completely randomized design study, to reduce the initial variation (8 replicates per treatment with 14 animals each). They were divided into 3 groups: W: water consume, PS: permeate serum consume (1/100), LP: permeate serum fermented with *L. plantarum* (1/100) (2x106 CFU / ml). All groups were fed with balanced food cereal. During the test the signs of morbidity and mortality of the animals were assessed daily. Weight, feed consumption, feed conversion and production efficiency factor (PEF) parameters were recorded weekly The study was conducted for 21 days. Slaughter of animals at days 7, 14 and 21 were performed. Blood samples were collected, serum was obtained to determine the levels of total plasma protein, albumin, globulin, albumin /globulin, liver enzymes (GPT, GOT, G-GT), urea, creatinine by commercial kits. Later autopsies were performed. Liver, spleen and bursa of Fabricius were removed and weighed in sterile conditions. The relative weight of these organs was obtained by comparing them with the weight of slaughtered birds. Furthermore, the bursa weight/spleen weight ratio was calculated. The presence of antimicrobial residues in poultry organs analyzed, and a histological sample of both intestine and liver was carried out. The results showed that there was no mortality in any of the treatments, and no statistically significant changes in production parameters evaluated. While organs tested had translocation in all groups (W, PS, LP), hepatic and renal function enzymes showed no changes that would show infectious processes or pathology in the animal. Histological examination showed no damage in the intestinal and hepatic tissues. We concluded that the *L. plantarum* CIDCA 83114 is a microorganism that is not pathogenic for broilers and also does not affect their normal growth functions and weight gain over time. This study provides a starting point for future research on the interactions with potential pathogens.

Código de Resumen: BF-023

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EFECTO DE LA LIOFILIZACIÓN SOBRE LAS PROPIEDADES FERMENTATIVAS Y MICROBIOLÓGICAS DE LOS GRÁNULOS DE KEFIR

LYOPHILIZATION EFFECT ON THE FERMENTATIVE AND MICROBIOLOGICAL PROPERTIES OF KEFIR GRAINS

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The effect of the kefir grains lyophilization on the physicochemical, microbiological and sensorial properties of the milk kefir was studied by our group. We lyophilized CIDCA AGK1 kefir grains (using UHT milk as cryoprotector) and stored them during 6 months at 4ºC. The different tests were made comparing fresh and lyophilized grains at months 0, 1, 3 and 6. The milk fermentation kinetics was assessed by inoculating fresh milk with kefir grains 10% v/v, followed of incubation at 30 °C. The pH drop was measured every hour. The microbiological composition was assessed during storage, by accounts of lactic acid bacteria and yeasts. Serial dilutions were made from the fermented products, and 100 ul from these dilutions were inoculated on MRS® and YGC® medium for the account of lactic acid bacteria and yeasts respectively. The physicochemical properties as protein composition, lipid content and acidity were assessed too. Cell free supernatants (CFS) were obtained from the fermented products by centrifugation and filtration. The CFS were used to study the antimicrobial activity against *E.coli* and *Salmonella* sp. We made a sensorial study in order to determine if there were differences between the fermented milk with lyophilized and fresh kefir grains. We found that LAB and yeasts survived the lyophilization process as well as the storing time, keeping their viability. The final account of LAB and yeasts from lyophilized grains after six months of storage was the same that the obtained with fresh grains;these microorganisms fermented lactose from the milk and produced lactic and acetic acid. CFS obtained from lyophilized and fresh grains didn't show significant differences on the inhibitory activity against *E. coli* and *Salmonella* sp. Finally, there was no sensorial difference between the kefir obtained with lyophilized and fresh grains. Additionally, the fermented milk obtained with fresh and lyophilized grains got a high score, near9, in the sensorial acceptability test. We concluded that the lyophilization process followed of a 6 months of storage doesn´t affect the fermentative, microbiological, bromatological, antimicrobial and sensorial properties of the kefir grain CIDCA AGK1. It gives us a perspective for a more thorough study of different drying strategies for the kefir grains.

Código de Resumen: BF-024

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

BIOSÍNTESIS DE COMPUESTOS ANTI-HCV MEDIANTE BIOCATALIZADORES TERMÓFILOS

DEVELOPMENT OF A THERMOPHILE BIOCATALYST FOR ANTI-HCV COMPOUND BIOSYNTHESIS

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Molecules with pharmaceutical value have been synthesized using chemical methods over the years. However, the use of biocatalysts has replaced these techniques because of their efficiency and selectivity in mild conditions of reaction, becoming those industrial processes greener. Thermophile biocatalysts have been widely studied as source of enzymes with high stability, selectivity and efficiency. The use of whole thermophilic microorganisms in sustainable bioprocesses allows cofactor regeneration and extend enzyme half life. Therefore, biocatalysts

can be stabilized by immobilization procedures. Cell entrapment is the most used technique for whole cell immobilization. The immobilized biocatalyst could be reused without activity loss improving operational stability. Furthermore, the development of this immobilized biocatalyst facilitates downstream operations, biocatalyst recovery and scale up of the bioprocess. In this work, the use of *Geobacillus kaustophilus* strain for Ribavirin biosynthesis in one-pot reaction is described. This nucleoside analogue is widely used for chronic Hepatitis C treatment in combination with peginterferon alfa. We have been able to optimize some parameters of reaction for Ribavirin biosynthesis as substrates ratio (1:4, uridine:TCA), temperature (30°c), number of cells (1x10 10) and pH (7) conditions. Convertion rates of 70 % for free cells at 6 h were obtained. Additionally, several supports were tested for *Geobacillus* immobilization as agar, agarose and acrilamide. Agarose thermogel was the best matrix for microorganisms stabilization with a final yield of 50 % at 24 h.

BIODIVERSIDAD

2 al 4 de Julio de 2014
Hotel 13 de Julio
Mar del Plata, Argentina

Código de Resumen: BD-001

Sección: Biodiversidad

Modalidad: Oral

BIODIVERSIDAD Y DINÁMICA MICROBIANA DURANTE LA ELABORACIÓN DE VINOS CHILENOS Y SU ROL EN LA PRODUCCIÓN DE AMINAS BIÓGENAS

MICROBIAL BIODIVERSITY AND DYNAMICS DURING WINEMAKING OF CHILEAN WINES AND THEIR ROLE IN BIOGENIC AMINE PRODUCTION

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Currently, Chile ranks fifth worldwide in wine exports. Wine is the product of interactions between species of fungi, yeasts and bacteria. These microorganisms carry out important processes in winemaking, as the alcoholic and malolactic fermentation, led by yeast and lactic acid bacteria, respectively. However, some compounds derived from microbial metabolism may be hazardous to the health of consumers, such as biogenic amines. The aim of this work was study biodiversity and dynamics of the main microorganism related to winemaking (yeasts, lactic and acetic bacteria) and their role in biogenic amine production.
For this purpose, five Chilean vineyards of different geographical areas was studied (cabernet sauvignon grape). Samples were taken during winemaking process (grapes, alcoholic fermentation, malolactic fermentation and bottled wine). Microbial diversity and dynamics was assay by scanning electron microscopy (SEM), culture on selective media and Denaturing Gradient Gel electrophoresis (DGGE). Furthermore, total and individually biogenic amines concentration was determined (phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermine and spermidine) using High Performance Liquid Chromatography (HPLC). The results show low diversity of microorganisms at species-level, several of them being able to synthesize biogenic amines. Most bacterial isolates belonging to the genera *Lactobacillus* and *Pediococcus*, whereas yeasts mostly belong to the genus *Saccharomyces*. DGGE results show higher species richness in alcoholic and malolactic fermentation, whose dominant groups are yeasts and lactic acid bacteria, respectively. Putrescine, cadaverine, spermine and spermidine were found in all vineyards, being putrescine the most abundant in all cases (average concentration, 78.6 mg/L). The average biogenic amines concentration found in the samples ranging on 23.20 to 205.76 mg/L. The concentrations of amines in certain wines were superior to levels established as safe for the consumer (i.e., over 100 mg/L of some amines may cause migraine). It was observed from the results that both microorganisms and grape geographical origin influenced the content of biogenic amines in wine. The identification of the dominant organisms in this process as well as determining their possible role as producers of biogenic amine in Chilean wines, can help to strategies development to control the winemaking process, thus final product will be according to the demands of quality and safety of the main world markets.
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Código de Resumen: BD-002

Sección: Biodiversidad

Modalidad: Poster

HONGOS MICOTOXIGÉNICOS Y MICOTOXINAS EN SILAJES DE MAÍZ EN LA PROVINCIA DE BUENOS AIRES

TOXIGENIC FUNGI AND MYCOTOXINS IN CORN SILAGE IN BUENOS AIRES PROVINCE

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The corn silage is an important fermented feed for cattle dairy and beef by allowing the forage supply in times of low forage production and improves the nutritional quality of diets. The growth of filamentous fungi in fermented feed reduces the nutritional value of them and may result in the production of mycotoxins. However, mycotoxin production may occur in the field, during post harvest, storage, processing or feeding under appropriate environmental conditions. Although more than 100 mycotoxins have been identified, less than 10 are of concern due to their natural occurrence and toxicity. The aim of this study was to characterize the fungal population; particularly toxigenic species present in silage corn and detect the presence of mycotoxins.
Corn silage samples were analyzed in respiratory and fermentation stage reserves located in different regions in the province of Buenos Aires, Argentina. Each sample was suspended in sterile peptone water and plated on agar culture media dicloran glycerol (DG18) and dicloran peptone (DCPA). Successive peals from fungal colonies were perfomed and these presented different morphology. Pure isolates were identified using conventional taxonomy techniques based on morphological and biochemical characteristics. Simultaneously, the isolation frequency of toxigenic species was determined. Detection of deoxynivalenol (DON), zearalenone (ZEA) and total aflatoxins (AFLA) was performed by Thin Layer Chromatography (TLC).
The fungal genera identified, in both stages and locations, were: *Cladosporium* sp., *Fusarium* spp., *Neosartorya* sp., *Eupenicillium* sp., *Aspergillus* spp. (*A. citrinum*, *A. flavus*, *A. terreus*, *A. fumigatus*) and *Penicillium* spp. (*P. chrysogenum*, *P.crustosum*, *P. decumbens*, *P. olsonii*, *P. oxalicum*, *P. simplicissimum*, *P. thomii*, *P. funiculosum*). *A. citrinum*, *A. flavus*, *A. fumigatus* and *Fusarium* spp. as potential producers of mycotoxins are reported. The majority of the isolated identified correspond to *Fusarium* spp. (68.75%) followed by *A. flavus* (40.63%) and 12.5% for the species *A. citrinum* and *A. fumigatus*.
No DON, ZEA and AFLA were detected by the methodology used in any stage of silage or regions evaluated. Finally, the corn silage showed high contamination with micotoxin-producing fungi. Therefore, it is important the periodic monitoring of the forage storage, since these fungi could produce toxic metabolic compounds, which constitute a risk factor for human and animal health.

Código de Resumen: BD-003

Sección: Biodiversidad

Modalidad: Poster

HONGOS Y BACTERIAS ASOCIADOS A GRANOS DE MAIZ ALMACENADOS EN ATMOSFERAS AUTOMODIFICADAS

FUNGI AND BACTERIA ASSOCIATED WITH STORED GRAIN CORN IN SELF-REGULATED ATMOSPHERES

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Maize is an important crop that is used worldwide as human food, as a raw material for starch and ethanol production, and as animal feed. Because of its relatively high moisture and starch

contents maize is very susceptible to biological degradation. The microbial degradation of grains during storage depends principally on a combination of three factors: temperature, grain moisture content (m.c.) and O_2 level. Furthermore, these microorganisms are responsible for the alteration of important germinative properties of seeds and, in the case of moulds, for the potential formation of mycotoxins. The purpose of the present study was to characterize the microbial population present in maize kernels in self-regulated modified atmospheres during hermetic storage. Corn samples with 14.5, 16.5 and 18.5% m.c., stored in hermetically sealed containers at 15, 25 and 35ºC, were analyzed at the beginning of the hermetic storage period (21% O2) and when complete depletion of O_2 occurred due to aerobic respiration. The concentration of O_2 was determined with a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector. Each corn sample was suspended in sterile peptone water and plated in plate count agar for isolating the total aerobic bacteria, and for yeasts and molds on potato dextrose agar. Successive peals from colonies were perfomed and these presented different morphology. Pure isolates were identified using conventional taxonomy techniques based on morphological and biochemical characteristics. The higher the m.c. the shorter the time it took for the O_2 to be consumed during the aerobic respiration. At m.c. constant, O_2 was consumed faster at higher storage temperatures. Most of the fungi species isolated from corn grain samples under different condition of hermetic storage belonged to the genus *Penicillium*, including *P. citrinum*, *P. funiculosum*, *P. restrictum*, *P. nalgiovense*, *P. olsonii*, *P. raistrickii*, *P. oxalicum*, *P. islandicum* and *P. funiculosum*.
Also were identified species of genera *Eurotium*, *Aspergillus*, *Fusarium* and *Acremonium*, in this order of importance. The yeasts (*Debaryomyces hansenii*, *Candida parapsilosis* and *Rhodotorula mucilaginosa*) were the most frequent isolated group (58.33%), principally in high m.c. of grains and anaerobic conditions. The bacteria strains identified were: *Alcalígenes* sp., *Corynebacterium* sp., *Lactobacillus* sp., *Acetobacter* sp., *Bacillus* sp., *Streptococcus* sp., *Actinobacillus* sp. and Enterobacterias.
Most bacterial isolates identified correspond to fermentative bacilli that can grow in an environment without O_2 . The self-regulated modified atmospheres, by lowering the level of O_2, limit the amount of species of microorganisms that can alter the quality of the stored grain. Therefore, the hermetic storage of the grains has advantages related to reducing the microbial activity and preserving the quality of grain in comparison with conventional storage practices, specially for wet grains.

Código de Resumen: BD-004

Sección: Biodiversidad

Modalidad: Poster

PROBIÓTICOS NATIVOS PARA REDUCIR EL USO DE ANTIBIÓTICOS EN PRODUCCIÓN ANIMAL AGROECOLÓGICA

NATIVE PROBIOTICS TO REDUCE ANTIBIOTIC USAGE IN AGROECOLOGICAL ANIMAL FARMING

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In contraposition to antibiotics, probiotic microbes are considered safe and are traditionally used for human and animal consumption to improve their host intestinal microbiota and immunity balance.
In order to develop a probiotic formula aiming to reduce antibiotic usage in animal operations, we investigated the *in vitro* inhibition of multi-antibiotic resistant *Salmonella* serovar Choleraesuis and *Escherichia coli* from septic and diarrheic piglets, by native-autochthonous isolates from porcine faeces in good sanitary status from Argentinean farming operations.
Twenty out of 82 (25%) highly inhibitory strains were selected, even superior to usual antibiotic used as growth promoters.
Selected strains were consequently evaluated for their bile tolerance in order to select those able to survive within the intestinal environment, and the resistance to antibiotics routinely used

in porcine production. Eight bile and antibiotic resistant strains were selected, and the ability to adhere to porcine gastric mucin and the intestinal epithelial cell line Caco-2/TC7 ($1,2x10^6$ – $1,9x10^7$ CFU/cm^2) was tested. The adhesion displayed by these mutants was similar to the adhesion exhibited by commercial strains claimed to be probiotics. Furthermore, inhibition between selected strains was checked, showing no growth inhibition among them.
The inhibitory power against 14 different *Salmonella* spp. strains is now being tested in order to further characterize this probio-sanitary spectrum against a broad spectrum of *Salmonella* isolates from pig and poultry production units, in order to evaluate their potential usage as antibiotic replacers.
The relevance of this study relies in showing the importance of using native-authocthonous probiotic microorganisms –no commercial, nor genetically modified, and adapted to a particular production system-, to be used as antibiotic replacers. This trend will pave the avenue for development of environmentally friendly animal farming and the prevention of zoonotic diseases caused by multi-resistant aggressive microbes.

Código de Resumen: BD-005

Sección: Biodiversidad

Modalidad: Poster

MICROORGANISMOS FOTOSINTÉTICOS EN LOS CULTIVOS DE ENRIQUECIMIENTO DE BIOFILMS DE LOS SISTEMAS GEOTERMALES DE DOMUYO Y CAVIAHUE-COPAHUE, NEUQUEN, ARGENTINA

PHOTOSYNTETIC MICROORGANISMS IN BIOFILM ENRICHMENT CULTURES FROM GEOTHERMAL DOMUYO AND CAVIAHUE–COPAHUE SYSTEMS, NEUQUÉN, ARGENTINA

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Photosynthetic microorganisms have gained a lot of attention in recent years because of their potential applications in biotechnology. They have been identified as a rich source of biologically active compounds and they have been considered as a very promising source of alternative energy. In addition to these applications, these microorganisms are also used in aquaculture, wastewater treatment, food, fertilizers and production of secondary metabolites including exopolysaccharides, vitamins, toxins, enzymes and pharmaceuticals.
Microalgae and cyanobacteria play a major role in the nitrogen, carbon, and oxygen dynamics of a wide variety of aquatic environments. In this work the geothermal areas of Domuyo and Caviahue-Copahue have been selected for the research of photosynthetic microorganisms that could present distinctive properties useful for different biotechnological processes such as bioremediation and biofuel production. Three microbial mats in Las Máquinas spring (Biofilm A: 40ºC, pH 8; Biofilm B: 50ºC, pH 7; Biofilm C: 43ºC, pH 7) placed in Copahue geothermal system, one from Domuyo spring (Biofilm D: 40ºC, pH 7) and other from Caviahue area (Biofilm E: 32ºC, pH 4) were chosen for microbial enrichment and identification.
Enrichment cultures under autotrophic conditions were done from the five biofilms. Medium BG 11 at pH 7 was selected for this purpose and the cultures were grown under fluorescent lightning at 30ºC. The photosynthetic microorganism diversity of the samples was analyzed by cloning a 16S rRNA gene region using cyanobacterial specific probes (CYA359F–CYA781Ra/CYA781Rb). The sequences obtained were compared with those in NCBI database using BLAST.
Several photosynthetic microorganisms were identified including cyanobacteria and microalgae. Even though PCR dependent techniques are not quantitative, in Biofilm A the most abundant species seemed to be *Scenedesmus obliquus.* This microalga has been reported for biodiesel production and wastewater treatment processes*. Leptolyngbya sp*. cyanobacterium was also identified in this sample. Enrichment cultures from Biofilm B and D had unique phototrophic species, *Synechococcus elongates* and *Synechocystis sp*. respectively. These cyanobacteria, belonging to *Chroococcales* order, are oxygenic phototrophs being the main source of primary production in oligotrophic aquatic environments and are able to grow at a wide range of light

intensities. As regards biotechnological applications, both species have been used in the production of biofuel and different bioactive compounds. In enrichment corresponding to Biofilm C, *S. obliquus* and *S. elongatus* were found. *Chlorella sp*. was identified in the culture but in a lower proportion. In Biofilm E culture, three different sequences corresponding to uncultured species were found together with the already mentioned *S. elongatus*. Most photosynthetic microorganisms found are very versatile and have great biotechnological potential we are currently studying.

Código de Resumen: BD-006

Sección: Biodiversidad

Modalidad: Poster

ANÁLISIS MICROBIOLÓGICO DE SAL ENTREFINA QUE SE UTILIZA EN EL PROCESO DE SALAZÓN DE PESCADO

MICROBIOLOGICAL ANALYSIS OF SALT USED IN THE PROCESS OF SALTING FISH

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The fish salting process includes salt like main input. The salt comes from open pit saltwork and has a typical microflora. Considering the own characteristics of the salt, these bacteria are adapted to hypersaline environment. The characteristics of this input is important due to more than 30% of salt, with respect to the raw material, is used in the different stages of this process.
Halophilic bacteria, molds and yeasts are natural contaminants of salt. Depending on their origin, the counts and species of microorganisms may vary. Their presence is important from the point of view of manufacturing process, since according to their characteristics can cause deterioration and in some cases toxicity of final product.
According to their salt requirements, bacteria may be classified as: extreme, strict, moderate or weak halophilic.
The fish salting process is characterized by the inactivation of the native flora of fish, which may be deteriorating or pathogens, caused by the decrease in water activity (Aw). Thus, the halophilic bacteria constitute the flora typical of these products.
Therefore, prior to use salt, it is important determine the microbiological quality, which can oscillate according to the climatic conditions before time of collection.
In this work, samples of salt (two samples of salt from Rio Negro Province that showed pink color, typical of the pigments generated by halophilic bacteria) to be used in the process of salting fish were analyzed with the aim of isolating the present bacteria, determining the optimal percentage of salt for growth and some of the features of importance to the salting process. Dilutions were prepared in sterile broth (ICMSF, 1983). We proceeded to the spread of 0.1 mL surface in solid culture media Agar IRAM-Milk (IRAM, 1988), Agar Pollock-Milk (Holt, 1989) and Gibbons (Bergey´s, 1989). All of these prepared with a final NaCl concentration of 15 % W/V (150 g NaCl/L) and 20 % W/V (200 g NaCl/L) and incubated 21 days at 35 ° C.
Were determined the following tests: morphology, Gram stain, catalase test, citrate, motility, fermentation of glucose, lipolysis, proteolysis and growth at different concentrations of salt (10, 15, 20 and 25 % W/V) in agar IRAM.
18 strains were isolated, 14 were originally isolated in 20% and 4 to 15 % NaCl. The strains analyzed showed that 71 % have pigments, varying in intensity from weak pink to red; 94% were gram-negative; 39% bacilli and 71% cocci; 89 % catalase positive; 33 % mobile strains; 22 % were lipolytic, 28 % proteolytic and the total of strains were negative citrate and glucose fermentation. For growth in different salt concentrations, 50 % were positive to 10 % W/V, 94 % to 15%, 100 % to 20 % and 78 % to 25 %.
According to the results, the storage conditions of the salt (temperature and humidity) are very important in order to minimize the growth of those bacteria whose characteristics denote its deteriorating power.