XI CONGRESO ARGENTINO DE MICROBIOLOGÍA GENERAL

5 al 7 de Agosto de 2015
Córdoba, Argentina

SAMIGE
Asociación Civil de Microbiología General

Diseño gráfico **Arq. Juliana A. Páez**
www.amberearquitectura.com
+54 351 155 571 957
Córdoba | Capital

Comisión Directiva Saliente:

Presidente: Néstor Cortez
Vice-Presidente: Osvaldo Yantorno
Secretaria: Marcela Ferrero
Pro-Secretaria: Diana Vullo
Tesorero: Claudio Valverde
Pro-Tesorera: Daniela Russo
Presidente Saliente: Ángeles Zorreguieta

Nueva Comisión Directiva:

Presidente: Osvaldo Yantorno
Vice-Presidente: Eleonora García-Véscovi
Secretaria: Diana Vullo
Pro-Secretario: Claudio Valverde
Tesorera: Daniela Russo
Pro-Tesorero: Leonardo Curatti
Presidente Saliente: Néstor Cortez

Comisión Organizadora Local

Andrea Smania (CIQUIBIC-CONICET, UNC)
M. Gabriela Paraje (IMBIV-CONICET, UNC)
Paulina L. Páez (FCQ-UNC)
José Echenique (CIBICI-CONICET, UNC)
H. Alex Saka (CIBICI-CONICET, UNC)
Claudia Sola (CIBICI-CONICET, UNC)
Carlos E. Argaraña (CIBICI-CONICET, UNC)

Colaboradores Locales

Alejandro Moyano (CIQUIBIC-CONICET, UNC)
Alín Tobares (CIQUIBIC-CONICET, UNC)
Victoria Borgogno (CIQUIBIC-CONICET, UNC)
Lucía Margara (CIQUIBIC-CONICET, UNC)
Antonella Colque (CIQUIBIC-CONICET, UNC)
Nubia Yandar (CIBICI-CONICET, UNC)
Melisa Quinteros (FCQ, UNC)
Viviana Cano (FCQ, UNC)
Pamela Bustos (FCQ, UNC)
Giuliana Mosconi (IMBIV-CONICET, UNC)
María Ángel da Silva (IMBIV-CONICET, UNC)
Juliana Marioni (IMBIV-CONICET, UNC)
Ana Lía Egea (CIBICI-CONICET, UNC)
María Emilia Panzetta (CIBICI-CONICET, UNC)

Comisión Evaluadora

Nancy López (FCEyN, UBA)
Diana Vullo (UNGS FCEyN, UBA)
Daniela Russo (FIL, IIBBA)
Claudio Valverde (UNQ)
Osvaldo Yantorno (CINDEFI-CONICET, UNLP)
Karina Herrera Seitz (IIB-CONICET, UNMdP)
Marcela Ferrero (PROIMI-CONICET, UNT)
Alex Saka (CIBICI-CONICET, UNC)
Alejandro Moyano (CIQUIBIC-CONICET, UNC)

Pares Evaluadores

Héctor Álvarez, Mariana Allievi, Carlos Argaraña, Eleonora Campos, Mónica Delgado, José Echenique, Julieta Fernández, Mariela Monti, Federico Sisti, Alejandro Viale, Roberto Paggi, María Inés Giménez, Débora Nercessian. Mario Baigorí, Licia Pera, Verónica Colín, Raúl Raya, Paulina Páez, Maria Alejandra Pereyra

Las siguientes Instituciones han financiado y auspiciado la organización del XI Congreso Argentino de Microbiología General

Consejo Nacional de Investigaciones Científicas y Técnicas

CONICET

Agencia Nacional de Promoción Científica y Técnológica

AGENCIA
NACIONAL DE PROMOCIÓN
CIENTÍFICA Y TECNOLÓGICA

American Society for Microbiology

AMERICAN
SOCIETY FOR
MICROBIOLOGY

International Society for Microbial Ecology

isme

Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba

UNC SECyT

Facultad de Ciencias Químicas de la Universidad Nacional de Córdoba

Facultad de Ciencias Exactas, Físicas y Naturales de la Universidad Nacional de Córdoba

Academia Nacional de Ciencias

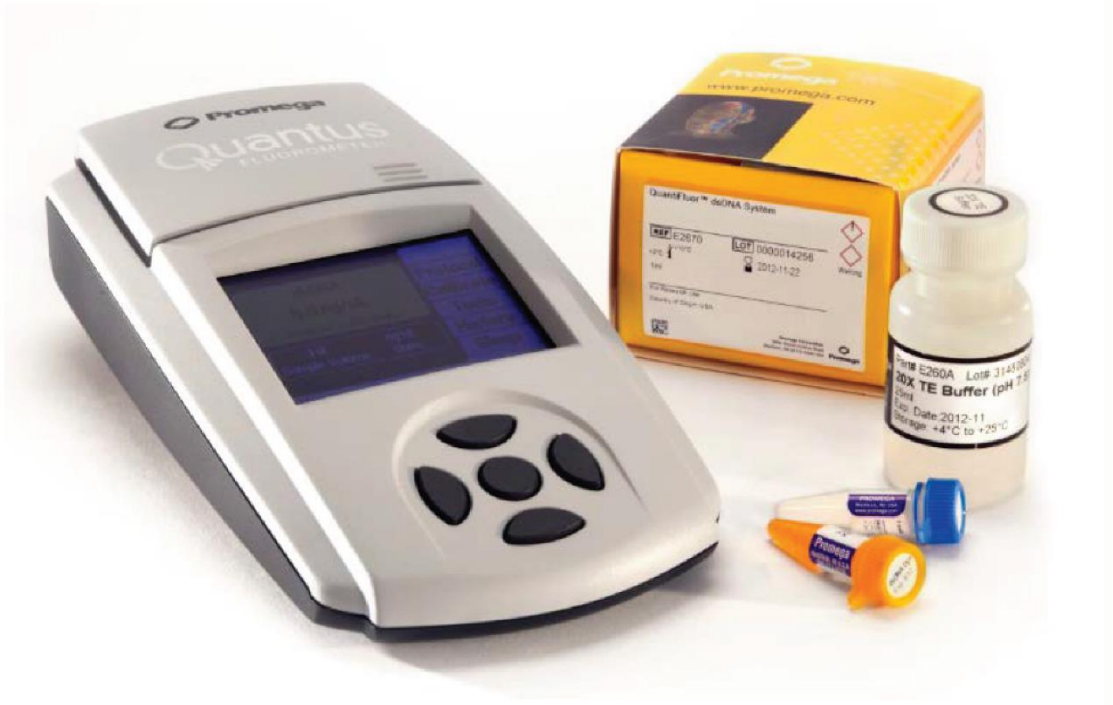
Legislatura de la Provincia de Córdoba

La Comisión Organizadora Local agradece muy especialmente la colaboración, trabajo y permanente disposición de Daniela Russo y Karina Herrera Seitz.

Promega

Quantus™ Fluorometer

Mide dsDNA, ssDNA y RNA.
Económico y fácil de usar!



Biodynamics

Biodynamics SRL ● ☎ (11) 4383-3000
info@biodynamics.com.ar ● www.biodynamics.com.ar

Lobov Científica

SOLUCIONES INTEGRALES PARA LABORATORIOS

www.lobov.com.ar

Visítela!
Suscríbase al newsletter

ENCUENTRE TODO LO QUE BUSCA

Ofertas
Productos
Servicios

Seguinos en facebook®
www.facebook.com/lobovcientifica

Av. Franklin D. Roosevelt 5828 - C1431BZV - Buenos Aires - Argentina
Tel: (54-11) 4574-3500 - Fax: (54-11) 4574-3600 - lobov@lobov.com.ar

INSUMOS BIOLOGICOS CORDOBA

de Viviana Grabois

DISTRIBUCION EXCLUSIVA EN CORDOBA DE

- *REACTIVOS Y/O INSUMOS PARA AREAS DE INVESTIGACON DIAGNOSTICO E INDUSTRIA, MARCA PB-L,*
- *INSUMOS DESCARTABLES PARA BIOLOGIA MOLECULAR Y CULTIVO CELULAR, MARCAS Y*

SOLUCION

SERVICIO AL CLIENTE

Contacto:
Tel y fax: (0351) 484-5210
Tel Móvil: 3517060967
Correo electrónico: insumos.biologicos.cordoba@gmail.com

Miércoles 5 de Agosto	Jueves 6 de Agosto	Viernes 7 de agosto
Salón de Actos	Sala de las Américas	Salón de Actos
	9:00-10:15 **Comunicaciones Orales**	9:00-10:15 **Comunicaciones Orales**
	10:15-11:00 Café	10:15-11:00 Café
	11:00-13:00 **Simposio "Synthetic Biology and Biosustainability"** Pablo Nikel (CNB, España) Gustavo Schujman (INMET, Rosario) Claudio Dunan (BIOCERES, Rosario)	11:00-13:00 **Simposio "Emerging viruses with epidemic potential"** Ariel Pereda (INTA, Buenos Aires) Gabriel Iglesias (Leloir, Buenos Aires) Gabriela Barbás (LCPC, Córdoba) Adrián Díaz (Inst. Virología, Córdoba)
13:00-17:30 **Registro y recepción**	13:00-14:30 Receso	13:00-14:30 Receso
	14:30-17:30 **Posters y café**	14:30-17:30 **Posters y café**
17:30-18:30 **Acto Inaugural**	17:30-19:00 **Comunicaciones Orales**	17:30-18:45 **Comunicaciones Orales**
18:30-19:30 **Conferencia inaugural** Luis Actis (Miami University, USA)		
19:30-21:30 **Cocktail de Recepción**	19:00-20:00 **Conferencia plenaria** Fernando Soncini (IBR, Rosario)	19:00-20:00 **Conferencia de Clausura** Hugo Lujan (UCC, Córdoba)
	20:00-22:00 **Asamblea General**	
		20:00-23:00 **Ágape de Despedida**

PROGRAMA

MIÉRCOLES 5 de Agosto

13:00-17:30 Registro y Recepción

17:30-18:30 **ACTO INAUGURAL**

Néstor Cortez
Presidente de SAMIGE
IBR-CONICET, Rosario

Palabras de Bienvenida

Actuación del Coro de Niños Cantores de Córdoba
Instituto Domingo Zípoli
Director: Guillermo Pellicer

18:30-19:30 **CONFERENCIA INAUGURAL**

Luis Actis
Department of Microbiology
Miami University, Oxford, Ohio USA

"Understanding the interaction of *Acinetobacter baumannii* with the surrounding extracellular environment"

Chairperson: Ángeles Zorreguieta

19:30-21:30 **COCKTAIL DE RECEPCIÓN**

JUEVES 6 de Agosto

9:00-10:15 **COMUNICACIONES ORALES**

Chairpersons: Leonardo Curatti y Paulina Páez

9:00-9:15 **FM 001**
"1,8-cineole inhibits biofilm and planktonic cells of multidrug resistant *Klebsiella pneumonia*"
Estela M Galván[1], Nicolás Vàzquez[1], Silvia Moreno[1,2]
[1]Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA)-CONICET, Fundación Instituto Leloir. [2]Universidad Maimónides.

9:15-9:30 **IN 001**
"Role of the *Serratia marcescens* hemolysin shla in the invasion of epithelial cells"
Gisela Di Venanzio, Eleonora García Véscovi
Instituto de Biología Molecular y Celular de Rosario (IBR) CONICET-UNR.

9:30-9:45 **MM 003**
"A long-term experimental evolution study by comparative genomics: modulation of cdi-GMP has a key role in *Pseudomonas aeruginosa* adaptation to bimodal switching between biofilm and planktonic states"
Romina A. Tobares, Andrea M. Smania
CIQUIBIC-CONICET. Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

9:45-10:00 **MM 004**
"X-OME-Q: a web-based server for integrative omics analysis"
Germán F. Burguener[1], Ezequiel J. Sosa[1], Leonardo A. Lucianna[1], Leandro Radusky[3], Esteban Lanzarotti[3,2], Lucas De Felipe[3,2], Darío A. Fernández Do Porto[1], Adrián G. Turjanski[1,2], Marcelo Martí[1,3]
[1]Plataforma Bioinformática Argentina, Instituto de Cálculo, FCEyN, UBA. [2]Departamento de Química Inorgánica, Analítica y Química Física, INQUIMAE - CONICET, FCEyN, UBA. [3]Departamento de Química Biológica, FCEyN, UBA.

10:00-10:15 **MM 005**
"Two LuxR-type transcriptional regulators within a cyclic-lipopeptide gene cluster are novel targets of the post-transcriptional Gac/Rsm cascade in *Pseudomonas protegens* CHA0"
Patricio Sobrero[1], Julieta Frescura[1], Marc Ongena[2], Claudio Valverde[1]
[1]LBMIBS, DCyT, Universidad Nacional de Quilmes. [2]Université de Liege, Bélgica.

10:15-11:00	**CAFÉ**

11:00-13:00	**SIMPOSIO**

"Synthetic Biology and Biosustainability"

Chairpersons: Julia Pettinari y Carlos Argaraña

Pablo I. Nikel
Systems and Synthetic Biology Program
National Centre for Biotechnology (CNB-CSIC), Madrid, Spain

"Glycolysis in Pseudomonads: the surprising turn of an old pathway"

Gustavo Schujman
Ingeniería Metabólica S.A., INMET:-CONICET
Rosario, Argentina

"Fatty acid metabolism in *Bacillus subtilis:* from regulation to a new Metabolic Engineering company"

Claudio Dunan
Director de Estrategia Bioceres S.A
Argentina

"The role of microorganisms in the sustainable intensification of agriculture"

13:00-14:30	**RECESO**

14:30-17:30	**POSTERS y CAFÉ**

FM 003-FM 012
IN 010-IN 016
BB 004-BB 014
MS 003-MS 012
BF 004-BF 021
MM 007-MM 032
EM 002-EM004

17:30-19:00	**COMUNICACIONES ORALES**

Chairpersons: Karina Herrera Seitz y Alex Saka

17:30-17:45 **BF 001**
"Effects of global regulators on the production of organic acids and solvent tolerance in *Escherichia coli*"
Diego E. Egoburo, Manuel S. Godoy, María J. Pettinari
[1]*Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.*

17:45-18:00 **BF 002**
"Characterization of the CT domain of Slpa from *Lactobacillus acidophilus* ATCC 4356 and its use as an anchor to display heterologous proteins on the surfaces of lactic acid bacteria"
Pablo Waehner, Joaquina Fina Martin, Lucía Malone, Mariana Allievi, Julián Tarsitano, Mariano Prado Acosta, Sandra Ruzal, María Mercedes Palomino
Departamento de Química Biológica, FCEN-UBA, IQUIBICEN-CONICET.

18:00-18:15 **BF 003**
"Antilisterial peptides from spanish dry-cured hams: purification and identification"

Patricia Castellano[1], Leticia Mora[2], Elizabeth Escudero[2], Constanza Melian[3,1], Graciela Vignolo[1], Fidel Toldrá[2].
[1]Centro de Referencia para Lactobacilos (CERELA), Chacabuco 145, T4000ILC, Tucumán, Argentina. [2]Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Avda. Escardino 7, Valencia, España. [3]Universidad Nacional de Tucumán, Ayacucho 491, Tucumán, Argentina.

18:15-18:30 **BB 001**
"Microbiological diversity and functionality of a chronically hydrocarbon contaminated soil post chemistry oxidation"
Rocío Medina[1], Pedro M. David Gara[2], Janina A. Rosso[3], Marisa R. Viera[4], Maria T. Del Panno[1]
[1]CINDEFI (CONICET-UNLP). [2]CIOP (CIC-CONICET). [3]INIFTA (CONICET-UNLP). [4]CIDEPINT (CIC-CONICET).

18:30-18:45 **BB 002**
"Assessment of aflatoxin b1 in interacting mixed cultures of *Aspergillus* section *Flavi* and non-toxigenic *Aspergillus*"
Carla L. Barberis, Cecilia S. Carranza, Marina C Rodriguez, Carina E Magnoli
Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto.

18:45-19:00 **BB 003**
"A specific antifungal activity and biofilm formation induced by the interaction between *Bacillus subtilis* and *Setophoma terrestris*"
Andrea G. Albarracín Orio[1], Romina A. Tobares[2], Andrea Smania[2], Daniel A. Ducasse[3]
[1]Laboratorio de Biología Molecular, Facultad de Ciencias Agropecuarias, Universidad Católica de Córdoba. [2]CIQUIBIC CONICET. Dpto. de Química Biológica, FCQ - UNC. [3]Instituto de Patología Vegetal (IPAVE) INTA.

19:00-20:00	**CONFERENCIA PLENARIA**

Fernando Soncini
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina

"Copper homeostasis and bacterial virulence: *Salmonella*'s way"

.

Chairperson: Andrea Smania

20:00-22:00	**ASAMBLEA GENERAL**

VIERNES 7 de Agosto

9:00-10:15	**COMUNICACIONES ORALES**

Chairpersons: Daniela Russo y Alejandro Moyano

9:00-9:15 **IN 002**
"Functional analysis and transcriptional regulation of the o-oligosaccharyltransferase of *Ralstonia solanacearum"*
Paula Vicino, Elena G. Orellano, M. Laura Tondo
IBR-CONICET, FBioyF-UNR. Rosario, Argentina

9:15-9:30 **MM 001**
"New insights for growth at low temperatures revealed by RNA-seq in the antarctic bacterium *Pseudomonas extremaustralis*"
Paula M. Tribelli[1,2], Esmeralda C. Solar Venero[2], Martiniano M. Ricardi[3], Maria Gomez-Lozano[4], Laura J. Raiger Iustman[1,2], Søren Molin[4], Nancy I. Lopez[1]
[1]Dpto. QB, FCEyN, UBA, Bs As, Argentina. . [2]IQUIBICEN, CONICET, BsAs, Argentina . [3]IFIBYNE-CONICET FCEyN, UBA, BsAs, Argentina. [4]Novo Nordisk Foundation Center for Biosustainability, DTU, Denmark.

9:30-9:45 **MM 002**
"In vitro and in vivo chaperone activity of the phasin PhaP from *Azotobacter sp.* FA8"
Mariela P. Mezzina[1], Diana E. Wetzler[1], Nina Dinjaski[2], Auxiliadora M. Prieto[2], María J. Pettinari[1]
[1]Instituto de Química Biológica de la Facultad de Cs. Exactas y Naturales, UBA (IQUIBICEN-CONICET). [2]Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CIB/CSIC).
CIQUIBIC-CONICET, Dpto. de Qca. Biol., FCQ-UNC, Córdoba. Argentina.

9:45-10:00 **MM 006**
"The mismatch repair protein Muts controls Pol IV dependent-mutagenesis induced by subinhibitory concentrations of ciprofloxacin"
Lucia Margara, Carlos Argaraña, Mariela Monti

10:00-10:15 **EM 001**
"LIBReciencia: an IBR outreach project and Microbiology as a tool for teaching science"
Eleonora García Véscovi[1,2], el equipo de trabajo Libreciencia[1]
[1]Instituto de Biología Molecular y Celular de Rosario- IBR-CONICET. [2]Facultad de Ciencias Bioquímicas y Farmacéuticas - UNR.

10:15-11:00	**CAFÉ**

11:00-13:00	**SIMPOSIO**

“Emerging viruses with epidemic potential”
Simposio AAM-SAMIGE

Chairpersons: José Echenique y Oscar Taboga

Ariel Pereda
Instituto de Patobiología INTA – CONICET
OIE/FAO Network of expertise on animal influenza (OFFLU)

“Influenza A in pigs and humans, an endless story…from epidemics to endemics”

Gabriel Iglesias
Laboratorio de Virología Molecular. Fundación Instituto Leloir
CABA, Argentina

“Mechanisms of dengue virus-host cell interactions”

María Gabriela Barbás
Càtedra de Microbiologìa, Facultad de Ciencias Mèdicas, UCC
Laboratorio Central de la Provincia de Córdoba,
Córdoba, Argentina

“Emergency of Dengue and Chikungunya viruses in our region”

Luis Adrián Díaz
Laboratorio de Arbovirus y Arenavirus. Instituto de Virología "Dr. J.M. Vanella",
Facultad de Ciencias Médicas, UNC, Córdoba Argentina.

“Emerging arbovirus with epidemic potential in our region”

13:00-14:30	**RECESO**

14:30-17:30	**POSTERS y CAFÉ**

FM 013-FM 021
IN 003-IN 009, IN 013
BB 015-BB 026
MS 013-MS 022
BF 022-BF 040
BD 003-BD 009
MM 033-MM 054

17:30-18:45	**COMUNICACIONES ORALES**

Chairpersons: Gabriela Paraje y Claudia Sola

17:30-17:45 **FM 002**

"Antioxidant compound release by cinnamoyl esterases of probiotic lactic acid bacteria"

Matías I. Russo, Lucila Saavedra, Claudia Abeijón Mukdsi, Paola Gauffin Cano, Roxana Medina

Centro de Referencia para Lactobacilos, CERELA-CONICET.

17:45-18:00 **BD 001**

"Analyzing bacterial communities from microbial mats and sediments located in the Atacama desert"

Ana B. Fernandez[1], María C. Rasuk[1], Daniel Kurth[2], Manuel Contreras[2], Fernando Novoa[3], Daniel Poire[1], María E. Farias[1]

[1]Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas, PROIMI-CONICET, Argentina. . [2]Centro de Ecología Aplicada, Santiago, Chile. 3Universidad Nacional de la Plata. 4Centro de Investigaciones Geológicas, UNLP-CONICET, Argentina.

18:00-18:15 **BD 002**

"Extreme-halophiles: their role in the arsenic biogeochemical cycle"

María C. Rasuk[1], Omar F. Ordoñez[1], Mariana Soria[1], María E. Farias[1]

[1]Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas, PROIMI-CONICET, Argentina.

18:15-18:30 **MS 001**

"Microbiological, enzymatic and genomic characterization of a *Paenibacillus sp.* xylanolytic isolate"

S. Ghio[1], F. E. Piccini[2], M. Insani2, D. H. Grasso[1], E. Campos[2]

[1]Instituto de Suelos, CIRN, CNIA, INTA Castelar. [2]Instituto de Biotecnología, CICVyA, CNIA, INTA Castelar.

18:30-18:45 **MS 002**

"Application of MALDI-TOF mass spectrometry to the identification of endophytic bacterial communities associated with plants"

Florencia Alvarez[1,2], José Luis López[1], Antonio Lagares[1]

[1]Instituto de Biotecnología y Biología Molecular, Fac Cs. Exactas, Universidad Nacional de La Plata. [2]CEQUIBIEM, Facultad de Cs. Exactas, Dpto de Química Biológica, Universidad de Buenos Aires.

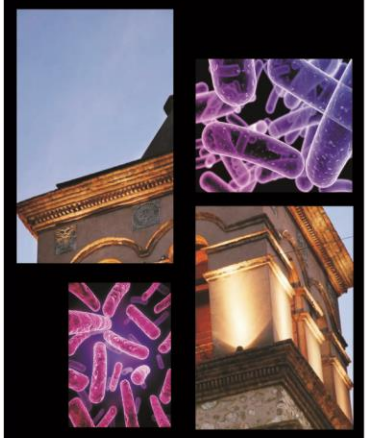
19:00-20:00	**CONFERENCIA DE CLAUSURA**

Hugo D. Lujan
Laboratory of Biochemistry and Molecular Biology, UCC
Center for Research and Development in Immunology and Infectious Diseases,
CONICET Córdoba, Argentina

"Development of an oral vaccine platform based on the protective and adjuvant properties of surface proteins of the intestinal parasite *Giardia lamblia*"

Chairperson: Eleonora García Véscovi

20:00-23:00	**ÁGAPE DE DESPEDIDA**



CONFERENCIAS PLENARIAS Y SIMPOSIOS

PLENARY LECTURES

UNDERSTANDING THE INTERACTION OF *ACINETOBACTER BAUMANNII* WITH THE SURROUNDING EXTRACELLULAR ENVIRONMENT

Luis Actis
Department of Microbiology, Miami University, Oxford, Ohio USA
E-mail: actisla@miamioh.edu

Acinetobacter baumannii persists under different conditions including those found in natural and medical environments and the human host. Thus, this facultative pathogen resists antimicrobial agents and host defenses, prospers under nutrient limitation, forms biofilms on abiotic and biotic surfaces, resists desiccation, and persists in water, soil, vertebrate and invertebrate animals and different food sources. However, the understanding of the mechanisms by which *A. baumannii* responds to these challenges is limited.

Our laboratory has shown that *A. baumannii* senses and responds to extracellular host-associated signals, such as iron limitation and the presence of mucin. Iron is not only an essential nutrient that is required for full virulence, but also a critical signal that controls the interaction with abiotic and biotic surfaces and the expression of functions such as phospholipase production, which could provide bacteria access to intracellular iron. Mucin, a complex glycoprotein product found in host secretions, is not only a target during *ex vivo* infections, but also a signal that controls global differential gene transcription via the BfmRS two-component transcriptional regulatory system. Biofilm assays, electron microscopy and transcriptomics showed that BfmRS plays a role in cell morphology, the production of cell appendages involved in attachment to abiotic and biotic surfaces and motility. In addition, the presence of red blood cells affects pili production independently of the erythrocytes' source.

A. baumannii also responds to extracellular signals, some of which are commonly found in medical environments. Our work has shown that ethanol, a disinfectant that serves as a carbon source for this pathogen, affects the production of exopolysaccharides, biofilm biogenesis, motility and virulence. These responses could be modulated by indole-3-acetic acid, a signaling molecule that plays a role in bacterial survival and resistance to host defenses such as those expressed in plants. *A. baumannii* also responds to ubiquitous signals such as light, although this is a non-photosynthetic bacterium. This response is mediated by the "short" BlsA sensor that does not contain predictable functions other than those associated with FAD binding. Transcriptomics showed that light and BlsA control a wide range of functions including iron acquisition, metabolic functions, production of adhesins and secondary metabolites, one of which controls cellular functions including motility, and gene regulators. Interestingly, the BlsA-mediated responses are also affected by temperature.

In summary, our work has shown that *A. baumannii* senses, "sees" and responds to extracellular signals through complex cellular and molecular processes, some of which could be expressed by unrelated bacteria. These responses are most likely responsible for the ability to *A. baumannii* to successfully persist in different environments and interact with the human host.

COPPER HOMEOSTASIS AND BACTERIAL VIRULENCE: *SALMONELLA*'S WAY

Fernando C. Soncini.
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina
E-mail: soncini@ibr-conicet.gov.ar

Copper is a critical component of proteins involved in a variety of cellular processes including cell growth, differentiation, and survival in organisms from bacteria to plants to mammals. However, it is also toxic even at low levels. The essentiality and at the same time toxicity of copper makes its active handling a vital skill for most organisms. As other organisms, bacteria have evolved specific copper homeostasis systems for maintaining a suitable intracellular concentration of this essential metal and at the same time, avoiding its toxic effects. Recent evidence indicates that copper actively contributes to the host innate immune response against bacterial infections and that along evolution pathogens have acquired specific mechanisms to deal with this intoxicant. Most enterobacterial species harbor the CusR/CusS two-component system to control envelope copper levels. CusR/CusS responds to a raise of periplasmic copper level by inducing the expression of a CBA-type efflux system that eliminates the excess of the metal ion from the periplasm. On the other hand, the bacterial cytoplasm is not predicted to require copper, and cells evolved different strategies to avoid the presence of this toxic ion in this compartment. Gram-negative species rely on a cytoplasmic transcription factor, CueR. Therefore, the independent detection of copper in each compartment provides a physiological advantage; allowing the maintenance of the appropriate quota in the envelope, and its avoidance within the cytoplasm. *Salmonella* lacks the *cus* locus, and instead, depends on CueP, a periplasmic cuproprotein encoded by a CueR-controlled gene. Different lines of evidence suggest that CueP is a major component of the *Salmonella* envelope copper-homeostasis, including its role in copper resistance in anaerobic conditions. This is also reinforced by our initial observation that *cueP* is mostly present in species in which the *cus* locus is either absent or incomplete. If CueP is required to maintain the periplasmic copper homeostasis and more important, to overcome its toxic effects when in excess, it can be hypothesized that its expression or activation requires the detection of periplasmic copper excess instead of been controlled by cytoplasmic metal ion levels. We observed that *cueP* induction requires the concerted activation of both CueR, by copper, and the CpxR/CpxA two-component system, by envelope stress. This co-regulation restricts expression of CueP only to cells encountering copper excess that causes envelope stress. As the number of specific envelope sensing signal-transduction systems present in a typical bacterial genome is limited, coordinated interactions between an envelope-stress detector and a signal-dependent cytoplasmic sensor contribute to expand the number and types of signals that can specifically fine-tune the damage responses in the periplasmic compartment.

DEVELOPMENT OF AN ORAL VACCINE PLATFORM BASED ON THE PROTECTIVE AND ADJUVANT PROPERTIES OF SURFACE PROTEINS OF THE INTESTINAL PARASITE *GIARDIA LAMBLIA*.

Hugo D. Lujan
Laboratory of Biochemistry and Molecular Biology. Catholic University of Cordoba and Center for Research and Development in Immunology and Infectious Diseases (CONICET). Argentina.
E-mail: hlujan@ucc.edu.ar

Despite the impact of world-wide vaccination programs, there is still a great necessity to develop novel, cheap and safe innovative vaccination strategies inducing long-lasting immunity. Since most infectious agents invade the organism via mucosal surfaces, adaptive mucosal immunity plays a central role in protecting the host against infections. Oral administration of vaccines represent a very attractive option, notably because it is non invasive and suitable for mass vaccination. However, the main impediment for oral vaccine development has been that orally administered antigens are easily destroyed by the gastrointestinal tract or potentially capable of inducing immune tolerance. The intestinal parasitic protozoan *Giardia lamblia* expresses at its surface variant-specific surface proteins (VSPs) that are extremely resistant to the low pH of the stomach as well as to intestinal proteases, allowing the parasite to survive in the harsh environmental conditions of the small intestine. We thus hypothesized that the expression onto virus-like particles (VLPs) of *Giardia* VSPs should shield these particles for oral administration. To obtain a proof of principle and, simultaneously, to develop a potential vaccine candidate, we used Influenza Hemagglutinin (HA) as a vaccinal antigen. Our results clearly demonstrated that *Giardia* VSP can protect vaccinal antigens in the gastrointestinal track for oral administration of vaccines, generating strong T and B cell-mediated protective responses. The development of this universal platform for oral delivery of vaccines should have a broad application to different infectious diseases.

SYMPOSIA

"Synthetic Biology and Biosustainability"

GLYCOLYSIS IN PSEUDOMONADS: THE SURPRISING TURN OF AN OLD PATHWAY

Pablo I. Nikel
Systems and Synthetic Biology Program, National Centre for Biotechnology (CNB-CSIC), Madrid, Spain
E-mail. pablo.nikel@cnb.csic.es

Metabolic pathways for glucose catabolism in bacteria are far more diverse than the classical biochemistry textbook view suggests. Glycolysis encompasses several biochemical processes, such as the Embden-Meyerhof-Parnas (EMP) and the Entner-Doudoroff (ED) pathways. While the linear, canonical EMP pathway prevails in Enterobacteria, many microorganisms have opted for alternative routes. One such example is the ubiquitous soil bacterium *Pseudomonas putida*, which has pre-endowed metabolic, physiological, and stress-endurance traits optimal for a number of biotechnological applications. *P. putida* operates an ED-based glycolysis, and the linear EMP pathway has been considered non-functional mainly due to the absence of the catabolic 6-phosphofructo-1-kinase. Since the EMP pathway has a higher ATP yield from glucose than the ED route, the former is preferable from an industrial point of view, specially for biotechnological processes aimed at biomass generation. Activating an EMP-based glycolysis in *P. putida* is therefore of paramount interest, but partial efforts (e.g., introducing a 6-phosphofructo-1-kinase activity from *Escherichia coli*) have failed so far. Understanding the combination and regulation of biochemical reactions that constitute the central carbon metabolism in this bacterium is a requisite for launching *P. putida* as an useful microbial cell factory. A systems level, multi-omic approach was adopted to unveil the regulation of catabolic and anabolic pathways in *P. putida* KT2440 when cells grow on glucose as the sole carbon substrate. The cyclic operation of a set of biochemical reactions, converting trioses back to hexoses phosphate, defines the core carbon metabolism in this strain. Such atypical feature in the glucose processing pathways has relevant consequences in the redox balance mainly at the level of NADPH regeneration - and it helps explaining the prevalence of the ED pathway in Pseudomonads from an evolutionary perspective. Furthermore, this particular metabolic architecture provides a new view to implement flawless, experiment-driven metabolic engineering strategies in environmental Gram-negative bacteria.

FATTY ACID METABOLISM IN BACILLUS *SUBTILIS:* FROM REGULATION TO A NEW METABOLIC ENGINEERING COMPANY

Gustavo Schujman
Ingeniería Metabólica S.A. (INMET S.A.-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Argentina.
E-mail: gustavo.schujman@indear.com

The biosynthesis of membrane lipids is an essential pathway for virtually all bacteria. Despite its potential importance for the development of novel antibiotics, and as precursor of high valued compounds, little is known about the underlying signaling mechanisms that allow bacteria to control their membrane lipid composition within narrow limits.

Our group has been studying fatty acid metabolism in bacteria for many years, specially in *B. subtilis*. This soil bacterium is the paradigm of Gram positive bacteria. Although non pathogenic (Generally Recognized As Safe by the FDA), *B. subtilis* is related to several disease causing organisms. Also, this bacterium is widely used for industrial production of enzymes, it is able to grow with a plethora of carbon sources and tolerates relatively harsh environmental conditions.

Fatty acid biosynthesis is a repetitive cyclic process fed by malonyl-CoA. We characterized the condensing enzymes of *B. subtilis*, which catalyze the first step of the cycle, and found that their expression was transcriptionally regulated. We identified and studied the transcriptional repressor FapR that regulates not only the expression of the condensing enzymes but of a larger regulon involving most fatty acid and initiation of phospholipid biosynthesis genes. We performed genetical and biochemichal experiments that demonstrated that malonyl-CoA is the metabolic link that modulates FapR activity to adequate fatty acid biosynthesis to the metabolic state of the bacteria. We provided strong evidence highlighting the role of FapR as a target for novel antibiotics, not only in *B. subtilis*, but in several related pathogenic bacteria containing the same regulation scheme. Additionally, we identified long-chain acyl-ACPs, the end product of fatty acid elongation cycle as potent inhibitors of fatty acid biosynthesis.

With all the accumulated knowledge on primary metabolism and fatty acid biosynthesis of *B. subtilis*, we explored the possibility of turning the bacterium into a cell factory of fatty acid derived compounds of industrial relevance. Even more, we were interested in converting industrial waste or low cost byproducts, into high value goods. To this end, we incorporated several genes of other microorganisms, optimized for proper transcription and high-level expression, into the genome of *B. subtilis*, and succeeded in the production of fatty acid ethylesters (biodiesel) from raw industrial glycerin.

With this first success and the collected experience, we started a pioneer biotechnological company in Argentina called Inmet (Ingeniería Metabólica) together with Bioceres, a company with great management skills. To this end, we mounted several technological platforms to address the Synthetic Biology, Metabolomics, Fermentation and Downstream Processing needs of our projects. This transition and our current goals will be discussed in this presentation.

THE ROLE OF MICRORGANISMS IN THE SUSTAINABLE INTENSIFICATION OF AGRICULTURE

Claudio M. Dunan
Director of Strategy, BIOCERES S:A:, Argentina.
E-mail: claudio.dunan@bioceres.com.ar

The growth of the global population and its purchasing power are creating an unprecedented demand for agricultural outputs that will have to be supplied with higher resource use efficiency and lower dependence on fossil fuels. Seed biotechnology and biological based crop protection and nutrition are key technologies for the early developing low carbon agriculture.

The rate of adoption of bio based agro inputs is doubling the growth of traditional products. The biopesticide market is expected to grow from the current USD 2.2 billions to USD 4.5 billions. The agricultural inoculants market is expected to reach USD 1 billion by 2020.

Biopesticides have lower toxicity, lower risk of pest resistance and lower environmental impact than conventional pesticides.

The possibility of a new global agreement on cutting carbon emissions to be reached in Paris 2015 would accelerate the adoption of the bio based agricultural inputs.

“Emerging viruses with epidemic potential”

INFLUENZA A IN PIGS AND HUMANS, AN ENDLESS STORY…FROM EPIDEMICS TO ENDEMICS

Ariel Pereda
Instituto de Patobiología INTA - CONICET - OIE/FAO Network of expertise on animal influenza (OFFLU), Argentina.
E-mail: pereda.ariel@inta.gob.ar

Throughout the history of diseases there are several reports of sudden onset of respiratory disorders. But only in the twentieth century it began to be reported and better understood those respiratory diseases caused by influenza viruses, including the ecology and the involvement of pigs in the transmission and adaptation of these viruses. Four Influenza pandemics occurred: 1918-1919; 1957-1958; 1968-1969, and 2009-2010. There were also warnings of possible pandemics threats, that was the case of Fort Knox in 1976, in China in 1977 where a limited pandemic affecting children under 23 years old, and finally in 1997 and 1999 with the emergence of a virus of high pathogenicity for birds (H5N1).
But if we study the last pandemics, we can see that viruses emerge with certain characteristics and in any case they share a common source, but in all cases the pandemic virus was then established as endemic in the population. A clear example of this establishment was the last pandemic (2009-2010), with a traceable porcine origin and been then the main circulating subtype in humans and in pigs.
With the aim of providing a local approach of the human-animal interface in Influenza viruses, it will be detailed the viruses founded in pigs in Argentina, as well as their potential relation to viruses circulating in humans. Argentina has recently reported the presence of human lineage viruses in pigs. Argentine viruses are distinguishable from similar subtypes in North America and represent independent events of transmission from human to pigs (Reverse Zoonosis). In late 2008, a fully human H3N2 was isolated from pigs with clinical signs of respiratory disease and fever. Experimental infection showed that the virus transmitted efficiently among pigs, and inoculated pigs showed lesions characteristic of influenza, suggesting that this virus was fully adapted to pigs. This particularity shows the potential to keep subtypes in the pig population, which can be a future risk factor for public health. Also, it was isolated in 2009 (as the the second report in the world), a pandemic virus from pig samples. Then in 2009 and in 2010 Argentina reported the isolation of viruses with rearranged internal pandemic genes and surface genes (HA and NA) from pigs but with human origin. In 2011 another reassorted virus was isolated containing surface genes of the fully human H3N2 virus first isolated in 2008 and all the internal genes from the pandemic virus. The clinical signs observed in all these cases were typical of influenza.
It is postulated that the lack of vaccines and characteristics of pig production in Argentina can contribute to the emergence of these new reasserted viruses. Continued surveillance of the swine population in Argentina and elsewhere is warranted to better understand the ecology of influenza viruses in these hosts and to prevent the emergence of viruses with pandemic potential.

MECHANISMS OF DENGUE VIRUS-HOST CELL INTERACTIONS

Gabriel Iglesias, De Maio FA, Byk LA, Mondotte JA, Samsa MM, Alvarez C and Gamarnik AV.
Laboratorio de Virología Molecular. Fundación Instituto Leloir, CABA, Argentina. CONICET
E-mail: giglesias@leloir.org.ar

Dengue virus (DENV) is an arthropod borne pathogen that causes a significant burden of disease in tropical and subtropical countries. DENV is a positive-sense RNA virus belonging to the *Flavivirus* genus of the *Flaviviridae* family. Its genome consists of a single, positive-strand RNA of about 11 kb that encodes a single ORF flanked by highly structured 5′ and 3′ untranslated regions. The ORF is translated in a unique polyprotein that produces 3 structural proteins (C-prM-E) and 7 non-structural proteins (NS1-2A-2B-3-4A-4B-5). Due to their minimal genome, DENV requires many host factors for their replication. DENV, like other plus strand RNA viruses, induces a profound rearrangement of cellular membranes to provide platforms for viral replication. DENV RNA synthesis occurs in membranous structures called vesicle packets that function as viral genome factories, which are in close association to viral particle morphogenesis sites in the ER membrane. During this process, the viral capsid protein (C) is responsible for recruiting the viral genome during viral encapsidation, forming a nucleocapsid that buds into the ER lumen, acquiring membranes and the structural proteins E and prM. The new viral particle travels through the secretory pathway to be released by exocytosis.

During infection the C protein is detected both in the cytoplasm and the nucleus of the host cell. Inside the nucleus it has been shown to accumulate in the nucleolus. In the cytoplasm we discovered that C localizes on ER-derived organelles known as lipid droplets (LDs). We found a link between dengue virus replication and LDs. Dengue infection increases the amount of LDs per cell and pharmacological inhibition of LD formation greatly reduces viral replication. Specific amino acids on the □2 helix of C were found to be crucial for both accumulation of C on LDs and DENV infectious particle formation.

To understand the function of the C protein on LDs in dengue virus infected cells, we studied the mechanism by which C is transported from virally-modified ER membranes to LDs. Using chemical inhibitors, siRNA, heterologous protein expression, and confocal microscopy, we found that C transport depends on the host vesicle trafficking system. In this regard, we defined the requirement of a non-canonical function of GBF1/ARF/COPI transport system. The process was found to be independent of components of the COPII system and C transport did not require Golgi integrity.

In summary, we showed that DENV co-opts LDs for replication and uses a cellular transport system for viral capsid protein trafficking to these organelles. This knowledge provides valuable information for targeting host proteins for antiviral strategies.

EMERGENCY OF DENGUE AND CHIKUNGUNYA VIRUSES IN OUR REGION

María Gabriela Barbás
Laboratorio Central de la provincia de Córdoba, Ministerio de Salud
Cátedra de Microbiología, Facultad de Ciencias Médicas, Universidad Católica de Córdoba, Argentina
E-mail: mgbarbas2001@yahoo.es

Arbovirus (arthropod borne virus) infections are a group of emerging and reemerging diseases difficult to control in the entire world. Dengue and Chikungunya fever belong to this group of diseases, and share besides the transmission mechanism and control actions, clinical features and Argentina´s laboratory diagnostic network that allows integrated monitoring.

Rapid detection of of Dengue and Chikungunya viruses is essential to take appropriate control measures. In order to detect the occurrence of a clinical case, prevent the spread of the disease and its impact on population health, a syndromic surveillance strategy was implemented. This strategy allows, in addition to monitoring the known diseases, detect other unknown diseases which may be important for public health system. The timely etiologic diagnosis of dengue and chikungunya viruses is essential to take necessary measures to control and monitoring cases.

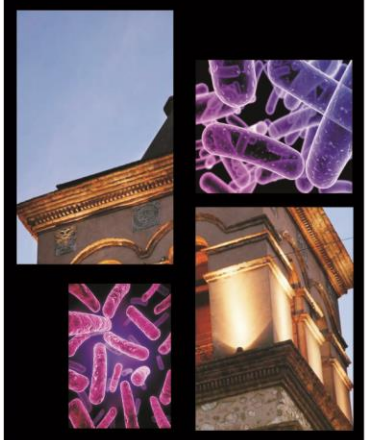
. A significant increase in the number of dengue cases has seen in the last twenty years in the Region of the Americas. Since the reintroduction of dengue in Argentina in 1997 and 2008, provinces of Salta, Jujuy, Misiones, Formosa and Corrientes have reported indigenous cases and all dengue serotypes were detected. In the first half of 2009 it was saw the largest outbreak of dengue in the epidemiological history of Argentina, 26,923 cases were officially recorded.

In Cordoba, first cases of the disease were detected in 2000 and a total of 11 imported cases were notified until 2008. In 2009, the first autochthonous dengue outbreak was registered. Viral circulation of Den-1 serotype was detected. During this outbreak, the province reported 1334 cases of febrile syndrome suspected of dengue. One hundred and seventy five were confirmed, 98 native and 77 imported. During the years 2010, 2011 and 2012, 287, 58 and 138 cases of acute febrile syndrome were reported, respectively. Six cases were confirmed in 2010, 3 cases in 2011 and 5 cases in 2012. In 2013 the second dengue outbreak occurred in the province. In 2015 a dengue outbreak occurred in certain districts of the capital city.

EMERGING ARBOVIRUS WITH EPIDEMIC POTENTIAL IN OUR REGION.

Luis Adrián Díaz
Laboratorio de Arbovirus y Arenavirus. Instituto de Virología "Dr. J.M. Vanella", Facultad de Ciencias Médicas, (UNC). Instituto de Investigaciones Biológicas y Técnicas, (IIBT-CONICET), Argentina.
E-mail: adrian.diaz@conicet.gov.ar

Arboviruses do not represent a based related phylogenetic group but they are all transmitted by arthropods (ArBoViruses = arthropod-borne viruses). A total of 50 arboviruses pathogenic for animals (including humans) have been reported, belonging to the families *Asfarviridae* (genus *Asfivirus*), *Bunyaviridae* (Cache Valley -CVV-, Oropouche -OROV- viruses), *Flaviviridae* (genus *Flavivirus*: Dengue -DENV-, St. Louis encephalitis -SLEV-, Yellow fever -YFV-,West Nile -WNV-, Zika -ZIKV-), *Orthomyxoviridae*, *Rhabdoviridae*, *Reoviridae* and *Togaviridae* (genus *Alphavirus*: Chikungunya -CHKV-, Eastern, Western, and Venezuelan equine encephalitis viruses -EEEV, WEEV, VEEV-, Mayaro viruses -MAYV-).
Transmission of arboviruses from a viremic to a susceptible vertebrate host (birds, rodents, monkeys, humans) is based on biological transmission through the bite of an infected arthropod. This biological transmission requires an active viral replication in the arthropod vector. As a results of its evolutionary history and coadaptation process some arbovirus are vector-host specific (DEN, CHK, YF) and others vector-host generalist (SLEV, WNV, VEEV, EEEV, WEEV). That means they can be amplified and transmit by a wide range of arthropod vector and vertebrate host species altering their receptor specificity and antigenicity.
Through biological evolution and cultural development, human beings were able to modify the environments according to their needs. Thus, deforestation has produced new areas for agriculture, livestock, farming activities and urbanization. These anthropogenic activities have produced great changes to host and vector communities and population abundance, sometimes driving emergence and reemergence of arboviruses. For those arboviruses that humans are amplifiers, the high mobility and interchange make possible the introduction and dispersion of arbovirus to new geographic areas. Except for *Asfivirus*, all known arboviruses are RNA viruses. Thus, they have an intrinsic capacity to mutate and adapt rapidly to new biological contexts or to acquire new biological features. Accordingly, they can spill over a new vector and host or become more virulent for humans and other animals.
For most arboviruses humans and domestic animals are dead end host and they do not represent an essential part of their life cycle. Focusing our arbovirus control measures on humans and livestock immunization will not erradicate arbovirus activity. We need to understand how human and environmental changes affect the arbovirus transmission activity in order to create more healthy landscapes. This is a crucial issue to face the emergence and the potential epidemic activity of Arbovirus.
Emergence of arboviruses is a multicausal event rather than a single-cause event. In this lecture will explore causes that promote epidemic emergence for arboviruses of human and veterinary medical concern for our region (CHKV, MAYV, OROV, SLEV, VEEV, WNV, ZIKV).



FISIOLOGÍA MICROBIANA

Código de Resumen: FM-001

Sección: Fisiología Microbiana

Modalidad: Oral

1,8-CINEOLE INHIBITS BIOFILM AND PLANKTONIC CELLS OF MULTIDRUG RESISTANT *Klebsiella pneumoniae*

E.M. Galván[1], N. Vázquez[1], S. Moreno[1 2].

[1] *Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA)-CONICET, Fundación Instituto Leloir.* [2] *Universidad Maimónides.*

egalvan@leloir.org.ar

Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. In the last years, the emergence of bacterial resistance to antibiotics encourages the research of new antimicrobials from the reservoir of medicinal plants and special attention has been paid towards plant essential oils and polyphenols. Earlier we reported the antibacterial activity of *Rosmarinus officinalis* (rosemary) polyphenols which inhibited the human pathogen *Klebsiella pneumoniae*. Most studies focused on the antimicrobial properties of phytochemicals against planktonic cells, but there are limited reports evaluating their efficacy against biofilms.Here, we evaluate the efficacy of 1,8-cineole (main monoterpene of rosemary essential oil) against biofilm and planktonic cells of several carbapenem-resistant*Klebsiella pneumoniae*, which is an emerging multidrug-resistant nosocomial pathogen. The antibacterial activity against planktonic cells was studied by broth microdilution technique. The SYTOX green assay, a fluorescent nucleic acid stain, was used to assess the integrity of the bacterial plasma membranes. Biofilms were Developer *in Vitro*on microtiter plates and quantified by crystal violet stain. Results showed that 1,8-cineole inhibited planktonic nosocomial multidrug resistant-*K. pneumoniae* strains at 1-4% (v/v). A strong permeabilization effect on the plasma membrane of both non-nosocomial *K. pneumoniae* strain (increment in the fluorescence of 50-60%) and multidrug resistant *K. pneumoniae strains* (an increment of 30-35% in the fluorescence) was observed after treatment with 1,8-cineole at 4% (v/v) for 24 h. In order tostudy the effect of 1,8-cineole on biofilm disruption, biofilms were allowed to form in M9 minimal medium for 24 h and then treated with the compound another 24 h. Our data indicated that *K. pneumoniae* strains were good biofilm producers. Interestingly, a clear anti-biofilm effect was observed with 1,8-cineole in a concentration-dependent manner (0.003 to 4%, v/v), disrupting both susceptible and multidrug-resistant*K. pneumoniae* strains at least in a 30-50%. In conclusion: our *in Vitro*findings suggest that 1,8-cineole may be useful for the prevention and/or treatment of health care-associated*K. pneumoniae* infections.*In-vivo* studies using animal models and clinical trials are needed to further evaluate its potential as antibacterial agent

Código de Resumen: FM-002

Sección: Fisiología Microbiana

Modalidad: Oral

ANTIOXIDANT COMPOUND RELEASE BY CINNAMOYL ESTERASES OF PROBIOTIC LACTIC ACID BACTERIA

M.I. Russo[1], L. Saavedra[1], C. Abeijón Mukdsi[1], P. Gauffin Cano[1], R. Medina[1].

[1] *Centro de Referencia para Lactobacilos (CERELA) - CONICET.*

matiasr21@gmail.com

Hydroxycinnamic acids (HA), such as caffeic (CA), ferulic (FA), *p*-coumaric and sinapic acids, are commonly found in fruits, vegetables and grains ester-linked to vegetable cell wall polymers, and can not be absorbed under these complex forms. HA are phenolic compounds that exhibit antioxidant and chemoprotective properties, and they are released by cinnamoyl esterase (CE) enzymes. Hydrolysis of ester bonds carried out by CE and the subsequent release of HA is the first step required for the bioavailability and metabolism of these acids. CE were detected in human and animal intestinal mucosa and gut microbiota. There is few information about CE of probiotic lactic acid bacteria (LAB). The use of probiotic bacteria with CE activity able to stimulate the release of antioxidant compounds in the gut, can be applied for treatment and/or prevention of pathologies associated with malnutrition. The aim of this study was to detect CE activity in two probiotic LAB, and evaluate the release of antioxidant compounds [ferulic acid (FA) and caffeic acid (CA)] from the hydrolysis of hydroxycinnamates (HC) [ethyl ferulate (EF) and chlorogenic acid (ChA), respectively]. *Lactobacillus fermentum* CRL1446 and *Lactobacillus acidophilus* CRL1014, strains isolated from dairy products and human intestine, respectively, were selected for their probiotic properties. CE activity was evaluated on agarized MRS medium (with glucose omitted) supplemented with 4.5 mM ethyl ferulate (MRS-EF). Strains were streaked on the medium and incubated at 37 °C for 24 h. In addition, kinetics of HC consumption and HA production by both strains were evaluated in MRS-EF and MRS-ChA broth media containing 1.5 mM EF or ChA, respectively. Strains were inoculated at 2% (v/v) and incubated at 37 °C for 24 h. Samples were taken at 0, 3, 6, 9, 12 and 24 h of incubation, and culture supernatants were obtained by centrifugation (10000 rpm at 4 °C). HC and HA concentrations in supernatants were determined

by HPLC. CE activity was detected in both strains by appearance of a clear zone around the colonies grown on MRS-EF plates. At the end of incubation period, *L. fermentum* CRL1446 hydrolyzed all EF and ChA present in the media, producing 0.72 mM FA and 0.75 mM CA, respectively. The production rate was 0.032 mM/h for FA and 0.031 mM/h for CA. *L. acidophilus* CRL1014 also hydrolyzed all HC present in the media, releasing 1.15 mM FA and 0.75 mM CA. The production rate was 0.097 mM/h for FA and 0.034 mM/h for CA. Released HA were not further metabolized by these strains, since no other metabolic products were detected up to 48 h incubation. On the basis of these results, *L. fermentum* CRL1446 and *L. acidophilus* CRL1014 can be proposed as potential probiotic strains able to release antioxidant HA. *In vivo* studies are being carried out to elucidate the effect of oral administration of these strains on the host oxidative status in a murine model of malnutrition.

Código de Resumen: FM-003

Sección: Fisiología Microbiana

Modalidad: Poster

MACROMOLECULAR OXIDATION CAUSED BY CITRIC ACID IN BACTERIUM

M. Rodríguez Varela[1], I. Albesa[1], V. Aiassa[1 2].

[1] Departamento de Farmacia, Fac de Cs Qcas, UNC, Córdoba, Argentina. [2] Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA), CONICET, UNC.

viraiassa@gmail.com

Citric acid (CA) is a weak organic acid it is a natural preservative which occurs naturally in citrus fruits and is also used to add an acidic or sour taste to foods and drinks. In biochemistry, the conjugate base of CA, citrate, is important as an intermediate in the CA cycle, which occurs in the metabolism of all aerobic organisms. Commonly is used in the food, cosmetic and pharmaceutical industries for its pleasant taste and low toxicity. Also, it has been used for its topical antibacterial effect against *Pseudomonas aeruginosa* multiresistant. Studies show that a greater concentration of 40 mM significantly reduced populations of *Cronobacter sakazakii* and *Salmonella enterica* serovar Typhimurium. According to the above, the objective of our study was to evaluate if the antimicrobial effect of CA could be due to oxidative damage and consequent macromolecular bacterial oxidation. The minimum inhibitory concentration (MIC) to CA in *Staphylococcus aureus* 29213 and *Escherichia coli* 35218 was determined using the standard tube dilution method as outlined by the Clinical Laboratory Standards Institute. As markers of protein oxidation, advanced oxidation protein products (AOPP) can be determined spectrophotometrically by a colorimetric assay with potassium iodide and acetic acid, while nitrosylation be evaluated by Saville assay. To determine the degree of lipid oxidation in bacterium cells used malondialdehyde (MDA) with thiobarbituric acid which leads to the formation of a colored product which absorbs at 535 nm. In all assays were used CA concentrations of 20 (sub MIC), 40 (MIC) and 80 (supraMIC) mM. *S. aureus* and *E. coli* showed an CA MIC of 40 mM. As regards the oxidation of macromolecules, it was observed that in *S. aureus* the oxidative damage was mediated by protein oxidation and nitrosylation while lipid peroxidation was not observed. In contrast in *E. coli*, a Gram negative bacterium with more lipid exposure, only lipid peroxidation was observed at the different concentrations assayed CA. The primary antimicrobial action of CA is its ability to inhibit the growth of many bacteria. The undissociated form of CA penetrates the cell membrane and enters the cytoplasm where it reduces the intracellular pH and disrupts the transmembrane proton-motive force. In addition, CA acts as a chelating agent and inhibits the growth of bacteria by chelating divalent metal ions. The present investigation about the macromolecular oxidation caused by CA may foster the study of the oxidative injure of proteins and lipids as the other factor involved in the action of CA. The results of this study may contribute to a better understanding of antimicrobial mechanisms of CA.

Código de Resumen: FM-004

Sección: Fisiología Microbiana

Modalidad: Poster

INTERACTION BETWEEN BIOPROTECTIVE LACTIC ACID BACTERIA AND *Escherichia coli* O157:H7 DURING GROWTH AS PLANKTONIC CULTURES IN A MEAT BASED MEDIUM

A. Orihuel[1], L. Saavedra[1], S. Fadda[1].

[1] Centro de Referencia para Lactobacilos (CERELA-CONICET).

orihuelalejandra@gmail.com

The main cause of product recalls associated with fresh meat is contamination with *Escherichia coli* enterohemorrhagic (EHEC) and related enteric pathogens. In Argentina, hemolytic uremic syndrome, mainly caused by EHEC, is the most common cause of acute renal failure and the second cause of kidney transplantation in children. Therefore, EHEC is a serious threat to public health and a major concern for the sustainability of the meat industry. In addition, and considering the increased demand for food with high standards of hygiene and sensory quality, the search for solutions is imperative. In this context, the use Lactic Acid Bacteria (LAB) as bioprotective cultures is known and well documented for certain pathogens. However the efficiency of

LAB and its metabolites to inhibit EHEC has been little explored. The aim of this study was to evaluate the inhibitory effect of bioprotective LAB strains on EHEC during incubation in a meat model system (MC). Four LAB strains, mostly isolated from meat products, with proven bioprotective properties were selected: *Lactobacillus* (*L.*) *curvatus* CRL 705; *L. sakei* CRL1862; *Enterococcus* (*Ent.*) *mundtii* CRL35 (isolated from artisan cheeses) and *L. plantarum* CRL 681. The pathogenic strain studied was *Escherichia coli* O157: H7 NCTC12900. This strain is a natural isolate which does not produce enterotoxins, but has intact other virulence factors. Each combination LAB-EHEC was evaluated by inoculating the MC with 10^6 CFU/ml of LAB and 10^4 CFU/ml of *E. coli* and incubated under gentle stirring at 25 °C for 96 h. Samples were taken to analyze pH evolution and viability of both microbial groups using selective agar media. Previously to the study of LAB-pathogen interaction, the growth of each microorganism individually in MC under the same conditions was carried pout. The results showed good growth of LAB in the MC as pure cultures, the maximum viability was between $2{,}0.10^8$ and $9{,}8.10^8$ CFU/ml depending on the strains. When *E. coli* NCTC12900 was cultivated individually, an optimal growth curve in MC was observed reaching the stationary phase at 24 h ($1{,}8.10^8$ CFU/ml). When the co-cultures (LAB-EHEC) were analyzed, a decreased growth rate of LAB in the presence of EHEC was observed (1 to 2 log units less than in single culture condition), however their acidifying capacity was unaffected. Interestingly, the growth of the EHEC strain in co-culture was affected considerably. The strains CRL 681 and CRL 35 were able to totally reduce the population of *E. coli* during the time while the other analyzed strains showed variable inhibitory effects. Based on these results, we propose *Ent. mundtii* CRL 35 and *L. plantarum* CRL 681 as potential bioprotective strains capable to inhibit EHEC in meat. Currently, more detailed studies about the molecular mechanisms involved in the microbial interaction are in progress, in view to provide the meat industry with an efficient biotechnological solution to mitigate EHEC in meat products.

Código de Resumen: FM-005

Sección: Fisiología Microbiana

Modalidad: Poster

MOTILITY OF *Escherichia coli*: TRANSITION FORM SWIMMING TO SWARMING

G.F. Fier[1], D.H. Hansmann[1], R.C. Buceta[1 2].

[1] Instituto de Investigaciones Físicas de Mar del Plata (UNMdP and CONICET), Argentina. [2] Departamento de Física, Facultad de Ciencias Exactas y Naturales - Universidad de Mar del Plata, Arg.

guidoarg@gmail.com

It is well-known that*E.coli*is a microorganism capable of moving individually (*swimming*) and collectively (*swarming*). Research groups working with *E.coli* colonies have observed that bacteria which prepare for swarming undergo remarkable physical changes like length growth and hyper flagellation. Further, it turned out that the typical swimming motility called “Run and Tumble” is not observed in swarming bacteria colonies. Based on this observation many authors draw the conclusion that *E.coli* shows a different individual behavior (motility) after changing from “swimming” to “swarming” motility. This conclusion is, however, based on dynamics which are dominated by “collision” between bacteria and may conceal the intended movement of the individual. We address the question of *individual* bacterial dynamics in swarming *E.coli* colonies using simulations of self-propelled rigid bodies with short range interactions. Based on experimental data we tune our simulation to reproduce swimming motility using agents who perform “Run and Tumble”. Using our *swimming* simulation, we study the transition from bacterial swimming to bacterial swarming raising the agent density and the driving force of the agents. The dynamic observables measured during the simulation are in good agreement with experimental data found in swarming *E.coli* colonies. From our simulation, we conclude that one can not rule out that bacteria intend to perform “Run and Tumble” even in swarming colonies

Código de Resumen: FM-006

Sección: Fisiología Microbiana

Modalidad: Poster

CHARACTERIZATION OF DUAL-SPECIES BIOFILMS DEVELOPED BY MICROORGANISMS INVOLVED IN POLYMICROBIAL CATHETER-ASSOCIATED URINARY TRACT INFECTIONS

C. Mateyca[1], L. Ielpi[1], E.M. Galvàn[1].

[1] Fundación Instituto Leloir - IIBBA (CONICET).

celeles@live.com.ar

Urinary catheterization is frequent in hospitals and long term care facilities. Bacteriuria associated with prolonged catheterization is polymicrobial. Establishment of biofilms constitutes a bacterial strategy for persistence on catheter surfaces. We aim to study whether microbial community interactions in dual-species biofilms developed by uropathogens could be beneficial or detrimental to the species involved.

First, we performed a retrospective study at a public hospital in CABA to assess the local epidemiology of pathogens involved in catheter-associated polymicrobial bacteriuria. The more prevalent associations found were *Klebsiella pneumoniae-Escherichia coli* (Kp-Ec), *E. coli-Enterococcus faecalis* (Ec-Ef), *K. pneumoniae-E. faecalis* (Kp-Ef), and *K. pneumoniae-Proteus mirabilis* (Kp-Pm). Next, we studied the ability of these co-isolated species to grow as single- and dual-species biofilms, as well as planctonically, in artificial urine medium (AUM). Biofilms were formed on a siliconized surface allowing bacteria to attach for 3 h, and then replacing AUM every day. At different times, biofilms were disrupted mechanically and colony forming units (cfu) were assessed by plating on selective antibiotics. All species attached to the surface (5-30 x 10^3 cfu/cm^2) and developed single-species biofilms, whose cell number increased over time and was maintained for 7 days. A 1000-fold increment in cell number was reached by both *K. pneumoniae* and *E. coli* biofilms, whereas the maximal increase in cell number reached by *E. faecalis* and *P. mirabilis* biofilms was 10-fold. The association Kp-Ec did not affect biofilm growth by *K. pneumoniae* but resulted in *E. coli* being outnumbered by *K. pneumoniae* after 72 h. In Ec-Ef dual-cultures, *E. coli* ability to form a biofilm was not altered, however, a 10-fold decrease in *E. faecalis* adherence to the surface, and a subsequent delay on biofilm development, was observed. Regarding Kp-Ef, characteristics of biofilm formation were identical to single-species biofilms. Planktonic growth of *E. coli*, *E. faecalis*, and *K. pneumoniae* in dual-species cultures were similar than single-species cultures. The Kp-Pm combination resulted in the impairment of *K. pneumoniae*, but not *P. mirabilis*, to attach to the surface and develop a biofilm. However, *P. mirabilis* behavior was similar in dual- and single-species biofilms. Kp-Pm liquid cultures showed a negative effect on *K. pneumoniae* growth and viability.

Our findings evidenced a variety of effects occurring after two bacterial species being together into a biofilm. Establishment of Kp-Ec biofilms resulted in a detrimental effect over *E. coli*, while interactions in Ec-Ef co-cultures diminished the adhesion to the surface of *E. faecalis*. In addition, Kp-Pm interactions impaired *K. pneumoniae* biofilm. The results suggest that species behavior in dual-species biofilm depends on the identity of the two partners involved.

Código de Resumen: FM-007

Sección: Fisiología Microbiana

Modalidad: Poster

IMMUNOMODULATING EFFECT OF β-LACTOGLOBULIN HYDROLYSATES BY *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL 656

M. Pescuma [1], L. Saavedra[1], E.M. Hebert[1], A. Orihuel[1], F. Mozzi[1], G. Font de Valdez[1].

[1] *Centro de Referencia para Lactobacilos (CERELA-CONICET).*

orihuelalejandra@gmail.com

The main whey protein β-lactoglobulin (BLG) is one of the major milk allergens affecting especially to children under 3 years old. Peptide and protein allergenicity can be assessed by their ability to stimulate IL-4 production by lymphocytes. In this work, the anti-allergy immune response of BLG hydrolysate obtained by *L. delbrueckii* subsp. *bulgaricus* CRL 656 (BLGh) by inhibition of IL-4 and stimulation of IFNg (Th1 cell-related cytokine) and IL-10 (immunosuppressive cytokine) production by murine splenocytes *in vitro* was evaluated. Three groups of BALB/c female mice aged from 3 weeks were used for lymphocyte proliferation tests. Mice were fed with whey-protein-free diet (AING soy, ICN, Illkirch, Francia) for two generations and mice from the second generation aged 3 week old were immunized with 1 mg of BLG (group1), BLGh (group 2) or phosphate buffer (group 3) using 0.5 mg choleric toxin as adjuvant once a week during 5 weeks. Spleens were removed from mice of all groups and were pressed separately through cellular sieves. Cells from each group were incubated separately in presence ConA (cell proliferation stimulation) and BLG, BLGh or buffer (control). Cell proliferation was assessed by using a MTT kit (Promega), IL-4 production was determined by ELISA, and INFg and IL-10 by flow cytometry using an inflammation kit (CBA kit, BD). No differences in cell proliferation and IL-10 concentrations in mice from group 1 were noticed while no IL-4 was detected. INFg concentrations were lower in presence of BLG and BLGh (2,1-1,9 pg/ml, respectively) than when icubating with buffer (57,9 pg/ml). On the other hand, mice from group 2 showed a lower IL-4 concentration when icubating with BLGh than with BLG (312 and 434 ng/ml, respectively) while INFg concentrations were higher (3.7 and 1.3 times) when incubating with BLG. Control mice (group 3) showed higher cell proliferation when cells were incubated with BLGh (1.2 times) although IL-4 production was lower (2.2 times) than when incubated with BLG. The concentration of the pro-immflamatory cytokine INFg was higher when incubating with buffer (112.2 pg/ml) than with BLG (41.9 pg/ml) or BLGh (89.5 pg/ml); however, IL-10 production was only higher when incubating with BLGh. Results showed that BLGh had a lower immune response than BLG in control mice due to the lower IL-4 production and higher IL-10 and INFg concentration observed. Moreover, when spleen cells from mice primed with BLGh were incubated with BLG, IL-4 concentration was lower than in control mice (1.3 times) and IL-10 and INFg poduction was higher (1.4 and 4.7 times). BLGh administration had an immunomodulating effect on BLG allergenic response; nevertheless, it was not enough to prevent IL-4 production.

Código de Resumen: FM-008

Sección: Fisiología Microbiana

Modalidad: Poster

INFLUENCE OF LACTIC ACID BACTERIA ON METABOLIC, ENDOCRINE AND IMMUNOLOGICAL PARAMETERS IN AN ANIMAL MODEL OF MILD CALORIC RESTRICTION

E. Fabersani[1], M.I. Russo[1], R. Ross[2 3], L. Saavedra [1], S. González [1 2], P. Gauffin Cano[1 3].

[1]Centro de Referencia para Lactobacilos (CERELA) - CONICET. [2]Universidad Nacional de Tucumán - Facultad de Bioqca., Qca., y Farmacia. [3]Universidad del Norte Santo Tomás de Aquino (UNSTA).

matiasr21@gmail.com

Caloric restriction (CR) is defined as a reduction in energy intake below the amount of calories that would be consumed *ad libitum*. CR induces changes in gut microbiota composition and the immune response, which affect the host health. Leptin, a pleiotropic hormone mainly produced by the adipose tissue, regulates multiple homeostatic functions such as food intake, reproductive and immune functions, besides basal metabolism.

The aim of this study was to evaluate the effects of a CR diet and lactic acid bacteria (LAB) administration on leptin levels, metabolic and immunological parameters in a murine model of mild CR.

Animals were daily fed with a restricted diet (25% less than the daily ration) for 45 days, in order to reach a degree of mild malnutrition (10-25% body weight loss compared to control group). Different groups of mice received *Lactobacillus (L.) fermentum* CRL1446, *Lactococcus (Lc.) lactis* CRL1434, and *L. casei* CRL431 strains resuspended in the drinking water at the dose of 10^8 cells / mL / day / mouse. We determined leptin, glucose, cholesterol and triglyceride levels and serum cytokines (TNFα, IL-6 e IL-10) at 0, 20 and 45 days. Body and fat weight as well as adipocyte area were also determined.

The studied strains had different effects on leptin levels. *L. casei* CRL431 induced significant increase in leptin levels, reaching values similar to control. By contrast, *L. fermentum* CR1446 and *Lc. lactis* CRL1434 produced a significant decrease in leptin levels. There is a positive correlation between leptin concentration and pro-inflammatory cytokines and fat weight. The effects of LAB administration on metabolic parameters were more evident at 45 days of CR diet. *L. fermentum* CRL1446 showed hypocholesterolemic and hypoglycemic properties.

Our results suggest that different strains may have different functional roles and applications in diverse pathologies. LAB that induce increase in leptin levels could have positive influence on caloric-restricted individuals. Therefore, modulation of circulating leptin levels may be considered as a promising novel strategy to intervene on nutritional status.

Código de Resumen: FM-009

Sección: Fisiología Microbiana

Modalidad: Poster

HIGH SENSITIVITY TO H_2O_2 BY *Saccharomyces cerevisiae* CELLS EXPRESSING *Yarrowia lipolytica* STEROL CARRIER PROTEIN-2

A.R. Gianotti[1 2], C.Y. Scott[1], M.R. Ermácora[1 2], R.G. Ferreyra[1 2].

[1]Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes. [2]Grupo Vinculado de Biología Estructural y Biotecnología IMBICE-CONICET-CCT La Plata.

rferreyra@unq.edu.ar

The dimorphic yeast *Yarrowia lipolytica* represent a model eukaryotic organism in areas as protein secretion, peroxisome biogenesis, cellular differentiation, hydrophobic substrates utilization, and several other application fields. The entire sequence of the six *Y. lipolytica* chromosomes has been determined. Sterol Carrier Protein-2 (SCP-2) is a nonspecific lipid transfer protein that has been implicated in the transfer, uptake, and metabolism of cholesterol, branched-chain fatty acids, acyl-CoA conjugates, and other lipids. SCP-2 module can be found as a component domain in multidomain proteins or as single domain polypeptides in all forms of life. SCP-2 structure and function has been studied mostly in mammals and next in insects. It has been generally found that the main function of this protein is in the peroxisomal degradation of lipids. We have previously shown that *Y. lipolytica* SCP-2 (YLSCP-2) is a 128-amino-acid basic protein inducible by fatty acids, that it is located in the yeast peroxisomes and able to bind and transfer a variety of lipids to membranes by a collision-mediated mechanism. YLSCP-2 tridimensional structure was recently solved in our lab (PDB code 4JGX). X-ray diffraction of the protein crystals shows the lipid binding site as a large system of interconnected tunnels and surface pockets partially occupied by palmitate. Despite having such detailed structural information on this protein, very little is known about SCP-2 function in plants, yeast and prokaryotes. Intriguingly, *S. cerevisiae* and *Schizosaccharomyces pombe* are the only fungi known to lack SCP-2 or any related domain; they also have difficulties to adapt growing on lipids. For those reasons, we expressed the YLSCP-2 gene in *S. cerevisiae* in order to evaluate the physiological function of this protein in this context. We found that cells expressing the protein are more sensitive to H_2O_2 compared to parental *S. cerevisiae*, which lacks SCP-2. This behavior was seen by parallel growth (measured by O.D.

and viability) on minimal liquid media (YNB-glucose) at subletal H_2O_2 concentration. Besides, growth of cells on the same solid media at different H_2O_2 concentrations was determined. Electron microscopy shows YLSCP-2 yeast cells morphology profoundly altered, when grown on subletal H_2O_2 level. Catalase marker, measured in total cell extracts by H_2O_2 degradation and zymograghy, is also increased over the parental strain, in cultures grown on the same condition. So, we hypothesized that YLSCP-2 may be involved in oxidative stress pathways, kidnapping peroxidized lipids generated by H_2O_2 and disseminating those to different structures in the cells. *Y. lipolytica* null mutants for the YLSCP-2 gene are also being generated to evaluate the consequences of its absence in this yeast.

Código de Resumen: FM-010

Sección: Fisiología Microbiana

Modalidad: Poster

PHYSIOLOGICAL PROCESSES OF YEAST CULTURES FOLLOWED BY CHANGES IN CELL SURFACE POTENTIAL

L.L. Lavaisse[1,2], A.H. Hollmann[1], M.N. Nazareno[2], A.D. Disalvo[1].

[1]Laboratorio de Biointerfases y Sistemas Biomiméticos, CITSE- UNSE- CONICET. [2]Laboratorio de Antioxidantes y Procesos Oxidativos, CITSE- UNSE- CONICET.

lulavaisse@gmail.com

Traditional techniques used for monitoring the viability and the growth of cell cultures such as measurements of turbidity of cell suspensions (as absorbance) exhibit some limitation because provides information of the bulk of the cell culture reporting an averaged state considering the whole cell population, without distinguishing alive cells from dead cells. On the other hand, the colony forming units (CFU) method that reports viable cells is very sensitive but depends on plating conditions and is relatively time-consuming. Finally, methylene blue staining distinguishes between alive and dead cells, but does not offer the same level of sensitivity. In contrast, zeta potential determination by optical microscopy provides information about the state of a single cell because it is able to measure cell surface charges in individual cells of the suspension. The goal of this work is to determine changes in the surface properties of cells in suspensions following the zeta potential and relate it to the physiological states. For this purpose *Saccharomyces cerevisiae* isolated from instant dry yeast commercial powder was chosen as a biological model system. Yeasts were grown in standard medium (Sabouraud glucose broth). At different incubation times, culture samples were collected to evaluate yeast growth, performing CFU, OD_{600nm} and following variations in the electrophoretic mobility of cells in an electric field. Data obtained show that changes in optical density during exponential growth phase are concomitant with a change in zeta potential values. A clear correlation between optical density and the zeta potential as a bulk measure was observed. A closer inspection of the zeta potential of individual cells allowed establishing that a distribution of cells with different zeta potentials exists in each sample. This suggests that different metabolic stages converge at each time of culture when averaged values are taken by turbidity. After the exponential phase, two main jumps in zeta potential values are observed. The first one matches with the transition from exponential to stationary phase, and the second one with the transition of late stationary to death phase. These results were confirmed by viability staining in an inverted fluorescence microscope and colony counting plate. It is concluded that zeta potential is a valuable tool for following the growing of suspended cells. It denotes that cells in different metabolic states coexist in a culture, revealing a delicate balance of populations. In comparison to turbidity it allow to anticipate the beginning of the death phase. The surface changes observed are possibly related to metabolic changes taken place during its growing. It is expected that the alteration of this balance predicts the changes on the growth cell phase.

Código de Resumen: FM-011

Sección: Fisiología Microbiana

Modalidad: Poster

IDENTIFICATION OF YEASTS ISOLATED FROM DETERIORATED STRAWBERRY JUICE OF THE NORTHWEST ARGENTINE

C.V. Vallejo [1], O.D. Delgado [2], A.M Strasser de Saad[1], G. Rollan[3], M.J. Rodríguez Vaquero[1].

[1]UNT-CONICET - Facultad de Bioquímica, Química y Farmacia, Tucumán, Argentina. [2]CITCA, Facultad de Cs. Exactas y Naturales UNCA, Catamarca, Argentina. [3]Cerela .

mariajo@fbqf.unt.edu.ar

Yeasts can resist extreme conditions better than bacteria, so they play an important role as spoilage of foods and beverages. Procedures used for yeast identification including cellular morphology and fermentation and assimilation tests, sometimes lead to ambiguous results because of phenotypic variability of the strain. Whereby, molecular approaches are consistently used for yeast identification. Tucumán is the leading producer of strawberries in northern of Argentina and strawberry juices are highly consumed in this country; however there are no reports available about the identification of yeasts present in deteriorated

Argentinean strawberry juices. The aim of this work was the isolation of yeasts present in deteriorated strawberry juice and their identification in order to find out in the future a possible control compound or solution to this problem. Strawberries with no apparent physical or microbial damage were washed and milled in processor to make 1 liter of juice which was placed in sterile flasks until signs of deterioration appeared. After 10 days of elaboration, strawberry juices showed the first signal of detriment. Samples of deteriorated juice were plated onto YMPG agar medium supplemented with chloramphenicol (1%) (YMPG-C) and aerobically incubated for 48 h at 28°C. Yeast colonies were isolated from streaked YMPG plates. The isolated yeasts were characterized by microscopic and macroscopic morphology; classification in *Saccharomyces* or non-*Saccharomyces* yeast; sporulation assay; pseudomycelia formation; growth at different temperature; formation of amylaceous compounds, sugar fermentation, DNA extraction, RFLP, Microsatellite analysis, amplification partial sequencing of ribosomal RNA genes and DNA sequence analysis. Two different colony morphotypes were obtained which were separated into two groups. All the isolated yeast were non-*Saccharomyces* and amylaceous compounds production was not detected. The major differences between both groups were colonies appearance, cellular shaped, growth at 37 °C, carbon source fermentation and assimilation. The RFLP analysis of the ITS1/NL-4 amplicons from the strainsVRV-1, VRV-4, VRV-5, VRV-7, VRV-8, VRV-11, VRV-14 y VRV-16 (Group I) showed a similar profile, while the fragments from the strains VRV-2, VRV-3, VRV-6, VRV-9, VRV-10, VRV-12, VRV-13, VRV-15, VRV-17 y VRV-18 (Group II) resulted in a different pattern. The microsatellite amplification patterns obtained with primers (GAC)5 and (GTG)5, allowed us to confirm the division in two groups. The sequence analysis of the rDNA fragment obtained from the isolates of Group I showed a close phylogenetic relationship whith *Hanseniaphora osmophila*, whereas the same analysis applied to the Group II showed that isolates yeasts are closely related to *Starmerella bacillaris*. The identification of the isolated yeast could be useful to prevent juice deterioration by controlling these harmful microorganisms.

Código de Resumen: FM-012

Sección: Fisiología Microbiana

Modalidad: Poster

PHYTOSTEROLS FROM WINERY WASTE AS CONTROL AGENT OF CITRIS CANKER

M. J. Rodríguez Vaquero[1], S.M. Sosa Marmol[1], F. Saguir[1].

[1]*CONICET-UNT. Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán..*

mariajo@fbqf.unt.edu.ar

Wine production is one of the most important agricultural activities throughout the world, but generates a great amount of residues. In recent years, authorities look for viable solutions for valorizing waste products. Phytosterols are widely distributed in the vegetal kingdom with biological activities. Citrus canker is a disease affecting citrus species caused by *Xanthomonas citri sp citri.* The aim of this work was the recovery of phytosterols present in winery wastes, the development of techniques to determine the phytosterol concentration in wastes, the investigation of the effect of phytosterols extracted from winery wastes (PEWW) on the viability of *Xanthomonas citri sp citri in vitro* and *in vivo* assaysand the influence on biofilm formation by this bacterium. Results were compared with those obtained with pure phytosterols (B-sitosterol, stigmasterol and stigmastanol) and copper oxychloride, drug used to control the plant illness, but its use is restricted actually. The presence and concentration of phytosterols in waste samples was carried out using t*hin layer chromatography and* gas chromatography-mass spectrometry. The viability of bacteria was investigated *in vitro*, using solid and liquid plant-like medium XVM2 added with PEWW (200ml/L) and inoculated with *X. citri,* and *in vivo* assay inoculating the citric fruit treated with PEWW. Results showed that all waste samples content at least one phytosterols, being B- sitosterol the majority compound. *In vivo* and *in vitro* assays demonstrated that at low concentration, phytosterols extracted from grape skin and grape seeds possess antibacterial activity against *X. citri,* showed a reduction of one log cycle at least, in similar way as the individual phytosterols. The biofilm formation was reduced in presence of all samples. Our results demonstrated that the use winery waste, as a natural source of compounds is a promising environmentally friendlier method to control the presence of *Xanthomonas citri* in citrus.

Código de Resumen: FM-013

Sección: Fisiología Microbiana

Modalidad: Poster

SIDEROPHORE EXPRESSION INCREASED BIOFILM FORMATION IN *Burkholderia contaminans*

P.F. Martina[1 2], D.E. Galeano[1], C.I. Prieto[2], O.M. Yantorno[2], A.M. Bosch[2], J.A. Ferreras[1].

[1] *GRUPO DE INVESTIGACIÓN EN GENÉTICA APLICADA (GIGA), IBS-UNaM-CONICET.* [2]*CENTRO DE INVESTIGACIÓN Y DESARROLLO EN FERMENTACIONES (CINDEFI), UNLP-CONICET.*

pfmartina@hotmail.com

Bacteria belonging to *Burkholderia cepacia* complex (Bcc) are important opportunistic human pathogens that colonize lungs of cystic fibrosis (CF) patients producing long-term respiratory infections. Among Bcc bacteria, *Burkholderia contaminans* is the predominant species recovered from sputum samples of these patients in our country. CF is characterized by the absence of a functional chloride transporter known as cystic fibrosis transmembrane conductance regulator (CFTR) that is normally present in epithelial cell membranes, resulting in multiple organ system impairment. The respiratory tract is one of the most affected systems in CF patients where the defect in ion transport results in accumulation of highly viscous mucus. This environment is precious for the airway microbial colonization. In this context, biofilm formation from colonizing bacteria is considered an important virulence factor. Knowing the elements that stimulate or affect a productive establishment of a biofilm is crucial to learn how to control the infection. In the present investigation, we assessed the influence of siderophore on biofilm formation of *B. contaminans* isolated. Using defined iron limited and iron-rich minimal medium and, we examined the specific impacts of iron-III on biofilm formation for 109 clinical isolates sequentially recovered from sputum samples of CF and non-CF patients and 13 environment samples. We reported that *B. contaminans* is able to attach and grow on abiotic surfaces to form biofilms. Our results show that when the iron concentration is limiting, siderophore biosynthesis increases biofilm formation. Further research is necessary be carried out to address the molecular mechanisms of the iron limitation on siderophore production and biofilm formation in *B. contaminans.*

Código de Resumen: FM-014

Sección: Fisiología Microbiana

Modalidad: Poster

A DIET RICH IN SOYBEAN AGGLUTININ (SBA) ADMINISTERED TO BB CHICKS MODIFIES GUT MICROBIOTA AND BODY WEIGHT GAIN

J.D. Babot[1], E. Argañaraz Martínez[1 2], M.C. Apella[2 3], A. Perez Chaia[2 3].

[1]*CCT-CONICET-TUCUMAN.* [2]*UNIVERSIDAD NACIONAL DE TUCUMAN.* [3]*CERELA-CONICET .*

eloyam@fbqf.unt.edu.ar

The components of broilers diet contain secondary products of plant metabolism, lectins among them, which act as defence mechanism in vegetables. These proteins show remarkable resistance to heat, pH and proteolysis so they can overcome pre-treatments and digestion, and reach the intestine active. Dietary lectins may impair intestinal epithelia development and enzyme digestive activity with the consequent growth depression. Besides, changes in the intestinal microbiota may occur. In Argentina, poultry feeds contain high amount of soybean proteins, SBA among them. Classic diets contain 67-204 µg SBA/g of feed, a concentration that may not exert negative effects on the microbiota. The content of soybean protein on the diet could be higher but is limited by toxic effects attributed to SBA. Thus, the aim of this work was to purify SBA from soybean, elaborate a SBA-rich diet and evaluate its effect on caecal microbiota of BB chicks. To do this, soybean grains were peeled and crushed. Several ratios of grains/water and different $(NH_4)_2SO_4$ concentrations were evaluated to assess the conditions for the highest SBA yield and purity. SBA purity was determined by PAGE, and its titre by hemagglutinating activity. Semipurified SBA was sprayed over a regular diet for broilers to obtain a concentration of 217-354 µg SBA/g of feed. One-day-old BB chicks were daily fed this diet for 13 days (group S). A control group (group C) including birds fed the same diet but without the SBA supplement, was also evaluated. Animals´ weight and feed intake were daily assessed. Chicks were sacrificed before the start of the trial and on days 6 and 13, and caecal content samples were taken to assess microbiota composition by FISH using fluorescent probes, and fermentation products (FP) by HPLC. Birds of group C showed significantly higher weight than those of group S from day 7 on. Caecal microbiota showed predominance of *Lactobacillus* and *Enterococcus* on newly hatched chicks. *Bifidobacterium* population decreased on day 6 in chicks of group S, but recuperated on day 13, whilst it kept relatively constant in birds of group C. *Bacteroides* counts decreased on day 13 for animals of group S, but remained constant in birds of group C. Animals of both groups showed similar FP patterns on day 6 and 13. In conclusion, the higher amount of SBA in the feed led to a decrease in *Bifidobacterium* counts at the first week of chicks' life and the concomitant delay on body weight gain of birds. This suggest that intestinal microbiota of BB chicks may be reinforced with *Bifidobacterium* sp. to counteract the negative effect of a soybean diet with high lectin content.

Código de Resumen: FM-015

Sección: Fisiología Microbiana

Modalidad: Poster

IN VIVO EVALUATION OF A MIXED CULTURE AS PROTECTOR OF THE INTESTINAL EPITHELIUM OF BB CHICKS AGAINST THE NEGATIVE EFFECTS OF SOYBEAN AGGLUTININ (SBA)

J.D. Babot[1], E. Argañaraz Martínez[1 2], M.C. Apella[2 3], A. Perez Chaia[2 3].

[1]*CCT-CONICET-TUCUMAN.* [2]*UNIVERSIDAD NACIONAL DE TUCUMAN.* [3]*CERELA-CONICET.*

eloyam@fbqf.unt.edu.ar

Lectins, glycoproteins with high resistance to heat, proteolysis and pH, are among the components of the ingredients used for the elaboration of broilers feed. These proteins can specifically and reversibly bind to carbohydrates. Once ingested, they interact with superficial carbohydrates expressed on the surface of intestinal epithelial cells affecting epithelial development and digestive enzyme activities, thus delaying the bird growth. SBA lectin is a secondary metabolite of soybean and specifically binds to N-acetyl-galactosamine and/or galactose. *In vitro* capture of SBA by *Bifidobacterium infantis* CRL1395 was previously reported. Thus, the aim of this study was to evaluate the effect of the administration to BB chicks, fed with a diet supplemented with SBA, of a mixed culture constituted by 5 strains (*B. infantis* CRL1395, *Enterococcus faecium* LET 301, *Lactobacillus salivarius* LET 201, *L. reuteri* LET 210 and *Propionibacterium acidipropionici* LET 103) capable of binding different lectins (SBA, Con A and WGA). Towards this end, a combination of the 5 strains was incorporated into the drinking water (10^6-10^7 CFU/mL, each strain) and daily administered for 13 days to one-day-old BB chicks (group TS, n = 20). A control group (CS, n = 20) including BB chicks fed the same diet but without the bacterial mixture in the drinking water, was also evaluated. All birds were fed a diet supplemented with previously purified SBA to reach approximately 217-354 µg SBA/g of feed. Urea and creatinine in chick´s blood plasma, liver weight/body weight and spleen weight/body weight ratios, bacterial translocation to these organs, and the activity of several digestive enzymes were evaluated at days 6 and 13; jejunal mucosa integrity was studied at day 6. Concerning urea and creatinine in blood plasma, organs/body weight ratios and bacterial translocation to liver and spleen, there were no differences between animals of both groups. All chicks showed alterations in jejunal mucosa, nevertheless birds of group TS had higher overall integrity, showing less immune cells infiltration in lamina propria and no increase in cellularity of the epithelium covering the villi. In concordance to this, mucosa of animals from group TS evidenced significantly higher activities of alkaline phosphatase and leucineaminopeptidase than those of group CS. In conclusion, the administration of the mixed culture prevents some negative effects associated to SBA. Nevertheless, the results of this study indicate that greater protection could be reached through the administration of a higher dose of bifidobacteria in the probiotic mixture.

Código de Resumen: FM-016

Sección: Fisiología Microbiana

Modalidad: Poster

ANTIBACTERIAL ACTIVITY OF CIPROFLOXACIN MEDIATED BY OXIDATIVE STRESS IN A CO_2 ATMOSPHERE CONTROLLED

V. Cano Aristizábal[1], M.G. Paraje[2], S. Dukan[3], P.L. Páez[1].

[1]*Dpto. Farmacia. FCQ-UNC. Argentina.* [2]*IMBiV-CONICET. FCEFyN-UNC. Argentina.* [3]*Institut de Microbiologie de la Méditerranée, CNRS. Marseille, France.*

vcano@fcq.unc.edu.ar

Oxidative stress is caused by exposure to reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radical (OH•), which can damage proteins, nucleic acids, and cell membranes. In cells, CO_2 is a main by-product of metabolism. It also constitutes the main physiological pH-buffering system in higher eukaryotic organisms and is required for the growth of many microorganisms. The best known effect of increasing CO_2 concentration is global warming, but large increases in CO_2 concentration (to 1% or 10%) are also known to affect cellular biochemical reactions, leading to an increase in intracellular oxidative stress in human neutrophils, pulmonary inflammation in mouse and increased virulence or bactericidal activities of various pathogenic bacteria. However, physiological concentrations are not of this order of magnitude; hence, the probable, direct effects of CO_2 on living organisms at the predicted concentrations remain unclear. The objective of this work was study the bacterial response to oxidative stress generated by CIP in controlled atmospheres of CO_2. *Escherichia coli* ATCC 25922 was grown aerobically in Luria Bertani (LB) broth, at 37 °C, with shaking at 160 rpm. When the OD_{600} reached 0.5, cells were exposed to various concentrations of CO_2 (50 ppm or 5 %). LB agar plates with and without ciprofloxacin (CIP) 0, 2.56 and 256 µg/mL in the medium were allowed to equilibrate for 20 h at the CO_2 level to be tested. Serial dilutions of cell suspensions in phosphate buffer (0.05 M, pH 7.4) were prepared and *E. coli* cells were spread on LB agar plates and incubated in the presence of either 50 ppm or 5% of CO_2. Colonies were counted after incubation at the CO_2 concentration tested for 16 h at 37 °C. When the antimicrobial activity of CIP was evaluated against *E. coli* in the presence of CO_2, it was observed that the antibiotic activity decreased as the concentration of CO_2 is increased. At concentrations of 5% CO_2, CIP concentration necessary for bactericidal activity is 256 µg/mL. This value is well above the minimum bactericidal concentration of CIP against *E. coli* ATCC 25922. To evaluate the ROS participation in the mechanism of action of CIP, the procedure described previously

was performed in the presence of an inhibitor of the formation of $OH^{\bullet}$, 2,2 dipyridyl, which is an iron chelator and therefore an inhibitor of the Fenton reaction which is a major mechanisms of formation of $OH^{\bullet}$. The effect of CO_2 on oxygen toxicity disappeared in these conditions, providing further evidence for the hypothesis that CO_2 directly exacerbates CIP toxicity mediated by $OH^{\bullet}$. We show here that atmospheric CO_2 modify death rates due to oxidative stress mediated by CIP in *E. coli* in a dose-specific manner. This effect is correlated with a reduction in $OH^{\bullet}$ generated by CIP in a high concentration of CO_2. Thus, CO_2 modify ROS toxicity mediated by CIP.

Código de Resumen: FM-017

Sección: Fisiología Microbiana

Modalidad: Poster

EFFECT OF LUTEOLIN ON THE ANTIBACTERIAL ACTIVITY OF GENTAMICIN IN *Escherichia coli* AND *Staphylococcus aureus*

P.S Bustos[1], P.L. Páez[1], J.L. Cabrera[1], I. Albesa[1], M:G. Ortega[1].

[1] *Dpto. de Farmacia, IMBIV-CONICET. Fac. de Cs. Químicas, UNC, Córdoba, Argentina.*

pbustos@fcq.unc.edu.ar

Our research group has recently started a new line of research focused on the study of natural compounds that neutralize the toxic effects caused by Gentamicin (GEN) that are associated with an oxidative stress increase in human cells. Luteolin, a flavonoid isolated from fruits of *Prosopis strombulifera* var. *strombulifera* with antioxidant properties and scavenger capacity of free radicals, has demonstrated in previous studies of our group, a marked protective effect against the production of reactive oxygen species induced by GEN in human leukocytes. Thus, these results motivated us to study the effect of Luteolin on the activity of this antibiotic, in order to determine if its protective action on human cells can modify the antibacterial activity of GEN in different strains of *Escherichia coli* and *Staphylococcus aureus*. For this, a reference strain of *E. coli* ATCC 25922 and a clinical strain of *E. coli* resistant to GEN, a reference strains of *S. aureus* ATCC 29213 and a clinical strain of *S. aureus* resistant to GEN were used. The tube dilution method was used to determine the Minimum Inhibitory Concentration (MIC) of Luteolin on all strains of *E. coli* and *S. aureus*, while the checkerboard method was used to determine the interaction between Luteolin and Gentamicin employing combinations of these compounds at different concentrations in Mueller-Hinton broth. This way, it was observed that Luteolin submitted a MIC at the maximum concentration tested (125µg/ml) in both reference strains, while in clinical strains of *E. coli* and *S. aureus* resistant to GEN, Luteolin didn't show antibacterial activity. Furthermore, when GEN was combined with Luteolin for the inhibition of *E. coli* ATCC and *S. aureus* ATCC a synergistic effect was observed. In *E. coli* ATCC a synergism was observed ($FICI_{L+G}$ = 0.254) when the concentration of Luteolin was decreased 250 times below its MIC and of the antibiotic 4 times below its individual MIC, while in *S. aureus* ATCC the synergic effect was greater ($FICI_{L+G}$ = 0.133) than *E. coli* ATCC when the concentration of Luteolin decreased 125 times below its MIC and of the antibiotics 8 times below its individual MIC. Regarding the GEN resistant strains of *E. coli* and *S. aureus* the presence of Luteolin didn´t produce changes in the sensitivity of GEN. On this basis we can conclude that Luteolin significantly increases the antibacterial activity of GEN in ATCC strains of *E. coli* and *S. aureus*, while the combination of Luteolin with GEN did not alter the sensitivity of the resistant strains of *E. coli* and *S. aureus*. Therefore, and based on the results above, we can say that Luteolin proved to have a protective effect against oxidative stress induced by GEN in human leukocytes without modifying substantially the antibacterial effect of this drug against resistant strains and strengthening it against reference strains.

Código de Resumen: FM-018

Sección: Fisiología Microbiana

Modalidad: Poster

STUDY OF THE CAPABILITY TO FORM BIOFILMS OF TWO *Yersinia enterocolitica* STRAINS FROM DIFFERENT BIOSEROTYPES

N. Di Marco[1 3], D. Russo[4 5], A. Zorreguieta[4 5], C. Pungitore[1 3], C. Lucero Estrada[2 3].

[1] *Instituto de Investigaciones en Tecnología Química. CONICET, San Luis.* [2] *Instituto Multidisciplinario de Investigaciones Biologicas. CONICET, San Luis.* [3] *Facultad de Quimica, Bioquimica y Farmacia. UNSL, San Luis.* [4] *Instituto de Investigaciones Bioquimicas de Buenos Aires. CONICET, CABA.* [5] *Laboratorio de Microbiología Molecular y Celular. Fundación Instituto Leloir, CABA.*

nataliadimarc@gmail.com

Yersinia enterocolitica (*Ye*) is an important Gram-negative pathogen that is transmitted mainly through contaminated water and food. This bacterium species is classified in 6 biotypes (B) and in more than 57 serotypes (O). Five of the six biotypes (1B and 2 -5) are considered pathogens to human being due to the presence of a virulence plasmid pYV and several chromosomal virulence genes; these strains are confined to the gastrointestinal tract causing enteritis or diarrhea. The biofilm lifestyle of growth confers a protective advantage to bacteria, which is physiologically distinct from the planktonic counterpart of the same species, becoming more resistant to the host defense and adverse environmental conditions. *Ye* is able to synthesized two *N*-acil-homoserin lactones (AHSLs) or autoinducers. Biofilm formation, virulence and antibiotic resistance expression are influenced by *Quorum sensing* via the autoinducers.The aim of this work was to study the ability to form biofilm of two different *Ye* bioserotypes strains. *Y. enterocolitica* CLC001 B1A/O:7,8-8-8,2 is a deficient in pYV (pYV^-) strain and was isolated from food, while *Y. enterocolitica* WAP B1B/O:8, is a reference strain that carries the virulence plasmid (pYV^+) and was isolated from human feces. Biofilm formation on abiotic surfaces was assayed by the crystal violet technique using 96 wells polystyrene (PE) plates or glass tubes. Strains were incubated in TSB medium added with 0.25% glucose at 24°C. At different times, the planktonic and sessile growths were measured at OD 655 nm and 550 nm, respectively. After 24 h, *Ye* CLC001 strain developed stronger biofilms both on PE and glass surfaces in comparison with WAP strain (3.13 ± 0.27 vs 2.03 ± 0.21 ($p<0.01$) on PE and 1.96 ± 0.21 vs 1.31 ± 0.18 ($p<0.05$) on glass surfaces respectively). Then, the ability to produce lactones was quantified with the biosensor *Chromobacterium violaceum* which synthesizes violacein in presence of exogenous autoinducers. Co- cultures were performed in TSB for 24 h at 25ºC in shaking conditions. After the incubation period, the amount of released violacein was quantified at OD 585 nm. No differences in *Ye* AHSLs production were observed between both strains. Furthermore, the biofilm developed after 24h on a glass surface was observed by Laser Scanning Confocal Microscopy (LSCM). Briefly, *Ye* biofilms were fixed with paraformaldehyde and stained using propidium iodide. Both *Ye* strains were able to firmly attach to the glass chambered slide, establishing bacterial aggregates or microcolonies. Our results suggest that both pathogenic and non pathogenic *Y. enterocolitica* strains have the capacity to form biofilm onto different abiotic surfaces. Thus it could be considered as an alternative virulence trait.

Código de Resumen: FM-019

Sección: Fisiología Microbiana

Modalidad: Poster

BIOSYNTHESIS OPTIMIZATION OF TRANSITION METAL NANOPARTICLES

K. Crespo Andrada[1], J. Baronetti[1], M. Quinteros[2], M.A. da Silva [1], P.L. Páez [2], M.G. Paraje [1].

[1] IMBIV-CONICET- Fac. Ciencias Exactas Físicas y Naturales- Universidad Nacional de Córdoba. [2] Fac. Ciencias Químicas- Universidad Nacional de Córdoba.

karinacrespo@fcq.unc.edu.ar

The Metal nanoparticles (Nps) have gained interest in various areas of science and technology. Microorganisms, such as bacteria and fungi, have been exploited to synthesize these particles. Nonetheless, bacteria are preferred for Nps production over eukaryotic microorganisms due to easy of handling and genetic manipulation. The aim of this study was to optimize the intra and extracellular biosynthesis (IB and EB) of iron and zinc metallic Nps using bacterial strains such as *Escherichia coli* and *Pseudomonas aeruginosa* by modifying several physical- chemical parameters. *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were grown in Tryptic Soy Broth (TSB) for 18 h, at 37 °C kept under continuous agitation in an orbital shaker (200 rpm). For IB, 15 mL of this culture were mixed with 1.5 mL of the different metallic solution [FeSO4 (II), FeCl3 (III), Fe Citrate (III)), ZnSO4 (II), $ZnCl_2$ (II)] and incubated under shaking. Then, the solution was sonicated at 80W for 30 minutes and centrifuged at 8500 rpm for 5 min. For EB, the culture was previously centrifuged before mixing with the saline solutions and the sonication procedure was avoided. Different conditions of salt concentration (1 and 10 mM), incubation time (24, 48 and 72 h), temperature (37-50 °C) and pH (7 and 9) were assayed. Nps production was monitored by UV-visible in 200-800 nm range, observing the surface plasmon resonance (SPR). For *E. coli*, Fe Nps were formed both intra- and extracellularly at 1mM. However this production was not observed at 10 mM. The SPR peak was observed at 275 nm. The obtained solution presented a dark brown color. For *P. aeruginosa,* iron Nps were obtained only extracellularly at 1mM. Nps formation was detected with Fe Citrate, $FeSO_4$ in *E. coli* and FeCl3 in *P. aeruginosa* by increasing the pH to 9. The incubation times were from 24 to 72 h and not significant differences were observed in the SPR peak. In *E. coli*, a peak was observed at 275 nm both for intra- and extracellular synthesis with $ZnCl_2$. The solution had a white color. With $ZnSO_4$, the EB was only observed. In contrast, Zn Nps were synthesized extracellularly with both salts in *P. aeruginosa*, the peak was observed at 425nm and the solution presented a greenish-yellow color. Several parameters for the Fe and Zn Nps biosynthesis were optimized in *E. coli* and *P. aeruginosa* strains. The optimal conditions were obtained with *P. aeruginosa* at EB with $FeCl_3$ and $ZnSO_4$ 1 mM at 37 °C for 48 h. Future perspective: Potential biological activity of biosynthesized Nps will be evaluated by determining the antimicrobial and anticoagulant activity as well as application of bioremediation technology to contaminated soil and water systems.

Sección: Fisiología Microbiana

Modalidad: Poster

PHOTODYNAMIC INACTIVATION OF *Klebsiella pneumoniae* AND *Escherichia coli* BACTERIA USING TWO SYMMETRICAL SUBSTITUTED ZINC (II) PHTHALOCYANINES

R. Clementi[2], M. Mirettia[1], M. Baumgartnera[1], T. Tempesti[1].

[1] *INFIQC (CONICET). Dpto. Quimica Orgánica, FCQ - UNC, Ciudad Universitaria, Córdoba, Argentina..* [2] *Departamento de Microbiología, Hospital Policlínico Policial,Córdoba,Argentina .*

romina_clementi@hotmail.com

The widespread occurrence of antibiotic-resistant microorganisms makes clear the need for new antimicrobial treatments. Photodynamic therapy (PDT) as a method utilizes visible or ultraviolet light in combination with a photosensitizing agent to induce several phototoxic reactions, which results in cell damage or death. Photodynamic reaction involves a light absorption by a photosensitizer to excite the molecule to the excited singlet state. This excited state undergoes intersystem crossing to the long-lived triplet state, which can react with molecular oxygen inducing reactive species such as singlet oxygen, superoxide, and radicals. These reactive species can oxidize the surrounding bioorganic molecules leading to cell death. Phthalocyanines, which are characterized with far red wavelength absorption (>670 nm), long triplet life time (~1 ms), and high quantum yields of singlet oxygen generation (>0.2), have been studied as drugs in microbial photodynamic inactivation. The positively charged phthalocyanines can effectively photoinactivate both, gram-positive and gram-negative bacteria. The gram-positive are more susceptible to photoinactivation than gramnegative due to the morphological characteristics of their membranes. *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria are an emerging group of highly drug-resistant bacteria causing infections associated with significant morbidity and mortality. The photodynamic activity of two new cationic phthalocyanines (TMZnPc and TMAZnPc) and commercial ZnPc, was evaluated in KPC-producing bacteria and *Escherichia coli* ATCC 25922 bacteria. Both KPC-producing and *E. coli* ATCC 25922 were incubated with photosensitizers for 30 minutes in the dark at 37 °C. The photoinactivation of the microorganism was determinated for different irradiation times (10, 15 and 30 minutes). The TMZnPC phthalocyanine showed higher antibacterial properties than TMAZnPc phthalocyanine and ZnPC phtalocyanine for KPC-producing bacteria. The results was 3.9 log reduction (i.e. 99.9% cell killing), 2.6 log reduction (i.e. 99.8% cell killing) and 1.9 log reduction (i.e. 99.2% cell killing) respectively. For *E. coli* ATCC 25922 bacteria the results was 2.4 log reduction (i.e. 99. 7% cell killing) to ZnPc, 6.1 log reduction (i.e. 100% cell killing) to TMAZnPc and 7.1 log reduction (i.e. 100% cell killing) to TMZnPc. These results suggest that the zinc phthalocyanine cationic presented very interesting properties to be used in future as a photodynamic therapy in inactivation of bacteria-resistant and sensitive.

Sección: Fisiología Microbiana

Modalidad: Poster

FUNCTIONAL CHARACTERIZATION OF THE UBIQUITIN-DOMAIN PROTEIN Nmag_2608 OF THE HALOALKALIPHILIC ARCHAEON *Natrialba magadii*

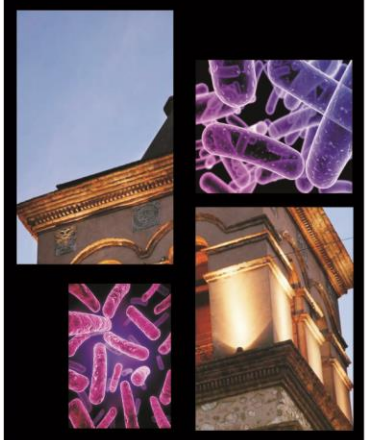
J.I. Solchaga[1], M.V. Ordóñez[2], D. Nercessian[1].

[1] *Instituto de Investigaciones Biológicas.* [2] *Instituto de Investigaciones en Ciencia y Tecnología de Materiales.*

juani_solchaga86@yahoo.com.ar

Ubiquitin is a small and extremely well conserved protein among the Eukarya domain but absent in the other two domains of life. It is transiently attached to different proteins where It regulates several cellular functions. Ubiquitin (Ub) displays a stable β-grasp fold and exhibit a conserved di-glycine motif in its C-terminus, essential to exert its function. Although Ub is restricted to eukaryotes, a superfamily of ubiquitin-like (Ubl) proteins is known to be present in all domains of life. In general, this group of proteins do not share high sequence identity with Ub, however, they display the β-grasp fold and often have the C-terminal di-glycine motif. The ubiquitin fold has also been found in larger multi domain proteins called Ubiquitin-like Domain-containing proteins (Ulds). They do not bind covalently with other proteins, however they can form non-covalent interactions with proteins containing either ubiquitin-associated or ubiquitin-like binding domains. Nmag_2608 is an Uld protein of the haloalkaliphilic archaeon *Natrialba magadii*. It was previously identified and characterizedin our laboratory as an extracellular protein with an ubiquitin-like domain, called P400. This protein is expressed and secreted to the extracellular medium specifically in early stationary phase. The aim of this work was to identify the physiological role of Nmag_2608 and the importance of P400 domain. For this, the ubiquitin domain P400 was heterologously expressed in E. coli and its purification was optimized. Given the extracellular localization of the protein, the possibility of an antimicrobial activity by P400r was evaluated. The detection of this activity was analyzed by growth inhibition of different microorganisms in liquid medium under the presence of P400. Results

shown here describethis analysis performed with 8 different halophilic microorganisms and two bacterial strains. We found that P400 exhibit antimicrobial activity against a diverse range of microorganism belonging to the haloarchaea family. None of the bacterial strains tested was sensible to the presence of P400. Also, our results indicate different degree of inhibition within haloarchaea species. The reason for this difference will be further analyzed, but they may be due to a more specific action of the molecule against microorganisms more commonly found in the same environment that *Natrialba magadii.* These results suggest that extracellular domain P400 of Nmag_2608 would act as antimicrobial peptide, regulating the competence with other microorganisms from its natural environment and giving *Natrialba magadii* an ecological advantage. This is the first report of an Ubiquitin-like domain protein with antimicrobial activity.



INTERACCIONES PROCARIOTA-EUCARIOTA

Código de Resumen: IN-001

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

ROLE OF THE *Serratia marcescens* HEMOLYSIN ShlA IN THE INVASION OF EPITHELIAL CELLS

G. Di Venanzio[1], E. García Véscovi[1].

[1] *Instituto de Biología Molecular y Celular de Rosario (IBR) CONICET-UNR.*

divenanzio@ibr-conicet.gov.ar

Serratia marcescens is an opportunistic pathogen important for public health. However, little is known about the factors and mechanisms that contribute to *Serratia* pathogenesis. The ShlA hemolysin is one of the major virulence factors and is responsible for the cytotoxic effect on erythrocytes and cultured cells. A hemolysin (*shlBA*) mutant strain showed a great increase of intracellular CFUs at late times post invasion in epithelial cells. Nevertheless and in contrast to the wt strain, no mutant bacteria were detected in the culture supernatant when gentamicin was eliminated from the assay at late times p.i. In addition, over 60% of the wt *Serratia*-containing vacuoles (SeCV) co-localize with galectin 8, a known marker of vacuolar damage, in a hemolysin-dependent manner. However, very few bacteria were detected in the cytoplasm of infected cells. The dissemination process seems not to involve the permeabilization of the plasmatic membrane. Moreover, the actin cytoskeleton seems to play an important role during this process, evidenced by the co-localization of the mutant strain with actin filaments, at late times p.i.. On the other hand, the wt and mutant SeCV recruit VAMP7, a key molecule of the vesicle fusion machinery, at late times p.i. In addition, a functional VAMP7 is necessary to allow the progression of the infection process, as evidenced by the lack of SeCV in cells expressing a dominant negative mutant of VAMP7. Based on these findings, we propose that a) ShlA is involved in the damage of the SeCV, allowing the escape, and thus the dissemination ofthe bacteria from the host celland b) VAMP7 is required for the progression of the bacterial infection of both, wt and *shlBA* mutant strains.

Código de Resumen: IN-002

Sección: Interacciones Procariota - Eucariota

Modalidad: Oral

FUNCTIONAL ANALYSIS AND TRANSCRIPTIONAL REGULATION OF THE O-OLIGOSACCHARYLTRANSFERASE OF *Ralstonia solanacearum*

P. Vicino[1], E.G. Orellano[1], M.L. Tondo[1].

[1] *IBR-CONICET, FBioyF-UNR. Rosario, Argentina.*

tondo@ibr-conicet.gov.ar

Protein O-glycosylation has become clearly established as a common posttranslational modification in bacteria. Glycans are often used to decorate several proteins at the bacterial surface such as the structural components of flagella and pili. Glycoproteins play diverse roles including adhesion, motility, immune evasion and host colonization. One of the proposed mechanisms of protein glycosylation in bacteria involves an oligosaccharyltransferase (OTase) that transfer short preassembled oligosaccharides to selected residues in the acceptor proteins. Although some OTases are specific to the pilin protein, others are classified as general OTases that target structurally and functionally diverse groups of membrane-associated proteins. *Ralstonia solanacearum* is a Gram-negative soil-borne β-proteobacterium which is the causal agent of bacterial wilting, one of the most devastating plant diseases in the world. The strong impact of this pathogen results from its worldwide geographical distribution and its wide host range; it affects more than 200 plant species including economically important food crops. In previous studies we have identified the product of the *RSc0559* gene of *R. solanacearum* GMI1000 as a functional OTase, capable of transferring oligosaccharides to the pilin protein in an *in vivo* system in *Escherichia coli* cells. In the present work, an OTase deficient mutant generated by a clean deletion of the *RSc0559* gene from the parental *R. solanacearum* GMI1000 strain was physiologically characterized. The OTase mutant exhibited similar growth kinetics than the wild-type strain in liquid BG medium and a normal colony morphology when grown on triphenyltetrazolium-supplemented agar plates. We found that the OTase mutant do not exhibit twitching motility and has an impaired ability to form biofilms on glass surfaces compared to the wild type strain. However, both strains exhibited comparable swimming motilities in soft agar plates. In order to assess the significance of the OTase for bacterial virulence, the interaction with host and non-host plants was also analyzed. During the interaction with tomato (host) plants the mutant strain exhibited a drastic reduction of virulence, with no development of typical wilting symptoms at 20 days post-infection. On the other hand, the OTase mutant was able to elicit the typical hypersensitive response when inoculated in tobacco and cotton (non-host) plants. Finally, we constructed transcriptional fusions between the OTase promoter and the *lacZ* gene, and we measured the β-galactosidase activity in mutant strains for different transcriptional regulators. By this approach we were able to determine that the expression of the OTase gene is controlled by main regulators of pathogenicity determinants of *R. solanacearum.*

Código de Resumen: IN-003

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

ROLE OF *Bradyrhizobium* sp. SEMIA 6144 NOD FACTORS IN THE PROTECTION OF PEANUT PLANTS AGAINST THE PHYTOPATHOGEN *Sclerotium rolfsii*

M.S. Figueredo [1], F. Ibañez[1], A. Fabra[1].

[1] *Universidad Nacional de Río Cuarto.*

solefigueredo@hotmail.com

Plants possess highly sensitive perception systems by which microbial signal molecules are recognized. The earliest event in the establishment of the rhizobia–legume association is a highly specific exchange of signal molecules. Plant roots exude signal molecules, mainly flavonoids, which induce bacterial *nod* gene expression, resulting in synthesis of bacteria-to-plant signal molecules, called Nod factors. It is interesting to note that the signaling occurring at the beginning of the N_2 fixation symbiosis involves exchange of flavonoides and chitin based compounds because the plants have a well-characterized ability to detect chitin fragments, elicitors of plant defense reaction and constituents of fungal cell walls, and, in response, to produce phytoalexins, often flavonoides. Previous studies in our laboratory demonstrated that the peanut symbiont *Bradyrhizobium* sp. SEMIA 6144 protect peanut plants against *Sclerotium rolfsii* through a mechanism that seems to involve the plant's systemic defense response. The aim of this study was to evaluate whether the *Bradyrhizobium* sp. SEMIA 6144 Nod factors are involved in the protection of peanut plants against *S. rolfsii*. In order to grow plants, we used a system consisting of two pots with sterile vermiculate put one above the other and connected by a hole made in the base of the upper pots. In this container, pregerminated seed peanut was placed so that its radicle was introduced into the lower pot (larger diameter) through the communicating hole. Two days after planting, the radicle was inoculated with the bacterial strains *Bradyrhizobium* sp SEMIA6144 or an isogenic *Bradyrhizobium* sp strain (V2) unable to synthesize Nod factors (Ibáñez and Fabra 2011). A week later, the plants were challenged with the pathogen putting a wheat seed infested with *S. rolfsii* mycelia. At 30 days post-pathogen challenge, disease symptoms were recorded and plants were harvested to determine their shoot and root dry weights. At 30 days post-pathogen challenge, the disease incidence was higher in plant inoculated with the mutant strain than in plants inoculated with *Bradyrhizobium* sp. SEMIA 6144, although it was lower than those that were only pathogen challenged. Furthermore, the shoot and root dry weights were in agreement with this result. Considering the results obtained, we concluded that the rhizobial Nod factors contribute to the protection of peanut plants against *S. rolfsii* by the microsmbiont *Bradyrhizobium* sp. SEMIA 6144.

Código de Resumen: IN-004

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

IMPORTANCE OF BACTERIA AND PLANT ANTIOXIDANT SYSTEM IN PEANUT-*Bradyrhizobium* sp. INTERACTION

V. Muñoz[1], F. Ibañez[1], A. Fabra[1].

[1] *Universidad Nacional de Río Cuarto, Facultad de Ciencias Exactas, Fisico-Químicas y Naturales, Depar.*

vaninamta@gmail.com

Peanut is a leguminous plant of great agroeconomic importance, capable of establishing a nitrogen-fixing symbiotic association with bacteria of the genus *Bradyrhizobium*. We recently demonstrated for the first time that the early events of the symbiotic interaction peanut-*Bradyrhizobium* sp. trigger an increase in reactive oxygen species (ROS) production (represented exclusively by a higher H_2O_2 content), which is a prerequisite for the efficient development of root nodules. We also demonstrated that Nod Factor (NF) modulates this response by enhancing the plant antioxidant machinery, specifically the activity of enzymes catalase (CAT) and total peroxidase (PX), contributing to the creation of adequate conditions for symbiosis development. The role of ROS is now widely accepted as well as the importance of plant and bacterial antioxidant system in signaling processes during the establishment of the symbiosis between rhizobia and legumes infected intarcellularly. However, in less characterized processes of rhizobial infection, such as intercellular invasion that occurs in peanut-*Bradyrhizobium* sp. interaction, the role of antioxidant system of both partners has not been elucidated yet. Thus, the aims of this study were a) to evaluate whether the increase in PX activity is due to the "*de novo*" induction of its synthesis b) to determine the requirement of the antioxidant system of *Bradyrhizobium* sp. SEMIA 6144 in the symbiotic interaction with peanut. In order to accomplish the first aim, *prx1* geneexpression, which encodes a peroxidase, was analyzed using qReal-Time PCR in peanut plants inoculated with *Bradyrhizobium* sp. SEMIA 6144 or *Bradyrhizobium* sp. V_2 (strain impaired in NF production). The results indicate that NF from bradirizobios peanut symbionts modulate oxidative burst occurring in the early stages of this interaction by increasing PX

activity, which is caused by the "*de novo*" induction of its synthesis. In order to achieve the second aim, PX activity from *Bradyrhizobium* sp. SEMIA 6144 was inhibited by adding salicylhydroxamic acid (SHAM) to the bacterial culture medium, and then its symbiotic phenotype was analyzed. SHAM is a specific, potent and irreversible inhibitor of enzymes with peroxidase activity. From the results obtained, it can be inferred that the antioxidant system of bradirizobios peanut symbionts, particularly the activity of PX, is important in order to counteract the oxidative burst occurring during the early stages of the peanut-*Bradyrhizobium* sp. interaction. Taken together, our results provide new insights into the role of plant and bacterial antioxidant system in the development of the symbiotic interaction established in legumes infected in an intercellular way.

Código de Resumen: IN-005

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

COLONIZATION PATTERNS OF THE N_2-FIXING BACTERIA *Burkholderia tropica* IN BARLEY

S.S. García[1], P.R. Bernabeu[1], J.L. Boiardi[1], M.F. Luna[1 2].

[1] *CINDEFI-CONICET, Facultad de Ciencias Exactas-UNLP, 50 e/115 y 116. La Plata, Bs. As.* [2] *CIC-PBA, La Plata, Bs. As.*

s.soledad.garcia@gmail.com

The use of biofertilizer and biocontrol organisms is being considered as an alternative or a supplementary way of reducing the use of chemicals in agriculture. Plant growth promoting bacteria (PGPB) are a group of microorganisms able to confer beneficial effects on plant growth and development, without causing damage neither to the host nor the environment. These microorganisms stimulate plant growth as a consequence of nitrogen fixation, production of phytohormones, enhancement of mineral availability and biocontrol of phytopathogens.PGPB have to colonize and grow on or around the roots for the establishment of an effective plant-microbe interaction. After this necessary step, some of them are able to enter roots by different mechanisms and establish endophytic populations. The study of colonization process and patterns will give a more detailed insight into plant bacterial interactions and is a critical prerequisite for the development of effective inoculants.Several diazotrophic plant-associated*Burkholderia*species have been described as promising candidates for biotechnological applications. In order to find PGPB for grasses, the aim of this work is to characterize the colonization pattern of theN_2-fixing bacteria*B. tropica*MTo-293 containing the marker gene Green Fluorescent Protein when barley seeds are inoculated with this bacterium and grown under gnotobiotic conditions.Colonization was monitored by plating bacterial suspensions from homogenized tissues (CFU/g fresh weight) and by microscopic localization of bacteria.*B. tropica*could be isolated from root surfaces (up to 12 log CFU/g fresh weight), from surface-disinfected and disrupted roots (4.0 log CFU/g fresh weight) and also from stems of inoculated barley plants. Microscopic assays showed a wide root surface colonization by*B. tropica*in these plants. This is in accordance with the high rhizoplane population densities found in all plants tested that are higher compared to*B. tropica*surface populations in other inoculated grasses. Microscopic studies showed colonizing bacteria on root hairs, lateral root emergence sites and root surfaces.These results show that inoculation of barley seeds with*B. tropica*led to an extensive root colonization of barley plants. Moreover, it is able to colonize other plants than its original host and also establish stable associations, at least under our experimental conditions

Código de Resumen: IN-006

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

CHANGES IN THE PRODUCTION OF MOLECULAR SIGNALS INVOLVED IN THE RHIZOBIAL COLONIZATION OF LEGUMES ROOTS IN PLANTS INOCULATED WITH A MICROBIAL CONSORTIUM

M.L. Tonelli[1], C. Magallanes Noguera[1], A. Fabra[1].

[1] *Universidad Nacional de Río Cuarto.*

mtonelli@exa.unrc.edu.ar

In previous studies we demonstrated that in peanut and soybean plants inoculated with the microbial consortium composed by a biocontrol agent (*Bacillus*sp. CHEP5) and a microsymbiont (*Bradyrhizobium*sp. SEMIA 6144 or *Bradyrhizobium japonicum* E109) in presence or absence of *Sclerotium rolfsii* and *Cercospora sojina*, respectively, the symbiotic behavior of both microsymbionts was improved. Moreover, the disease incidence or severity caused by the fungal pathogens was reduced when plants were inoculated simultaneously with the microsymbiont and the biocontrol bacteria.Taking into account these

results, the objectives of this work were a) to determine changes in the composition of flavonoids secreted by roots when the legumes grow in presence of fungal pathogens and beneficial bacteria, b) to evaluate the ability of bacterial mixed cultures to form biofilms.The flavonoids profiles from the legume root exudates were determined by HPLC. Preliminary results indicated differences in peanut root exudates composition, revealing an important production of daidzein and crysin in control plants. Meanwhile, in root exudates from plants inoculated with the microsymbiont or the microbial consortium it was detected the production of genistein. In root exudates from soybean plants challenged with the pathogen it was detected the production of crysin and luteolin, while in those inoculated only with the microsymbiont or the microbial consortium it was identified crysin.On the other hand, results obtained from the*in Vitro*biofilm formation assay revealed that the bacterial consortium showed a greater biofilm formation index compared to pure microsymbiont culture. However, the biofilm formation index of*Bacillus*sp. CHEP5 growing in pure culture was higher than in mixed cultures.From these results it is suggested that legumes flavonoids production is affected by the presence of the microbial consortia. Moreover, the ability to form biofilms appears to be increased in bacterial mixed cultures, compared to microsymbionts pure cultures. Since the production of biofilm confers a greater capability to colonize plant roots, this could turn in higher root microsymbiont colonization, explaining the improved symbiotic behavior under this condition.

Código de Resumen: IN-007

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

DEGRADATION OF PHENOLIC COMPOUNDS BY *Xanthomonas citri* subsp. *citri*: VIRULENCE, ADAPTATION AND ENVIRONMENTAL IMPLICATIONS

I. Kraiselburd[1], S. Beltramino [2], R. Assis [3], L. Moreira[3], E.G. Orellano [1].

[1] IBR-CONICET, FCBF-UNR, Rosario Argentina. [2] FCBF-UNR Rosario, Argentina . [3] DECBI y NUPEB-Universidade Federal de Ouro Preto, Ouro Preto, Brasil.

kraiselburd@ibr-conicet.gov.ar

Plants are constantly exposed to pathogens and they possess a complex set of defense responses to prevent the invasion and spreading of pathogenic microorganisms. Virulent pathogens modulate host processes in order to delay or counteract these defense responses. The production of phenolic compounds at the site of invasion plays an important role in plant innate defense as they can cause deleterious effects on the bacterial cell structure. *Xanthomonas citri* subsp. *citri* (Xcc) is the bacterium responsible for citrus canker, a disease that affects all commercial varieties of Citrus. The Xcc genome is completely sequenced and it includes several genes related to the degradation of phenolic compounds. In this work we present a study of the catabolic pathways of Xcc, potentially involved in the degradation of phenolic compounds. First we performed an *in silico* analysis of Xcc genome sequence using the Kyoto Encyclopedia of Genes and Genomes database, where we identified 98 genes associated with transport and degradation of phenolic compounds. A bioinformatic compilation and evaluation of these data allowed us to find the complete degradation route for vanillate, 4-hydroxybenzaldehyde, 4-hydroxybenzoate and 3,4-dihydroxybenzoate. We thus postulated that Xcc is able to detoxify these compounds and, as many of the pathways generate intermediates of the tricarboxylic acid cycle, they may act as an alternative source of carbon for the bacterial energy metabolism. In order to demonstrate the functionality of these routes and to evaluate the ability of Xcc to use these compounds as a carbon source, bacterial cells were grown in a minimal medium supplemented with 2 mM vanillate or 3,4-dihydroxybenzoate. Controls were performed in minimal medium supplemented with glucose. Bacterial populations were evaluated by the optical density at 600 nm and by analyzing the number of colony forming units in plates containing rich medium. Moreover, we performed a transcriptional evaluation of Xcc genes encoding central enzymes of these catabolic pathways by RT-PCR. Finally, to evaluate the role of the degradation of phenolic compounds in the bacterial virulence, modified strains of Xcc were generated, deficient in the genes encoding dioxigenase enzymes potentially involved in the degradation of these compounds. The presence and functionality of the above mentioned catabolic pathways is probably associated to the bacterial ability to detoxify the phenolic compounds released during the plant defense response, favoring the successful colonization of the host tissue. Gaining knowledge of these adaptive pathways is crucial to propose methods to combat pathogens. Moreover, as the degradation of phenolic compounds is carried out only by a reduced number of microorganisms expressing specific enzymes, the ability of Xcc to use these compounds as a carbon source represents an opportunity for the development of biotechnological strategies for the degradation of xenobiotics.

Código de Resumen: IN-008

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

ROLE OF PHYTOCHROMES FROM *Pseudomonas syringae* pv. *tomato* DC3000 IN THE BACTERIAL PHYSIOLOGY AND DURING NON-HOST PLANT-PATHOGEN INTERACTION

L. Moyano[1], A. Carrau[1], S. Petrocelli[1], W. Gärtner[2], E.G. Orellano[1].

[1] *Instituto de Biología Molecular y Celular de Rosario. IBR-CONICET-UNR, Rosario.* [2] *Max-Planck-Institute for Chemical Energy Conversion, Mülheim, Germany.*

moyano@ibr-conicet.gov.ar

All living organisms have evolved the ability to perceive and respond to light of different wavelengths whithin the visible spectrum. The light perception is achieved by photoreceptor proteins. The bacterial phytochrome (Bph) family possesses a linear tetrapyrrole (bilin) as chromophore. These photoreceptors can be photoconverted between red-absorbing (Pr) and far-red-absorbing (Pfr) states. Recent studies revealed the participation of these photoreceptors in bacterial virulence of plant pathogens, such as *Xanthomonas citri* subsp. *citri*, *Agrobacterium tumefaciens* and *Pseudomonas syringae* pv. *tomato*. *Pseudomonas syringa*e pv. *tomato* DC3000 (*Psto*) affects economically important plant species. The *Psto* genome contains two genes encoding red/far red light photoreceptors (*Bph1: PSPTO_1902* and *Bph2: PSPTO_2652*). The aim of this work was to study the role of *Psto* phytochromes in the bacterial physiology and during the interaction with non-host tobacco plants (*Nicotiana tabacum* cv. Petit Havana). For that purpose, we have constructed the Psto phytochromes knockout mutants: Δ*phy1* and Δ*phy2* and the double mutant strain Δ*phy1-phy2.* We also constructed the complemented strains cΔ*phy1* and cΔ*phy2.* Bacterial motility, biofilm formation, and type II secretion system were evaluated in these strains under different light conditions, demonstrating that the light and *Psto* phytochromes are implicated in the control of these features. Finally, we analyzed the interaction of *Psto* mutants and wild type strains in leaves of tobacco plants in different light treatments. The infiltration with the Δ*phy1* and Δ*phy2* strains caused a hypersensitive response in both white light and darkness. However, when the infiltrated tobacco plants were exposed to red light, HR symptoms were not observed. These results suggest that *Psto* phytochromes play a light-dependent role during non-host interactions.

Código de Resumen: IN-009

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

ROLE OF LIGHT IN THE PHYSIOLOGY AND VIRULENCE OF *Xanthomonas citri* subsp*. citri*

A. Carrau[1], L. Moyano[1], J. Tano[1], S. Petrocelli[1], E.G. Orellano[1].

[1] *Instituto de Biología Molecular y Celular de Rosario. IBR-CONICET-UNR, Rosario.*

moyano@ibr-conicet.gov.ar

Xanthomonas citri subsp. *citri* (*Xcc*) is a hemibiotrophic phytopathogen responsible for the citrus cancer, one of the most severe disease that affect citrus crops worldwide. Light is an important environmental signal for almost all living organisms. Light perception is achieved by photoreceptor proteins, which trigger the signal transduction cascades that regulate the light responses. The BLUF (Blue Light sensing Using Flavins) photoreceptors are flavin-binding proteins using FAD (Flavin adenine dinucleotide) as chromophore. In the *Xcc* genome there are two genes encoding putative BLUFs photoreceptors: Xcc2120 (*Bluf1*) and Xcc3278 (*Bluf2*). The aim of this work was to study the role of BLUF1 and BLUF2 photoreceptors in the bacterial physiology and in the pathogenicity process. We analyzed the expression levels of these genes by RT-PCR from bacterial cell suspensions under different light conditions. We found that the expression level of *Bluf2* gene was significantly higher than *Bluf1* gene, either under blue light or dark conditions. These results suggest that BLUF2 is a functional photoreceptor. Therefore, we decided to construct a mutant strain in this gene named *∆Bluf2*. We observed that the *Bluf2* gene is involved in bacterial morphology, in bacterial motility (swimming and swarming), and in the biofilm formation in a light dependent way. We also evaluate the role of this gene during the pathogenicity process. Upon the interaction with *Citrus sinensis* host plants, the *∆Bluf2* mutant strain showed a different infection pattern as compared with the wild type strain. Our results suggest that *Bluf2* is a functional gene and that would be involved in the bacterial physiology and in the pathogenicity process

Código de Resumen: IN-010

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

BIFIDOBACTERIA INCREASE PHAGOCYTIC ACTIVITY OF THP-1 CELLS

S.E. Assad[1 2], I.S. Rolny[2], J. Minnaard[1], P.F. Perez[1 2].

[1] *CIDCA,CONICET/CCT LA PLATA.* [2] *Universidad Nacional de La Plata.*

sabriassad@gmail.com

Bifidobacteria are potentially probiotic bacteria which colonize the intestine of infants and adults. One of the key steps in the immunomodulatory effects is the interaction of bacteria with phagocytic cells. Previous results showed that the interaction of bifidobacteria with cultured phagocytic cells is strain dependent and higher ratio of phagocytic cells internalizes *Bifidobacterium bifidum* CIDCA 5310 as compared with *B. adolescentis* CIDCA 5317. The present study aimed at evaluating the effect of these strains on the phagocytic activity of THP1 cells by using latex beads as a model of abiotic particles. Monocytic THP1 cells were differentiated with phorbol myristate acetate (PMA) 200 nm in DMEM (10% fetal bovine serum) for 3 days at 37°Cin 5% CO_2 atmosphere.*B. bifidum* CIDCA 5310 (hydrophobic strain) and *B. adolescentis* CIDCA 5317 (non-hydrophobic strain) were cultured (48h; 37°C) in MRS broth in anaerobic conditions. Heat treated (121°C - 15 min), UV treated (30 min) and untreated bacteria were suspended in DMEM and incubated with FITC-labeled latex beads and THP1 cells at multiplicity of infection (MOI)=10 bacteria/cell for 1h at 37°C 5% CO_2. Different beads/monocyte ratios (BM) were used. Association (phagocytosed + adhered beads) and phagocytosis were evaluated by flow cytometry by means of the uptake index (UI) = FL1(+) cells x mean fluorescence intensity. For quenching of non-internalized beads trypan blue was used. In addition, confocal laser microscopy was performed to gain further insight on the interaction of beads with THP1 cells. Beads uptake at BM=10 was significantly increased ($p<0.05$) in the presence of viable strain CIDCA 5310 (MOI=10) (UI: 1807 ± 265.41) as compared with controls without bifidobacteria (UI: 1007 ± 155.43). No effects were observed with viable strain CIDCA 5317 (UI: 1177 ± 131.62) nor in the presence of heat or UV-killed bacteria. In contrast, no changes in values of association were observed in the presence of viable bifidobacteria; i. e. CIDCA 5310 strain (UI: 3192 ± 632.22), CIDCA 5317 strain (UI: 2736 ± 412.76) and controls (UI: 3238 ± 379.22). At lower beads/monocytes ratios (BM=5) values of UI for association and uptake diminished 5 and 3 times respectively as compared with those for BM=10. In these conditions, the ability of strain CIDCA 5310 to increase bead uptake remained unchanged. Analysis of frequency distribution by confocal laser microscopy revealed that the most frequent number of beads internalized per cell was 1 but cells with 2, 3 and 4 internalized beads were also found. Ratio of cells with 1 or 2 internalized beads was higher in the presence of strain CIDCA 5310 as compared with controls. Our results suggest that bifidobacteria can modify the activity of professional phagocytic cells in a strain dependent manner. These findings could be related to the ability of modulate host´s response of these potentially probiotic bacteria.

Código de Resumen: IN-011

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

Herbaspirillum hutiense ISOLATED FROM ALGAE AS A WHEAT GROWTH PROMOTING BACTERIUM

J. Inchaurrondo[1], M. Do Nascimento[2 3], A. Arruebarrena Di Palma[3], L. Curatti[2 3].

[1] Universidad Nacional de Mar del Plata. [2] Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET). [3] Fundación para Investigaciones Biológicas Aplicadas.

lcuratti@fiba.org.ar

In present days, agriculture is associated with extensive use of fertilizers, pesticides and other chemical products, to improve crop productivity. However, extensive use of these agrochemicals is harmful to the environment, and often times, also to human's health. Thus, there is increasing interest in developing and improving biological alternatives as partial or complete substitutes for agrochemicals for the production of most crops. Our group is interested in developing the concept of plant growth promoting bacteria (PGPB) for the culture of aquatic microalgae mostly for bioenergy purposes. After three years of enrichment by successive dilutions of algae cultures in growth medium for autotrophic algae (no carbon source other than CO_2), some bacterial strains have been isolated. Most of them have been characterized for their algae growth promoting activities. The majority of the isolates belong to bacterial genera that are known for their PGPR activities, however most of them appear to correspond to different species of those genera. In this work we characterize in more detail several aspects of a strain of *Herbaspirillum hutiense.* This strain produces copious amounts of auxin'like substances and contrary to the most studied species of the genus, *H. seropediacea*, it appears not to be a N_2-fixer. Inoculation of wheat seeds with *H. hutiense* strain 15III, improved the percentage of seeds germination by 37 %. When inoculated onto groups of seeds on top of vermiculite substrate watered with NO_3^--lacking INTA 13 fertilizer solution, it produced a noticeable effect at 28 days on length and chlorophyll content of third leave, total weight of leaves and length and total weight of roots per pot. A similar growth promotion effect was observed when young seedlings (equalized by size) were incubated in the presence of the bacterium for two hours and then individually transplanted onto vermiculite substrate and watered with different dilutions of the INTA 13 fertilizer. The effect was consistent under a wide range of fertilizer dilutions. Moreover, growth promotion under fertilizer deficiency was also confirmed in pots containing different mixtures of perlite and soil, watered with tap water, but fertilized lightly once after 30 days of seedlings emergence. These findings suggest that algae/microalgae might serve as an alternative source of PGPRs for sustainable agriculture.

Código de Resumen: IN-012

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

THE ROLE OF DIFFERENT MICROORGANISMS IN THE LIFE CYCLE OF *Culex pipiens*, A WIDE SPREAD VECTOR OF PARASITES AND PATHOGENS

L.M. Díaz-Nieto[1], M.A. Perotti[2], C. D'Alessio[3 4], C.M. Berón[1].

[1] Instituto de Investigaciones en Biodiversidad y Biotecnología-CONICET, FIBA, Mar del Plata Argentina. [2] School of Biological Sciences, University of Reading, Reading, UK. [3] Fundación Instituto Leloir- IIBBA, CONICET, Argentina. [4] Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina.

leomdn@gmail.com

The Pipiens Assemblage is a widespread group of mosquito vectors that transmit several vertebrate pathogens and parasites. One member of this group, *Culex pipiens,* was detected as a competent vector of West Nile and Saint Louis encephalitis virus in Argentina. Like many other insects, mosquitoes interact with microorganisms throughout their life cycle. Recently, mosquito symbiont bacteria have been used in the development of new strategies for the control of human and animal parasites and pathogens carried by these insects. Some bacteria and yeasts have been identified in the gut of many insects and found to be transmitted vertically to their progeny by residing in special organs or by covering the egg shell that could be consumed by the hatching larva. In mosquitoes, some yeast species were detected during their life cycle but transmission to progeny was not proven. The aim of this work was to study the role of several microorganisms in different developmental stages of the mosquito-vector *Cx. pipiens*. Groups of neonates' larvae were fed with cyanobacteria, microalgae, yeasts and mosquito native bacteria to analyze the nutritional quality of these microorganisms. *Cx. pipiens* larvae can use yeast as an efficient food source allowing the complete mosquito development, showing the shortest developmental time and the highest survival rate. The insects reached the adult stage after 25 days when they were fed with yeasts showing similar survival rate as fish food as control diet. However, when fed with microalgae, they could only reach up to larval stage III after 25 days of treatment, showing also a decreasing survival rate after 10 days. When fed with cyanobacteria and native bacteria, the larvae survival rate decreased drastically, dying on day 15th at larval stage II. To analyze if the yeast *Saccharomyces cerevisiae* is transmitted along oviposition and the developmental stages of the insect, larvae were fed with GFP-labelled cells. The growth of GFP-labelled yeast in specific culture medium and the presence of the GFP gene by PCR were evaluated during the entire mosquito developmental stages including the offspring eggs. We determined that *S. cerevisiae* could not be transmitted to the eggs. However, we identified two bacteria species present in the eggs that could be inoculated by females during the oviposition providing the first food for the neonate larvae. Finally, we carried on oviposition tests offering females different substrates inoculated with specific microorganisms. The two native bacteria present in the eggs, *Klebsiella* sp. and *Aeromonas* sp. were chosen by mosquito females for oviposition but yeast substrates were not chosen. The native bacteria identified in this work could provide the first progeny intake but it might not be sufficient to complete the entire mosquito developmental stages. The function of these bacteria in the *Cx. pipiens* life cycle is being further investigated.

Código de Resumen: IN-013

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

BIFUNCTIONAL CATALASE KatG OF *Xanthomonas citri* subsp. *citri* RESPONDS TO HYDROGEN PEROXIDE AND CONTRIBUTES TO UV RESISTANCE AND EPIPHYTIC SURVIVAL ON CITRUS LEAVES

M.L. Tondo[1], M.L. Delprato[1], I. Kraiselburd[1], M.V. Fernández Zenoff [2], M.E. Farías [2], E.G. Orellano[1].

[1] IBR-CONICET, FBioyF-UNR. Rosario, Argentina. [2] PROIMI-CONICET. San Miguel de Tucumán, Argentina.

tondo@ibr-conicet.gov.ar

Xanthomonas citri subsp. *citri* (Xcc) is a Gram-negative obligate aerobic bacterium that infects citrus plants. During its life cycle Xcc is constantly exposed to hydrogen peroxide produced either by normal aerobic metabolism or as a part of the plant defense response against microbial invasion. In order to survive and colonize plant tissues Xcc must overcome hydrogen peroxide toxicity, and catalases are enzymes employed for its detoxification. We have previously shown that three catalase genes are effectively expressed in Xcc growing cells, being one of them the bifunctional catalase-peroxidase KatG. In this study we evaluated the physiological role of KatG and its relevance for Xcc virulence by the construction and characterization of a *katG*

deficient mutant strain. We found that the Xcc *katG* mutant exhibit basal levels of catalase activity considerably lower than wild-type cells, and does not induce this activity when exposed to sub-lethal levels of hydrogen peroxide. In addition, the *katG* mutant was found to be extremely sensitive to hydrogen peroxide and does not increase its resistance after pre-adaptation with low doses of the oxidant. The extreme sensitivity of Xcc *katG* to oxidative stress was also evidenced by a significant build-up in cellular peroxides levels after treatment with low concentrations of hydrogen peroxide. The mutant strain also displayed reduced resistance to UV-A and UV-B radiation than wild type cells, and an impaired ability to develop biofilms on glass surfaces. In the interaction with orange plants, the *katG* mutant produced typical canker lesions when infiltrated directly in the apoplast space and was able to multiply inside plant tissues with similar kinetics of growth and to the same extent as wild-type cells. However, the mutant strain exhibited reduced epiphytic survival on host leaves, indicating that KatG may be important for the epiphytic state of Xcc prior to entry into the apoplast and colonization, which in term would determine the probability of disease occurrence.

Código de Resumen: IN-014

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

STUDY OF THE *Xanthomonas citri* subsp. *citri* TYPE THREE PROTEIN SECRETION SYSTEM IN CITRUS CANKER

C. Vranych[1], A. Piazza[1], G. Sgro[1], J. Ottado[1], N. Gottig[1].

[1] *Instituto de Biología Molecular y Celular de Rosario.*

cecilia_vranych@hotmail.com

Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (*Xcc*), is one of the most severe diseases affecting citrus production worldwide. Plant-pathogenic bacteria colonize their hosts through the secretion of virulence effector proteins which depends on the type III protein secretion system (TTSS), present in many other pathogenic bacteria. This system (named *Hrp pilus*) mediates the translocation of effector proteins across the bacterial membrane through the plant cell wall and the plasma membranes, and elicits the hypersensitive response (HR). *Xcc* mutants in TTSS are unable to colonize vegetable tissues. HrpE protein is a principal component of the *Xcc Hrp pilus.* Although the role and mechanisms of HrpE in the assembling, secretion and translocation of proteins in *Xcc* is well described, little is known about other functions of HrpE in plant-pathogen interactions. At the moment, there is no evidence about possible plant proteins that may interact with HrpE. In order to identify citrus proteins that could interact with HrpE, we performed yeast two hibrid assays. We detected interactors belonging to the following categories: enzymes participating in phytohormone production, membrane proteins and proteins involved in stress, degradation mechanisms, redox reaction, cell rearrangement and apoptosis. In order to analyze the physiological importance of these protein-protein interactions that we found *in vitro*, *Arabidopsis thaliana* mutant plants in the different genes that encode the identified proteins were used to evaluate the response to HrpE proteins. These results could help us determine if these identified proteins are involved in the recognition of bacterial HrpE protein. This approach would allow us to select plant proteins and then evaluate them in *Citrus*. To conclude, this study is useful to enable the characterization of new plant proteins involved in citrus canker and will contribute to the understanding of infection processes and plant-pathogen interactions.

Código de Resumen: IN-015

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

COMPARATIVE EFFECTS OF CHITOSAN OLIGOMERS AND CELLOBIOSE ON THE CONTROL OF THE PHYTOPATHOGENIC FUNGUS *Fusarium solani* f. sp. *eumartii*

A.Y. Mansilla[1], C.V. Tonón[1], J.R. Mendieta[1], L. Albertengo[2], M.S. Rodríguez[2], C.A. Casalongué[1].

[1] *Instituto de Investigaciones Biológicas, CONICET-UNMdP, Mar del Plata, Argentina.* [2] *LIBAQ-INQUISUR, CONICET-UNS, Bahía Blanca, Argentina.*

amansill@mdp.edu.ar

Fusarium spp are plant pathogens and soil saprophytes that cause a broad spectrum of diseases. *Fusarium solani* f sp *eumartii* (*F. eumartii*) is an economically important pathogen for potato and tomato crops. Currently, control of *Fusarium* spp relies on toxic and synthetic fungicides increasing public concern regarding environmental contamination and proliferation of resistance. Chitosan is a natural, non toxic copolymer of glucosamine and N-acetylglucosamine obtained by chitin deacetylation. This biopolymer and its oligosaccharides have potential applications in agriculture with regard to eliciting plant defense mechanisms and controlling plant diseases. On the other hand, cellobiose is a sugar obtained by cellulose degradation and its chemistry structure is similar to chitosan oligomers. The aim of this work is to give insights into the properties and mechanisms of action of chitosan oligomers (ChO) and cellobiose on the phytopathogenic fungus *F. eumartii*. Chitin was isolated from shrimp shells waste (*Pleoticus mülleri*). Chitosan was prepared by heterogeneous alkaline deacetylation of chitin and ChO were obtained by

oxidative degradation. Cellobiose was purchased from Sigma-Aldrich (USA). Our findings revealed that, ChO and cellobiose-pretreated tomato seedlings were significant protected against *F. eumartii* infection compared with control. Fungal lesion area and the inoculi remaining were significantly reduced in pre-treated seedlings. In order to provide a clue into the mechanism mediated by ChO and cellobiose on disease control, chitinases were analyzed as defense markers in tomato seedlings. Two isoforms of chitinases were accumulated in ChO and cellobiose-treated seedlings evidencing activation of tomato defense responses of both compounds. Additionally, the effect of ChO and cellobiose on the mycelial growth was assessed. Meanwhile, ChO exerted inhibitory effects on *F. eumartii* growth; cellobiose did not show antimicrobial properties. Validation of inhibitory action of ChO on cell viability was demonstrated using propidium iodide as an intercalating agent excluded from viable cells. All these findings pointed out that the assayed ChO has a great potential for controlling *F. eumartii* disease in plants. Its useful action could be due by, at least, two different mechanisms: antimicrobial and elicitor properties in tomato plants.

Código de Resumen: IN-016

Sección: Interacciones Procariota - Eucariota

Modalidad: Poster

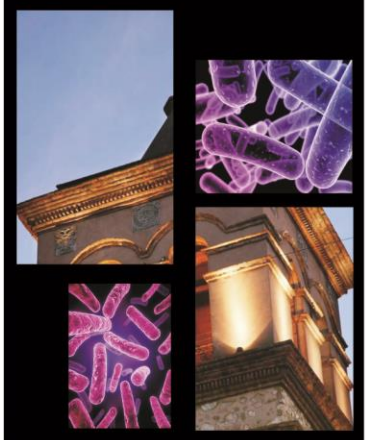
OVEREXPRESSION OF SODA GENE ON *Mesorhizobium loti*: EFFECTS OVER FREE-LIVING AND SYMBIOSIS

P. Gonzalez[1], R. Lascano[2], M. Melchiorre[1].

[1] *INTA - CIAP - IFRGV - Córdoba - Argentina..* [2] *UNC - CONICET - Córdoba - Argentina..*

gonzalez.pablojavier@inta.gob.ar

Drought and salinity conditions are the major factors affecting nitrogen fixation by legume-rhizobium symbiosis. A response to these stress conditions is the increase of intracellular ROS leading to activation of antioxidant system to ensure cellular homeostasis. One of these antioxidant components is the enzyme superoxide dismutase able to catalyze superoxide forming peroxide hydrogen and water. Under the premise that overexpression of sodA gene (mlr7636) in *M. loti* MAFF303099 improve tolerance to oxidative stress and performance in symbiosis we decide to overexpressing *sodA* constitutively under *PnptII* promoter of pFAJ1708 plasmid. Our study revealed that *M. loti* sod carrying plasmid pFAJsod with five-fold increase in SOD activity in periplasm, respect to *M. loti* 1708 (empty plasmid), showed an increased tolerance to superoxide and hydrogen peroxide in bacterial killing assay. The influence of overexpression of *sodA* gene on symbiotic performance of *M. loti* was investigated using *L. japonicus* MG20 in pouch nodulation assay. There was no significant difference between the average numbers of nodules formed on the root of plants inoculated by the *M. loti* MAFF303099, *M. loti* 1708 and *M. loti* sod strains in unstressed conditions. However, nodulation assays in stress condition differed in their sensitivity to the salt treatment. Despite the number of nodules was reduced under salt stress condition in presence of 150 mM NaCl in the three strains compared to untreated control, nodulation efficiency of the *M. loti* sod strain was drastically affected in salinity with an inhibition of 90% in nodules when compared to the other strains at the same condition. In conclusion, our results showed that *sodA* overexpression conferred to *M. loti* tolerance to oxidative conditions in free-living conditions but had a negative impact on the symbiotic interaction with *Lotus japonicus* in salinity conditions.



BIORREMEDIACION Y BIOCONTROL

Código de Resumen: BB-001

Sección: Bioremediación y Biocontrol

Modalidad: Oral

MICROBIOLOGICAL DIVERSITY AND FUNCTIONALITY OF A CHRONICALLY HYDROCARBON CONTAMINATED SOIL POST CHEMISTRY OXIDATION

R. Medina[1], P.M. David Gara[2], J.A. Rosso[3], M.R. Viera[4], M.T. Del Panno[1].

[1]*CINDEFI (CONICET-UNLP).* [2]*CIOp (CICCONICET) .* [3]*INIFTA (CONICET-UNLP) .* [4]*CIDEPINT (CIC-CONICET).*

marisa.rviera@gmail.com

In situ chemical oxidation (ISCO) is increasingly used for the remediation of soil containing organic contaminants such as polycyclic aromatic hydrocarbons (PAH). However, the impact on the soil microbial community has not been thoroughly elucidated. The aim of the study was to analyze the effect of the ammonium persulfate application followed by a bioremediation process on the matrix, microbial community and the PAH removal of the soil. Chronically contaminated soil (*S*) was collected from a petrochemical area (214 ppm PAH). Ammonium persulfate (PS) was sprayed as aqueous solution on contaminated soil by three additions (1% wt/wt) every two days and incubated at 30°C (*SOx*). *S* and *SOx*were further incubated at 25°C, 25% moisture content, mixed and monitored for 28 days. These microcosms were named *SB* and *SOxB* respectively. The PAH concentrations were determined by GC-FID. No PAH elimination was detected in *SB*. A significant elimination (35%) was observed in *SOx* while no additional decrease was detected *SOxB*. Alkaline extraction was performed to obtain an aqueous solution of natural organic matter of the soil. The Total Organic Carbon contents (TOC, TOC-5000 Shimadzu) and the Fluorescence Excitation Emission Matrixes (FEEM, Perkin-Elmer LS-50B) were determined for *S*and *SOx*. FEEM of *S*presents two zones of emission. The zone on lexc ~ 320 nm and lem ~ 440 nm could be assigned to the presence of PAH. These emissions were absent in *SOx* in line with the PAH elimination, and a significant increment on TOC values was also detected. A significant decrease in the microbial counts was observed in *SOx*. The subsequent bioremediation only increased the heterotrophic bacterial population which suggested that the available organic carbon allowed the growth of this population. To evaluate the microbial activity, four enzymes lipase, aril sulphatase, urease and protease were analyzed. All of them were slightly expressed in *S* microcosms and only lipase activity was significantly increased in *SOx*. Seed germination test using *Lactuca Sativa* on water extracts was performed to evaluate the soil toxicity. The toxicity detected in *S* was exacerbated in *SOx* and it was not reversed in *SOxB*. The dynamics of the bacterial community structure, analyzed by 16S rRNA PCR DGGE, evidenced a great change due to the oxidation. The clustering among the *S* and *SOxB* profile bands suggested the tendency of *SOxB* to recover the original structure. The pyrosequence analysis showed that members of actinobacteria, bacilli and acidimicrobiia classes were the predominant populations in *SOx.* Members of the actinobacteria became the dominant population in *SOxB*. This group was considered as k-strategist microorganisms and a major component in the later stages of successions in bioremediated soils. The initial PAH elimination provoked by PS was not followed by an additional elimination under bioremediation condition. However, a microbial succession of generalist populations was observed

Código de Resumen: BB-002

Sección: Bioremediación y Biocontrol

Modalidad: Oral

ASSESSMENT OF AFLATOXIN B1 IN INTERACTING MIXED CULTURES OF *Aspergillus* section *Flavi* AND NON-TOXIGENIC *Aspergillus*

C.L. Barberis[1], C.S. Carranza[1], M.C. Rodriguez[1], C.E. Magnoli[1].

[1]*Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto.*

cbarberis@exa.unrc.edu.ar

Aspergillus species are important contaminant of several oilseeds as peanut in pre, post harvest and stored stage. Furthermore, *A. flavus* is the main species isolated from peanuts in Argentina followed by *A. niger* aggregate strains. A previous study shown that aflatoxin B_1 (AFB_1) levels in peanut destined to human consumption in Argentina exceed the acceptable maximum levels, being *Aspergillus flavus* and *A. parasiticus* the main aflatoxigenic strains isolated from peanut ecosystem. The aim of this study was to determine inhibition of AFB_1 production on interactive mixed cultures in solid medium, between ten non-toxigenic *Aspergillus* section *Flavi* and *Nigri* strains, respect to their ability to prevent AFB_1 production by *A. flavus* and *A parasiticus* strains. *Aspergillus flavus* (AFS 56) and *A. parasiticus* (APS 55) as active producers of AFB_1, and ten non-toxigenic tested strains of *Aspergillus* spp.: *A. niger* aggregate (5 strains), *A. flavus* (2 strains), *A. oryzae* (3 strains) isolated from soil destined to peanut crop, used as biocompetitive agents in this study. Medium containing 150 g of sucrose, 20 g of yeast extract, 10 g of soytone was made. The water activities (a_W) of the basic media were adjusted to 0.980 and 0.930 with known amounts of glycerol. Plates were inoculated centrally by needle single point with *A. flavus* and *A. parasiticus* strains, as controls. Interactive

cultures of each toxigenic strain (*A. flavus* AFS 56 and *A. parasiticus* APS 56) and each non toxigenic strain were co inoculated on the basic medium separated at a distance of 45 mm in every plate. Cultures were grown for 21 days at 28ºC. Radii of colonies were recorded daily. AFB_1 production was tested after 7, 14 and 21 days of incubation. For this, colonies plugs at 4 cm from the inoculation point were taken. AFB_1 production on each interacting mixed culture was determined by HPLC. The results showed that AFB_1 production was completely inhibited when *A. flavus* and *A. parasiticus* were inoculated on interactive mixed culture with all strains of *A. niger* aggregate. This fact was observed at two a_W assayed and from 7 days of incubation time ($p<0.0001$). Only one non-toxigenic strain of *A. oryzae* was capable to completely inhibit AFB_1 accumulation at all a_W and incubation time assayed, from 14 days of incubation. *A flavus* strains significantly reduced of AFB_1 production on 71.4% at 0.98 a_W and 7 days of incubation, and 42% at 0.93 a_W, respectively ($p <0.0001$). The results of this study suggest that, non-toxigenic *Aspergillus* strains isolated from soil could be effective AFB_1 inhibitors, as they are able to completely reduce AFB_1 production. Further studies could address the topic of AFB_1 degradation with the aim of developing corrective techniques that reduce AFB_1 accumulation as much as possible.

Código de Resumen: BB-003

Sección: Bioremediación y Biocontrol

Modalidad: Oral

A SPECIFIC ANTIFUNGAL ACTIVITY AND BIOFILM FORMATION INDUCED BY THE INTERACTION BETWEEN *Bacillus subtilis* AND *Setophoma terrestris*

A.G. Albarracín Orio[1], R.A. Tobares[2], A.M. Smania[3], D.A. Ducasse[2].

[1]Laboratorio de Biología Molecular, Facultad de Ciencias Agropecuarias, Univ. Católica de Córdoba. [2]CIQUIBIC CONICET. Dpto. de Química Biológica, FCQ - UNC. [3]Instituto de Patología Vegetal (IPAVE) INTA.

a.albarracinorio@conicet.gov.ar

Onion is the second vegetable crop produced worldwide. Among the major limiting diseases of onion is pink root. As its causal agent, *Setophoma terrestris* (*St*), is one of the most severe pathogens in Argentina, we decided to address the biocontrol as a mean to control it. We have previously isolated bacteria with antagonistic activity against *St* from soil samples under continuous onion culture. The most efficient isolate was characterized by MALDI-TOF as *Bacillus subtilis* subsp. *subtilis* (*Bss*). The activity of *Bss* against *St* was screened by dual cultures and antagonism evaluated by measuring the radial growth of the pathogen after 3-4 days of incubation. *Bss* showed a strong capacity of fungal growth inhibition. To assess if the inhibition was determined only by the release of diffusible extracellular bacterial metabolites, the antifungal activity of the cell-free supernatant of *Bss* cultures were evaluated. Interestingly, we observed a high fungal growth inhibition on plates containing cell-free supernatant of *Bss* previously grown in the presence of the fungus (*Bss post-St*). In contrast, no significant differences in the fungal growth were obtained between control plates and plates containing cell-free supernatant from *Bss* grown without previous contact with *St*. Moreover, no antagonistic activity against other onion fungal pathogens such as *Fusarium oxysporum* was found, indicating this activity was specific against *St*. In fact, before bacteria were overtaken by the mycelium of *F. oxysporum*, a sample of *Bss* from co-culture plates (*Bss post-Fo*) was recovered and antifungal activity of cell-free supernatant against *St* was tested. We observed no significant differences between control plates and those containing cell-free supernatant from *Bss post-Fo*. In addition, an enhance biofilm forming ability and an increment in pellicle formation in *Bss post-St* compared to *Bss* grown without previous contact with *St* was observed. Consistently, *Bss post-St* showed an inhibition of swarming motility. These results suggest that there may be an association between biofilm formation pathways and biocontrol. Finally, the antagonistic activity was also tested in interaction assays in onion plants cultivated *in vitro*. For plants not inoculated with *Bss* we observed a loss of vigor of the roots and dark coloration of tips roots when infected with the fungus. On the other hand, no abnormalities were found in roots of plants inoculated with *Bss*. Furthermore, we found a strong fungal growth inhibition, with no invasion of hypha inside the medium, and an increase of bacterial density, especially near the root system in tubes containing plants inoculated with *Bss*. Taken together, our results suggest that *B. subtilis* releases diffusible bacterial inhibitory compounds whose secretion is specifically induced by the interaction with the pathogen *S. terrestris* and that *B. subtilis* undergoes cell differentiation that results in biofilm formation after contact with the fungus

Código de Resumen: BB-004

Sección: Bioremediación y Biocontrol

Modalidad: Poster

STUDY OF BENEFICIAL PROPERTIES OF *Lactobacillus brevis* L52 TO BE CONSIDERED AS PROBIOTIC STRAIN

G. Gerbaldo[1], P. Asurmendi[1 2], L. Pascual[1], L. Barberis[1].

[1]Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto.. [2]Consejo Nacional de Investigaciones Científicas y Técnicas.

pasurmendi@exa.unrc.edu.ar

Probiotics have been defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. Generally, strains that belong to the Lactobacillus genus are good candidates to be considered as probiotics bacteria. A list of criteria has been described for the selection of specific lactobacilli strain as potential probiotic agent, and beneficial properties are some of these desired characteristics. Moreover, probiotics results an important strategy for reducing the incidence of post-weaning diarrhea, a main problem in swine production. Lactobacillus brevis L52 was isolated from brewer´s grains, a substrate used as an alternative swine feedstuff. The aim of this study was to evaluate the beneficial properties of L. brevis L52, a potential probiotic strain to be used in pig diet. Auto-aggregation test, surface hydrophobicity properties, acid and bile salt tolerance, antimicrobial activity and antibiotic resistance were determined. Lactobacillus brevis L52 showed 16% of auto-aggregation and aggregated with an ammonium sulfate solution of 1,5 mol/L; according to the salt aggregation test (SAT), strain L52 exhibits intermediate surface hydrophobicity properties. These characteristics suggest that L52 strain could form biofilms and colonize intestinal surface, which are a barrier against colonization by pathogenic microorganisms. Regarding to gastrointestinal condition tolerance, 92% and 30% of the total lactobacilli survived to pH 3 and pH 2, respectively. L52 tolerated all bile salt concentration assayed (0,1%, 0,3%; 0,5% and 2 %) and the maximum reduction lactobacilli count was of 10% with 2% of bile salt solution. Antimicrobial activity was tested by the streak-diffusion method, all Escherichia coli (n=15) and Staphylococcus aureus (n=15) strains were inhibited by L52. The mean inhibition halos were of 39 mm and 33 mm, respectively for each pathogen microorganism. Antibiotics commonly used to prevent post-weaning diarrhea in pigs were assayed. L52 cells were sensitive to ampicillin, tetracycline, gentamicin, kanamycin, and amoxicillin; and resistant to vancomycin and nalidixic acid. In conclusion, these preliminary results suggest that L. brevis L52 exhibits excellent properties to colonize intestinal epithelium, to pass through gastrointestinal tract and to inhibit pathogenic bacteria growth. Accordingly, L52 is an important candidate to be considered as potential probiotic agent for biocontrol diarrhea pathogens and improve pig health.

Código de Resumen: BB-005

Sección: Bioremediación y Biocontrol

Modalidad: Poster

MODE OF ACTION OF BACTERIOCIN L23 PRODUCED BY *Lactobacillus fermentum* ON *Listeria monocytogenes*

M.J. García[1 2], F. Ruiz[1 2], L. Pascual[1], L. Barberis[1].

[1]Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto.. [2]Consejo Nacional de Investigaciones Científicas y Técnicas.

mjgarcia@exa.unrc.edu.ar

The aim of this study was to evaluate the mode of action of bacteriocin L23 produced by probiotic lactobacilli on a susceptible strain of Listeria monocytogenes. Cell free culture supernatant (CFS) containing the bacteriocin L23 was added to a culture of L. monocytogenes in tryptic soy broth and listerial growth was evaluated. In order to obtain the CFS, L. fermentum L23 was cultured in MRS broth and incubated at 37°C under a 5% CO2 atmosphere for 20 h. Then, the supernatant was removed by centrifugation (7500 rpm min-1 at 4°C for 20 min) and neutralized with NaOH 1N to eliminate the inhibitory effects of the organic acids. Cultures of L. monocytogenes LM6 in early exponential phase were added with neutralized CFS containing the bacteriocin L23 at a final concentration of 120 antimicrobial activity and incubated at 37°C for 24 h. A culture of L. monocytogenes without bacteriocin was used as control. Samples were taken at different intervals and bacterial growth was examined by measuring OD (600 nm) and viable cell counts. Growth parameters such as lag time, maximum specific growth rate and generation time were calculated from the growth curve (OD vs time). Lag time (λ) and maximum specific growth rate (μ max) were calculated from linear regression of the data in the exponential growth phase. Generation time (G) was obtained using the equation G=log10(2)/ μ máx. The results showed that addition of bacteriocin L23 extended the lag phase of the culture of L. monocytogenes when compared with the control without bacteriocin. Control culture had a lag time of 2.97 h whereas the one added with L23 was 10.81 h. After this lag time, listerial culture without bacteriocin grew exponentially reaching the maximum OD value after 9 h of incubation. In contrast, OD values of the culture added with L23 remained constant for 10 h and after 14 h OD readings began to increase. However, the maximum OD value reached was significantly lower ($p<0.05$) than that observed with the control culture. Similar results were observed when bacterial growth was evaluated by viable cell counts. Control culture had a μ max of 0.28 h-1 and a G value of 1.08 h, whereas the one added with L23 were 0.08 h-1 and 3.68 h, respectively. These results showed that bacteriocin L23 decreased the bacterial growth rate and increase 3 times the generation time compared with the control. In conclusion, these findings demonstrated that bacteriocin L23 had a bacteriostatic mode of action on L. monocytogenes showing inhibition of this pathogen microorganism growth.

Código de Resumen: BB-006

Sección: Bioremediación y Biocontrol

Modalidad: Poster

INHIBITION OF *Listeria monocytogenes* GROWTH BY A BACTERIOCINOGENIC STRAIN OF *Lactobacillus fermentum* IN WHOLE MILK

M.J. García[1 2], P. Asurmendi[1 2], L. Pascual[1], L. Barberis[1].

[1]*Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto..* [2]*Consejo Nacional de Investigaciones Científicas y Técnicas.*

mjgarcia@exa.unrc.edu.ar

The objective of this work was to investigate the antimicrobial activity of a bacteriocinogenic strain of Lactobacillus, L. fermentum L23, on Listeria monocytogenes in whole milk. L. fermentum L23 was inoculated in 90 ml of sterilized whole milk at a final concentration of 5x106 UFC ml-1 and incubated for 10 h at 30°C. After this period, a suspension of L. monocytogenes LM6 in sterilized whole milk was added to lactobacilli culture at a final concentration of 2x104 UFC ml-1 and re-incubated for another 14 h at 30°C. Control cultures of L. fermentum and L. monocytogenes inoculated separately in sterilized whole milk were included. Samples were taken at different intervals and bacterial growth was measured by viable cell counts. Samples were serially diluted in peptone water and plated on MRS agar for the enumeration of L. fermentum and on Oxford agar for listerial counts. Results were expressed as log UFC ml-1. Maximum specific growth rate (μ max) and generation time (G) were calculated. Furthermore, pH values were measured using a calibrated pH meter. Results showed that L. monocytogenes cultured alone in whole milk showed an exponential growth with a μ max of 0.25 h-1 and G value of 1.2 h. Listerial counts increased from 4.38 ± 0.05 log UFC ml-1 at the beginning of the experiment to a maximum value of 8.75 ± 0.02 log UFC ml-1 reached after 21 h of incubation at 30°C. In contrast, listerial counts in the assay with L. fermentum varied from 4.36 ± 0.05 to 4.52 ± 0.10 log UFC ml-1 along the incubation time. There were no significant differences ($p<0.05$) between listerial counts registered at different times of the experiment, showing that L. fermentum
produced a constant inhibition of L. monocytogenes growth in whole milk. On the other hand, viable cell counts of L. fermentum L23 cultured alone in whole milk were not significantly different ($p<0.05$) with the one added with L. monocytogenes. These results suggest that the presence of Listeria spp. did not affect lactobacilli growth. The pH measurements showed that lactobacilli produced a decrease of milk pH from initial pH of 6.81 to 5.25 at the end of the experiment. Similar results of pH were observed when L. fermentum cultured in whole milk was added with L. monocytogenes after 10 h of incubation. In contrast, L. monocytogenes produced a little diminution of milk pH which varied from 6.84 to 6.70 along the incubation period. In conclusion, the results presented in this study demonstrated that L. fermentum L23 was able to growth and produce antimicrobial metabolites in whole milk which caused a constant inhibition of the growth of L. monocytogenes.

Código de Resumen: BB-007

Sección: Bioremediación y Biocontrol

Modalidad: Poster

MTT ASSAY AS AN EFFECTIVE METHOD TO EVALUATE ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS AGAINST *Aspergillus flavus*

B.X. Camiletti[1 3], C.M. Asensio[2], M.C. Prieto[1], L.E. Dubini[1], M.P. Martin[1], A. Ocampo[1], E.I. Lucini [1].

[1]*Microbiología Agrícola, Facultad de Ciencias Agropecuarias, UNC.* [2]*Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET).* [3]*Centro de Investigaciones Agropecuarias (CIAP-INTA).*

bcamiletti@agro.unc.edu.ar

Aspegillus flavus is one of fungi which invade crops as cereals and nuts, with the consequent deterioration and contamination with aflatoxins. Essential oils (EOs) are presented as a natural alternative to control this pathogen because they are recognized as safe for health and environment. Traditional methods that evaluate antifungal activity consist in measuring the reduction of hyphal extension and the results are expressed as percentage of growth inhibition. MTT bioassay is a rapid colorimetric technique, based on the fact that living cells change the colour of the yellow salt 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into purple formazan which is a colored derivative. The goal of this work was to evaluate the antifungal activity of EOs from Mentha x piperita L. (Mi), Tagetes minuta L. (Tm), Laurus nobilis L. (La), Origanum vulgare sspp hirtum (OCor), Origanum vulgare L. sspp. Vulgare (OCom), and Origanum x majoricum (OMen) against Aspergillus flavus using both the traditional and the MTT method in order to determine their correlation. Petri plates of 9-cm diameter were prepared with 20 mL of liquid culture medium (20% potato lixiviated; 2% glucose; pH 4.5). EOs were added to culture medium according to the desired final concentrations. Five-millimeter diameter agar discs were collected from fungi and placed on Petri dishes. Treatments were incubated at 25 - 30°C for 10 days. Diameter mycelium was measured and antifungal activity was expressed as percentage of growth inhibition (PGI). The minimum fungicidal concentration (MFC) values were

defined as the lowest concentrations in which the PGIs were equal to 100%. On the other hand, 1,5-mL eppendorf tubes were filled with RPMI 1640 medium (500 μL). EOs were diluted in DMSO and added to culture medium. Tubes were inoculated with a conidial suspension (1x104 conidia/mL) and incubated at 37°C for 48 hs. Then, 50 μL of MTT solution (5mg of MTT/mL) were added to each tube and incubation continued for 3hs. The content was removed and DMSO (500 μL) was added to extract the dye. After agitation, the optical density (OD) was measured with a spectrophotometer at 570 nm. The percentage of MTT conversion was calculated following the equation: (OD of tubes with EO/ OD of EO free tubes) x 100. MFC were considered to be the lowest concentration of EO showing at least 95% of reduction in the OD. A Pearson correlation analysis was done to calculate the correlation coefficient. The MFC for OCor, OCom and OMen were 800, 1000 and 700 ppm in the traditional method and 800, 1000 and 800 ppm in the MTT assay respectively. Mi and La showed the same MFC (3000 and 3200 ppm respectively) in both methods while Tm had a MFC in 3800 ppm in traditional method and 3600 in the MTT assay. The correlation coefficient between both methods was 0,86 (<0.05). MTT assay could be an efficient method to evaluate the antifungal activity of EOs against *Aspergillus flavus* because of its practicality and rapidity

Código de Resumen: BB-008

Sección: Bioremediación y Biocontrol

Modalidad: Poster

CHLORPYRIFOS TOLERANCE OF NON-TOXIGENIC *Aspergillus* section *Flavi* STRAINS

C.S. Carranza[1 3], C.L. Barberis[1 3], C.E. Magnoli[1 3].

[1] Universidad Nacional de Río Cuarto. [2] Facultad de Ciencias Exactas, Físico-Químicas y Naturales. [3] Departamento de Microbiología e Inmunología.

ceciliacarranza90@gmail.com

Argentina is an agricultural country that exports raw materials and agricultural inputs. The cropping system adopted widely by the producers requires a great economic investment in agrochemicals to ensure better crops and higher profit. The insecticide chlorpyrifos, a non-systemic organophosphorus compound, is applied twice or even more times during different stages of crop development. The intake of pesticides and their residues through food is the main concern of international organizations such as the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). There is a need to develop safe, convenient and economically feasible methods for pesticide remediation due to environmental concerns associated to the accumulation of pesticides in food products and water supplies. For this reason, several biological techniques involving biodegradation of organic compounds by microorganism have been developed. The microbial metabolism is probably the most important degradative process of pesticides in the soil environment. Fungi possess a number of advantages that can be exploited in bioremediation systems. The aim of this work was to evaluate the chlorpyrifos tolerance of no-toxigenic *Aspergillus* section *Flavi* strains isolated from soil located in the south of Córdoba Province, Argentina. Four strains (AM1, AM2, GM3 and GM4) were centrally inoculated in soybean extract agar conditioned at two different water activities (a_W) (0.980 and 0.995) and modified with increasing chlorpyrifos concentrations (0, 100, 200, 300, 400, 500, 600 and 700 mg/L). Plates were incubated at 28°C for 20 days. The radius of each colony was taken daily to obtain growth rate and lag phase. All the strains tested were able to grow at the highest insecticide concentration assayed (700 mg/L). At higher concentrations of insecticide, higher values of growth rate were observed at 0.980 than at 0.995 for the strains AM1, AM2 and GM4. At 0.995 this growth parameter remained constant in the lowest chlorpyrifos concentrations. At 0.980, reductions in growth rate when chlorpyrifos concentration increased were observed; except in GM4 strain. The reductions of growth rate observed along the experiment did not achieve 50% reduction as respect to control treatments; therefore it was no possible to define an effective concentration (EC_{50}). Significant increases in lag phases with increasing insecticide concentration were observed in all strains and a_W tested. At 0.995, the duration of the lag phases of the strain GM3 was larger than the ones of the other strains along the experiment. The most noticeable increase in this growth parameter was observed with 700 mg/L of chlorpyrifos (78%). These results show that no-toxigenic *Aspergillus* section *Flavi* strains are able to tolerate high chlorpyrifos concentrations and their growth parameters change significantly.

Código de Resumen: BB-009

Sección: Bioremediación y Biocontrol

Modalidad: Poster

IN VITRO DEGRADATION OF ENDOSULFAN BY *Aspergillus oryzae* ISOLATED FROM AGRICULTURAL SOILS

C.L. Barberis[1], C.S. Carranza[1], C.E. Magnoli[1].

[1] *Departamento de Microbiología e Inmunología. Universidad nacional de Río Cuarto.*

cbarberis@exa.unrc.edu.ar

Endosulfan is an insecticide and acaricide still used in agriculture in some countries but already banned in Argentina since 2013. The contamination of atmosphere, soils, sediments, surface, rainwaters and foodstuffs by endosulfan has been informed in several studies. This chlorinated pesticide persists in the environment for very long periods, having a half-life of 60–800 days. Biotic degradation is one of the most viable options for pesticides remediation in soil and water. *Aspergillus* section *Flavi* strains are one of the most frequent isolated in agricultural soils. The purpose of the present study was to evaluate the potential degradation of endosulfan by *Aspergillus oryzae* strains isolated from agricultural soils under different water activities (a_W) conditions on synthetic medium. Two strains of *A. oryzae* were used (AM1 and AM2). Czapek medium (CZ) was adjusted to 0.98, 0.95 and 0.93 a_W with the addition of glycerol. Aliquots (50 ml) of CZ without pesticide were added aseptically into sterilized conical flasks and were immediately inoculated with one agar plugs (3 mm.) taken from the margins of actively growing cultures of each strains in the appropriate agar media. Subsequently, inoculation flasks were placed in a shaking incubator (60 rpm) at 25 ºC and the fungi were allowed to grow in the absence of the pesticides for 3 days. After this period, all flasks were supplemented with 5, 10 and 20 mM of the pesticide. The corresponding controls were included (flasks without pesticides, flasks with pesticide without strains). Immediately after pesticide addition and at (2 h), 2, 5, 10, 15, 20 and 30 days, subsamples of the liquid media were removed and pesticide residues were determined by HPLC. All the treatments were done by triplicate and repeated three times. In general, both strains were able to degrade different concentrations of endosulfan. The highest percentage of degradation in both strains and all pesticides concentration were observed at 0.98 aw ($p < 0.0001$). After 30 days of incubation, AM1 at 20 mM and 0.98 a_W, was able to degradate 76% of the endodsulfan respect to control treatments ($p < 0.0001$). At 20 mM and 0.93 a_W degradation percentages (43%) decreased significantly ($p < 0.0001$). The degradation of endosulfan by AM 1 strain was significant from 24 h for all tested pesticide concentrations at 0.98 and 0.95 a_W. On treatments inoculated with *A. oryzae* AM 2, at 20 mM and 0.98 of a_W, endosulfan concentration decreased by 56%. At 0.93 a_W degradation become significant from 5 days of incubation, but was lower at 0.98 and 0.95 of a_W, with reductions percentage that did not exceed 30% in any of the treatments analyzed ($p < 0.0001$). *Aspergillus oryzae* strains isolated from agricultural soil degraded *in vitro* higher concentrations of endosulfan and could be considered as potential biological canditates for the development of remediation agents.

Código de Resumen: BB-010

Sección: Bioremediación y Biocontrol

Modalidad: Poster

GROWTH OF *Aspergillus* section *Flavi* STRAINS IN PRESENCE OF CHLORPYRIFOS AS SOLE SOURCE OF CARBON, PHOSPHOROUS OR NITROGEN

C.S. Carranza[1 3], C.L. Barberis[1 3], C.E. Magnoli[1 3].

[1] Universidad Nacional de Río Cuarto. [2] Facultad de Ciencias Exactas, Físico-Químicas y Naturales. [3] Departamento de Microbiología e Inmunología.

ceciliacarranza90@gmail.com

The province of Cordoba, Argentina, has suffered a great expansion of its cultivated surface in the last two decades and, accordingly, of the volume of pesticides and fertilizer applied. Chlorpyrifos- (O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothionate) is a broad-spectrum insecticide whose mode of activity is as a cholinesterase inhibitor. It is used to kill a wide variety of insects. It is also used as a soil treatment (pre-plant and at planting), as a seed treatment and as a foliar spray, directed spray and dormant spray. It has been speculated that the bioaccumulation ability of chlorpyrifos and other organophosphorus pesticides in living tissues may spell a potential environment risk to organisms and humans. Soil microbes have a primary catabolic role in the environment and contribute to the global cycling of carbon, nitrogen, sulfur, phosphorus and other elements through degradation of plants and animals residues. Fungi share the major part of the microbial biomass and are most versatile in decomposing organic residues. The aim of this work was to evaluate the growth parameters of no-toxigenic *Aspergillus* section *Flavi* strains isolated from agricultural soils on different media with chlorpyrifos as the sole source of carbon, phosphorous or nitrogen. Four strains (AM1, AM2, GM3 and GM4) were centrally inoculated on modified Czapeck Agar (CZ) media. Carbon (CZC), phosphorous (CZP) and nitrogen (CZN) source were replaced by chlopyrifos at different concentrations (10, 1 and 1.5 mM respectively). Control treatments of each strain were prepared on full CZ and Water Agar (WA) media. The highest values of growth rate were observed in the full CZ media and the lowest values were registered in the media where the insecticide replaced the carbon source (CZC). Regarding the modified media, this parameter was significantly higher in CZN than in CZP in all strain tested. The longest lag phases were observed in CZC medium. When chlorpyrifos replaced phosphorous source, no significant differences between this media and control (CZ) were observed. The lag phases in CZP media were significantly shorter than WA media. On the contrary, the duration of lag phases in CZN media were significantly longer than the lag phases observed in CZ. No significant differences were observed between the lag phases registered in CZN and the ones registered in WA media. These results show that all the strains tested were able to grow in media with the insecticide as the sole source of carbon, phosphorous or nitrogen. Also the lag phase results indicate an inductive process. In conclusion, these results raise the possibility that these strains have a potential degradation capacity.

Código de Resumen: BB-011

Sección: Bioremediación y Biocontrol

Modalidad: Poster

IDENTIFICATION OF PROTEINS INDUCED BY POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN ACTINOBACTERIAL STRAINS USING ONE AND TWO-DIMENSIONAL GEL ELECTROPHORESIS

N. Bourguignon[1], P. Isaac[1], M.C. Estévez[1], V.P. Irazusta[2], M.J. Amoroso[1 3], M.A. Ferrero[1 3].

[1]PROIMI-CCT Tucumán-CONICET, Tucumán. [2]INIQUI-CONICET-UNSa, Salta. [3]Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, UNT, Tucumán.

natyb37@hotmail.com

Among the most abundant environmental pollutants, polycyclic aromatic hydrocarbons (PAHs) are one of the major concerns because of their persistence and toxicity. A high diversity of actinobacterial strains with the ability to degrade PAH were studied because their metabolic versatility and high resistance to environmental stress conditions. *Rhodococcus* sp. 20, *Rhodococcus* sp. 016, *Rhodococcus* sp. P18, *Rhodococcus* sp. F27 and *Amycolatopsistucumanensis* AB0, isolated from contaminated marine sediment and soil, were able to growth and remove naphthalene, phenantrene or pyrene from the culture medium. Strains were cultured in minimum medium supplemented with and without PAHs. After 72 h of incubation at 30 °C and 180 rpm the pellets were harvested and disrupted by sonication or French press. Soluble cellular protein fractions were evaluated SDS-PAGE (1-DE). Analysis of 1-DE revealed variations in the protein composition showing the up-regulation of multiple proteins for the three PAH treatments compared with the uninduced control sample. A total of 23 proteins were identified including propane, methane monooxygenase, toluene hydroxylase, which are involved in metabolism of aromatic compounds; catalases, oxidoreductases, molecular chaperone GroEL and aldehyde dehydrogenase, were expressed to control oxidative stress during the hydrocarbons catabolism. We also found proteins related to carbohydrate metabolism (aldehyde dehydrogenase, acetone carboxylase, amidohydrolase, lydantoinase), and energy production (ATP synthase, 5-oxoprolinase). Some proteins were detected uniquely upon exposure to a specific PAH, for instance most of the induced proteins in *Rhodococcus* sp. 016 were detected with pyrene; whereas others were shared, like methane monooxygenase for *Rhodococcus* sp. F27; which indicates that induction triggers not only specific responses but common responses in these strains. In addition, 2-DE studies revealed that when *A. tucumanensis* was exposed to phenanthrene it produced an over-expression of 22 spots. The identity of spots was related with the proteins identified in 1-DE. It is important to note the repetitive presence of methane monooxygenase and toluene hydroxylase. Proteins involved in energy production (ATP synthase, RNA polymerase, GTP-binding protein), detoxifying enzymes (sugar ABC transporter, universal stress protein) and those related to active metabolism (succinyl-CoA synthetase, elongation factor Ts, acetyl-CoA synthetase, enoyl-CoA hydratase, dihydrolipoamide acetyltransferase) were also up-regulated during phenanthrene degradation. Our results have shown that the actinobacteria studied degrade PAH through monooxidation of aromatic rings. Also, they up-expressed proteins involved in defense against oxidative stress and others related with energy gain. This is the first study that addresses the composition of proteins to reveal the metabolism of phenanthrene in *A. tucumanensis*.

Código de Resumen: BB-012

Sección: Bioremediación y Biocontrol

Modalidad: Poster

PHARMACEUTICAL FORMULATIONS CONTAINING *Lactobacillus fermentum* AND Lactobacillus rhamnosus FOR VAGINAL INFECTIONS TREATMENT

A.L. Camilletti[1 2], P. Asurmendi[1 2], L.M. Pascual[1], L.I. Barberis[1].

[1]Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto. [2]Consejo Nacional de Investigaciones Científicas y Técnicas.

anitaa.camilletti@hotmail.com

Lactobacillus genus is very important in relation to human and animal health and some species are often used as probiotics. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Lactobacilli are the most predominant microorganisms in the vaginal microbiota of healthy women in reproductive age, protecting it from possible infections caused by pathogenic and opportunistic microorganisms. This antimicrobial activity of lactobacilli is based on many properties, including their specific adhesion to the vaginal epithelium, coaggregation with pathogen microorganisms, stimulation of local immune system, competitive exclusion of pathogen agents from vaginal epithelium and production of metabolites such as organic acids, hydrogen peroxide, bacteriocins and biosurfactants, these antagonist substances inhibit the growth of other microorganisms. The aim of this study was to investigate *in vitro* antimicrobial activity of *L. fermentum* L23 y *L. rhamnosus* L60, two probiotic strains, in different pharmaceutical formulations. These formulations were

prepared using lactobacilli strains and different concentrations of glycerol and gelatin. Inhibitory activity of the probiotic strains contained in the ovules was evaluated and quantified using well diffusion assay and radial streak method. Count of lactobacilli from the time of production of the ovule until six months after was determined using plate dilution assay by duplicate. As an indirect method to determine the vaginal epithelium adhesion capacity by biofilm production crystal violet staining was performed. Lactobacilli contained in Formulation N°1 remained viable for 40 days, while from Formulations N°3 and N°4 viable lactobacilli were recovered even after 180 days. The inhibitory activity of lactobacilli was maintained over time regardless of the count of each strain in each formulation. Strains contained in pharmaceutical formulations are biofilm producers, and they maintained this capacity through the duration of the present experience. In conclusion, probiotic strains contained in the pharmaceutical formulations maintained their antimicrobial activity, biofilm production and other beneficial properties through 180 days.

Código de Resumen: BB-013

Sección: Bioremediación y Biocontrol

Modalidad: Poster

EVALUATION OF CELL VIABILITY AND ANTIFUNGAL CAPACITY OF A BIOFUNGICIDE YEAST CONSERVED IN XANTHAN GUM AND GLYCEROL AT DIFFERENT TEMPERATURES

M. Nally [1 3], V. Pesce[1 3], L. Rodriguez Assaf [1 2], M. Toro [1 3], R. Martinez Beguerí[1 3], F. Vázquez[1 3].

[1] *Instituto de Biotecnología- FI- UNSJ.* [2] *Departamento de Biología- FCEFy N- UNSJ.* [3] *Departamento de Agronomía- FI- UNSJ.*

ramb3489@gmail.com

Fungal diseases are one of the main reasons of economic losses in viticulture. These diseases can be treated by different chemical, physical, cultural and / or biological controls. Antagonistic microorganisms are a sustainable alternative to synthetic fungicides. The biggest obstacle in commercializing of antifungal biological products is the development of stable formulations that permit the preservation of antagonistic activity of these biofungicides. The objective of this study was to determine cell viability and antagonistic activity of *S. cerevisiae* BSc203 biofungicide yeast conserved in xanthan gum and glycerol mix solutions at two different temperatures. Evaluation of cell viability: BSC203 (a biofungicide yeast against B. cinerea) was inoculated in 250 mL of distilled water (10^8 CFU/mL), with xanthan gum (5g/L) and glycerol (20 v/v) and without auxiliary (control), in static conditions. Yeasts were incubated at 4° C and 25 ° C. Samples of 100 µL were taken at 60 days. They were seeded on YEPD-Agar plates. Developed yeasts colonies were counted (CFU/mL). Antagonistic action of yeast against gray mold on grape berries (Redglobe): A single wound (3 mm diameter and 3 mm depth) was made at the equator of each fruit using the tip of a sterile dissecting needle. Twenty µL of the yeast suspension (10^8 cfu/mL) were pipetted into each wound. After 2 h, 20 µL of 10^4 *B. cinerea* conidia/mL in sterile distilled water were poured into each wound. Treated grapes were air dried and placed in plastic bags (with wet paper towels to maintain high humidity). At the end of the experiment, the incidence of gray mold on each infected grape was calculated as follows: Incidence (%) = (number of decayed wounds/number of total wounds) x 100%. Each experiment used eighteen berries per replicate and three replicates per treatment. At 4°C and 25°C, *S. cerevisiae* BSc203 preserved in liquid medium with glycerol and xanthan gum solution during 60 days showed a significantly lower loss of cell viability and antifungal capacity, in comparison with the control (distilled water). BSc203 in mix solution presented a higher number of viable cells at 4°C (74.4% viable cells) than 25°C (65.12% viable cells). BSc203 conserved in mix solution reduced 74.88% the fungal disease incidence. The biofungicide BSc203 conserved at 4 °C with glycerol xanthan gum in distilled water during 60 days provided protective effect of the yeast cells, also maintaining its antifungal capacity.

Código de Resumen: BB-014

Sección: Bioremediación y Biocontrol

Modalidad: Poster

PATHOGENIC MICROORGANISMS CAUSING BOVINE MASTITIS: ISOLATION, IDENTIFICATION AND SUSCEPTIBILITY TO BIOSYNTHESIZED SILVER NANOPARTICLES

A.P. Ferreyra Maillard[1], M.N. Gallucci[1], P.R. Dalmasso[1], M.S. Pellegrino[2].

[1] *CITSE, CONICET, Universidad Nacional de Santiago del Estero..* [2] *Genética Microbiana, FCEFQyN, Universidad Nacional de Río Cuarto.*

anike_fm@hotmail.com

Bovine mastitis is one of the infectious diseases with the most significant adverse economic impact to milk producers, and it can be caused by a wide variety of factors. However, mastitis is nearly always caused by bacteria, usually *Staphylococcus* (*S. aureus*) and *Streptococcus* (*S. agalactiae, S. uberis*), which account for over 90% of pathogenic microorganisms. Also, Gram negative bacteria, such as *Escherichia coli, Klebsiella* spp. and *Enterobacter* spp. have been reported to cause a variety of

symptoms of the disease. Currently, antibiotic therapy plays an important role in eliminating existing intramammary infections. Nevertheless, an inadequate application of these treatments leads to the development of antimicrobial resistance strains. In this scenario, nanotechnology provides opportunities for adjusting the biological properties of nanoparticles (NPs) to generate effective antimicrobials. The aim of the present work was to determine the effect of NPs against the main bovine mastitis pathogens. For this, a simple and economic method of biosynthesis of silver nanoparticles (AgNPs) from $AgNO_3$ using *Geoffroea decorticans* (chañar) aqueous leaf extract is presented. Green synthesis of the AgNPs was confirmed by UV-vis spectroscopy and they were characterized by transmission electron microscopy (TEM). Bacterial strains in milk samples collected from cows showing clinical signs of mastitis were isolated and identified for their biochemical properties (catalase test, Gram stain, CHROM agar) and by molecular techniques. For *Staphylococc*i pathogens, a PCR-RFLP analysis of the *groEL* gene and the profiles generated using restriction enzymes (*Alu*I, *Pvu*II, and *Hind*III) were used to confirm our identification results obtained by the conventional biochemical method. Biochemical and molecular evidence allowed to identify 12 *Staphylococcus* strains, 7 *S. aureus*, 2 *S. simulans*, 1 *S. haemolytic*us, and 1 *S. hycus* (only one strain cannot be identified by molecular techniques), and 10 *Streptococcus* strains: 8 *S. uberis*, 1 *S. dysgalactiae*, and 1 *S. bovis.* The *in vitro* susceptibility of the strains identified against the biosynthesized AgNPs was evaluated using disc agar diffusion and microplate techniques. The results obtained showed an excellent antibacterial effect of AgNPs for all of the mastitis-causing pathogens tested to levels of concentration (pM) about 6 orders of magnitude lower than those reported for conventional antibiotics (around μM). *Streptococcus* strains were inhibited using AgNPs concentrations up to 20 times lower than those needed to inhibit *Staphylococcus*. In comparison, higher levels of AgNPs concentration were requires to inhibite *S. aureus* than those necessary for other *Staphylococcus*. Thus, the biosynthesized AgNPs may be used as a novel unconventional antimicrobial strategy for treatment of infectious mastitis in cow due to the broad spectrum of their antibacterial properties.

Código de Resumen: BB-015

Sección: Bioremediación y Biocontrol

Modalidad: Poster

BIOCONTROL OF *Colletotrichum gloeosporioides* IN OLIVE FRUITS BY ANTAGONISTIC YEASTS

V.M. Pesce[1 3], G.P. Carrizo[1 3], M.C. Nally[1 3], M. Brizuela [1 4], L.A. Rodríguez Assaf[1 4], M.E. Toro[1 3], L.I. Castellanos de Figueroa[2], F. Vazquez[1 4].

[1]Instituto de Biotecnología. FI. UNSJ. San Juan. [2]Planta Piloto de Procesos Microbiológicos (PROIMI) - CONICET. Tucumán. [3]Dpto de Agronomía. FI: UNSJ. San Juan. [4]Dpto de Biología. FCEFyN. UNSJ. San Juan.

virgi_pesce@yahoo.com.ar

Anthracnose, caused by *Colletotrichum* sp., is the major fungal disease in all olive producing areas of the world. In Argentina, this disease was reported in Catamarca, La Rioja, Córdoba, Mendoza and San Juan. Biological control using antagonistic microorganisms has been developed as an alternative to synthetic fungicide treatment. Yeasts have characteristics that give them advantages over other microorganisms to exert biocontrol of pathogenic fungi. However, yeasts-like fungi (*Aureobasidium*) have been reported against *Colletotrichum* species in olive fruits. The aim of this work was to evaluate the ability of autochthonous yeasts to control *C. gloeosporioides* in mature olive fruits, at different concentrations. Methodology: 92 antagonistic indigenous yeasts were previously selected in *in vitro* tests: 45 yeasts from viticultural environments and 47 from olivicultural environments. An isolate of *C. gloeosporioides* from the collection of INTA IMyZA, Castelar was used as pathogen. The pathogenicity of *C. gloeosporioides* was evaluated in olive fruits. The fungus was inoculated at different concentrations (10^2 to 10^6 conidia/mL) in mature fruits, and incubated during 5 days at 25°C. Percentage incidence [(number of decayed wounds/number of total wounds) x 100] and the mean of lesion diameter were determined. A suspension of yeasts (10^8 cells/mL) and 10^5 conidia/mL of fungi were inoculated in wounded fruits for biocontrol at *in vivo* tests (5 days, 25°C). Wounded fruits inoculated with fungal spore suspension were included as negative controls. The incidence of disease and %severity [(mean of diameter lesion in treated fruits/mean of diameter lesion in negative control) x 100] was calculated. A minimum of 50% reduction in the incidence of the disease was considered as antagonistic activity. The influence of yeast concentration at 10^7 and 10^6 cells/mL on biocontrol activity was evaluated. All experiments were made by triplicate. Nine indigenous yeasts (*Pichia membranifaciens* BPm6; *Saccharomyces chevalieri* BSch25; *Torulaspora delbrueckii* BTd126; *Candida tropicalis* Bo13b; *Cryptococcus albidus* Bo86; *Pichia kudriavzevii* Bo91, Bo108; *Wickerhamomyces anomalus* Bo107, Bo156) reduced the incidence of *C. gloeosporioides.* Biocontrol efficacies of these yeasts were: 50%, 50%, 63.34%, 90%, 76.67%, 53.34%, 70%, 60% and 90%, respectively. All antagonistic yeasts (except *W. anomalus* Bo107) were significantly effective in reducing the lesion diameter at 10^8 cells/mL. Only *C. tropicalis* Bo13b was antagonistic at lower concentration (10^7 cells/mL) with 70% incidence reduction. It can be concluded from this study that indigenous yeasts were effective as *in vivo* biocontrol agents against *C. gloeosporioides*. Concentrations of antagonists significantly affected their biocontrol activity. This is the first report that informed autochthonous biocontrol yeasts against *C. gloeosporioides* in olive.

Sección: Bioremediación y Biocontrol

Modalidad: Poster

THE BIOFILMS FORMATION OF *Exiguobacterium* sp. S17 ON SYNTHETIC SUPPORTS AND UNDER THE INFLUENCE OF ARSENIC

O.R. Ordoñez[1], F. Zannier[1], V.H. Albarracín[1 2], M.E. Farías[1].

[1] *Laboratorio LIMLA-Planta Piloto de Procesos Industriales y Microbiológicos (PROIMI-CONICET) .* [2] *Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán.*

omar_federico@yahoo.com.ar

The high-altitude Andean Lakes (HAAL) are ecosystems located in the South American Andes. These ecosystems are unique due to their geographical characteristics, their broad range of extreme environments, as well their abundant biodiversity. The genus *Exiguobacterium* is one of the most widespread and representative genera on the HAAL, being detected by direct (pure culture isolation) and indirect (DGGE) techniques. This genera have been isolated or molecularly detected from a wide range of habitats including cold and hot environments with temperature between -12 and 55°C. This fact confers substantial interest to the genus as a potential model system to research attributes that may correlate with adaptation and evolution of organisms to diverse thermal regimes. *Exiguobacterium* sp. S17 is a high arsenic resistant polyextremophilic bacteria isolated from the stromatolites of L. Socompa. This strain is able to grow readily in laboratory and represents an attractive model system for the study of environmental stress. Previous studies showed that *Exiguobacterium* sp. S17 is able to resist to high arsenic concentration and to produce biofilm. The aim of this work was to assess biofilms formation by *Exiguobacterium* sp. S17 in different synthetic supports and to investigate the influence of arsenic (As[III] y As[V]) in their development. Determination and quantification of biofilms was measured using crystal violet 1% following the methodology proposed by Tomaras et al., (2003). Biofilms production was evaluated at different incubation times (24, 48 and 72 h) in LB_{50} media (without As) and in different synthetic supports: sterile glass tubes (15 x 125mm) and polypropylene (12 x 75 mm) and polystyrene plates (20 cm^3). The influence of As was investigated supplemented LB_{50} with arsenate (As[V]): 50mM, 100 mM, 150 mM, 200mM, 250mM and arsenite (As[III]): 2.5 mM, 5mM, 7.5 mM, 10 mM, 12.5 mM at the same time. ANOVA analyzes revealed that the optimal production of biofilms is achieved after 24 hours of growth and the highest biofilm production was obtained when using glass as support and adding arsenate (As [V]100 mM). No significant differences were observed when adding arsenite in comparison to control medium (without arsenic). The findings obtained in this work made an important contribution to the knowledge of the biology and ecology of the microbial communities of the HAAL in response to stress factors. Moreover, this method can be applied for the benefit of human and environmental health by establishing an experimental basis for a bioremediation method. Furthermore, we propose that HAAL is a source of novel bacterial species of biotechnological interest.

Sección: Bioremediación y Biocontrol

Modalidad: Poster

INSECTICIDAL POTENTIAL OF *Serratia* sp. ON LARVAE OF *Aedes aegypti*

J.C. Rondan Dueñas[1], A. Muñoz[1], A. Belaus[1], P.S. Vélez[1], M.E. Doucet[2], P. Lax[2].

[1] *Centro de Excelencia de Procesos y Productos (CEPROCOR), Córdoba.* [2] *IDEA (CONICET-UNC) y Centro de Zoología Aplicada, FCEFyN-UNC. Córdoba.*

jrondan@ceprocor.uncor.edu

Some arthropods are agents transmitting diseases for public health importance. At present, indiscriminate use of synthetic insecticides generates environmental pollution, insect resistance and human toxicity. An alternative for insect control consists of obtaining molecules of bacterial origin with insecticidal activity. Some species within the genus *Serratia* are pathogenic to insects. The present work evaluated the potential insecticidal effect of *Serratia* sp. LB-1 isolated from the external cuticle of an entomopathogenic nematode (*Steinernema* sp.) on larvae of *Aedes aegypti* (mosquito that transmits dengue and chikungunya viruses).Bacteria were multiplied on culture with brain-heart medium for 48 h. Concentration was determined by optical density; then the bacterial phase was separated from the supernatant by centrifugation. Bacteria were suspended in sterile water and a series of dilutions ($1x10^5$, $5x10^5$, $1x10^6$, $5x10^6$, $1x10^7$, $1.5x10^7$, $2.5x10^7$ UFC/ml) were performed. The supernatant fraction was diluted in the same proportion as each one of the mentioned concentrations. Ten III and IV-stage larvae were placed in tubes containing 4 ml of each dilution; mortality was evaluated at 24 and 48 h. The experiment was performed at 25ºC, with 8 replications per treatment. At 24 h, the highest mortality percentages were observed in the $1.5x10^7$ and $2.5x10^7$ supernatant dilutions, with values ranging between 50-100%. At 48 h, $1x10^7$, $1.5x10^7$, $2.5x10^7$ dilutions produced between 80-100%

mortality. None of the bacterial suspensions had larvicidal effect. The results show that secreted secondary metabolites produced by the strain of *Serratia* sp. would have potential for control of mosquito larvae of sanitary importance.

Código de Resumen: BB-018

Sección: Bioremediación y Biocontrol

Modalidad: Poster

Cr(VI) RESISTANCE AND REMOVAL BY INDIGENOUS MICROORGANISMS ISOLATED FROM SAN LUIS RIVER

M.F. Castro[1], C.D. Delfini[1], C. Almeida[1 3], R. Olsina[1 3], M.A. Martínez[2 4], L.B. Villegas[1 3].

[1]INQUISAL-CONICET, San Luis. [2]PROIMI-CONICET, Tucumán. [3]Fac. Qca. Bqca. y Fcia. UNSL. [4]Fac. Ciencias Exact. y Tec. UNT.

m.fernanda.c183@gmail.com

Chromium is used in different industrial processes and released into the environments. The removal of toxic Cr(VI) by microorganisms is a promising approach for Cr(VI) pollution remediation. In the present work, Cr(VI) tolerant microorganisms were isolated by sequential enrichment, identified and the Cr(VI) removal also was studied. Because Chorrillos River Basin (San Luis, Argentina) has deteriorated significantly due to the dumping of inadequately treated domestic and industrial effluents, was selected for this study. The site selected for the isolation (33° 19'49.73 "S, 66° 21'41.92" W) showed low organic content, pH between 6,0 - 8,0 and presented 75, 109, 628.9, 48.82 and 40 mg kg^{-1} of chromium, copper, zinc, lead and nickel respectively. From the river sediment sample, enrichment was carried out in the liquid medium EG en g/l: glucose,10; K_2HPO_4,0.25; KH_2PO_4, 0.125; $MgSO_4$,0.1 and yeast extract,1. The selection was carried out by three sequential steps using 50 ppm de Cr(VI) as selection pressure at 180 rpm and 30°C. The capacity to remove Cr(VI) by consortium was estimated by measuring remaining Cr(VI) concentration in the supernatant using colorimetric 1,5-diphenylcarbaxide every 24h. 50% Cr(VI) removal was the criteria selected to continue the next step. After 15 days, a mixed culture in which representatives of Eukaryotic domain (yeasts and filamentous fungi) and a single Prokaryote were found. According to the partial sequence of 26S rRNA gene, yeasts showed identity with *Wickerhamomyces* and *Candida*, while the fungus belongs to *Trichoderma* genus. Based on the 16S rRNA gene sequence, the bacterium was associated with more than 99% identity to *Pediococcus sp.* A wide variety of bacteria have been reported for reducing Cr(VI) to Cr(III), under aerobic and anaerobic condition. Since, there are very few reports of capacity to remove chromium and other metals by *Pediococcus sp.* and related genera. Therefore this bacteria was selected for Cr(VI) removal study. The *Pediococcus* activity against Cr(VI) was checked by determining the minimum inhibitory concentration (MIC) therefore tolerance and growth was evaluated in increasing concentrations of Cr(VI) (25, 50, 75 and 100 ppm) in EG medium for 7 days. Flasks were inoculated to a final concentration of 2×10^7 FCU ml^{-1} with an active overnight pre-inoculum and incubated at 30 ºC. Interestingly, this isolated in monoculture showed MIC of Cr(VI) was 75 ppm and was able to remove up to 67% ± 0.62 of Cr (VI) in EG with 25 ppm; 45% ± 0.23 in medium with 50 ppm; and 37% ± 0.66 and 31% ±1.61 in media containing 75 and 100 ppm Cr (VI), respectively. Our results show the potential of *Pediococcus* sp. to achieve removal of Cr(VI) at higher than those reported in the literature, so this isolated presents an interesting potential for the study of tolerance and removal of chromium and other metals, individually such as part of the mixed culture or consortium.

Código de Resumen: BB-019

Sección: Bioremediación y Biocontrol

Modalidad: Poster

BIOLOGICAL CONTROL OF *Penicillium* sp. CAUSING POSTHARVEST BLUE ROT OF TABLE GRAPE BY VITICULTURAL YEASTS

L.A. Rodríguez Assaf[1 3], V.M. Pesce[1 2], P.M. del Castillo[1], L.P. Pedrozo[1 3], M.C. Nally[1 2], M.E. Toro [1 2], L.I. Castellanos de Figueroa [4], F. Vazquez[1 2].

[1]Instituto de Biotecnología. FI. UNSJ. San Juan. [2]Dpto. de Agronomía. FI. UNSJ. San Juan. [3]Dpto. de Biología. FCEFyN. UNSJ. San Juan. [4]Planta Piloto de Procesos Microbiológicos - CONICET. Tucumán.

virgi_pesce@yahoo.com.ar

Table grapes are highly perishable and non-climacteric fruits susceptible to severe changes during postharvest. Low temperatures stimulate the development of fungal diseases caused by different species of *Penicillium*, as blue rot. In cold storage, prior to export, sulfur dioxide (SO_2) is used as the most common method for controlling postharvest fungal decay in table grapes. However, SO_2 residues are dangerous to people allergic to sulfites, and this compound is highly injurious to fresh fruits, causing bleaching of the berries and browning of the rachis in grapes. Among different biological approaches suggested in

the literature, the use of yeast as biocontrol agents shows great potential as an alternative method of postharvest disease control. There is little background on the control of *Penicillium* using yeast at low temperature, and there are no reports about biocontrol of these fungi in table grapes in cold storage conditions. The main objective of this study was to evaluate the ability of viticultural yeasts for the biocontrol of pathogenic *Penicillium* isolates, at cold storage conditions. Yeasts were isolated from fermenting musts at 12°C and healthy berries stored at low temperature (2 ± 1°C). Four virulent *Penicillium* sp. were previously isolated from rotten grapes. Yeasts were assayed *in vivo* for biocontrol activity at 2 ± 1°C during 4 weeks. A single wound was made at the equator of berries of Superior Seedless table grapes using the tip of a sterile dissecting needle. Twenty microliters of each yeast suspension in water (10^6 cfu/mL) were pipetted into each wound. After 2 h, 20 µL of 10^4 *Penicillium* sp. conidia/mL of sterile destilled water were poured into each wound. Treated grapes were air-dried and placed in plastic bags (with wet paper towels to maintain high humidity). Positive and negative controls were included. Percentage of disease incidence [% = (number of decayed wounds/number of total wounds) ×100] was calculated at the end of the experiment. A randomized complete block design was used (10 berries per replicate and three replicates per treatment). Yeasts that at least reduced 60% disease incidence were considered antagonistic. Ninety-six yeasts were isolated from viticultural environments. *In vivo* experiments showed that 18 isolates belonging to 4 non-*Saccharomyces* species (4 *Rhodotorula glutinis*, 9 *Cryptococcus laurentii*, 4 *Cryptococcus humicola*, 1 *Debaryomyces hansenii*) significantly reduced at least one phytopathogenic fungus. From all antifungal selected yeasts, 14 were isolated from fermenting musts, and 4 from surface of healthy grapes. It can be considered that several non-*Saccharomyces* species mainly from viticultural environments can inhibit fungi involved in blue rot. This study suggests that these yeasts may be novel important agents of biocontrol during the cold storage of table grapes for extended periods of time.

Código de Resumen: BB-020

Sección: Bioremediación y Biocontrol

Modalidad: Poster

BACTERIOCIN-PRODUCING LACTOBACILLI AS POTENTIAL BIOLOGICAL AGENTS FOR ANTI-*Candida* CONTROL

M. Monton[1], F. Ruíz[1 2], P. Asurmendi[1 2], L. Pascual[1], L. Barberis[1].

[1]Fac. Cs. Exactas, Fco.-Qcas y Nat. Dpto. Microbiología e Inmunología. UNRC.. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) .

pasurmendi@exa.unrc.edu.ar

In the last years, there has been a global increase of invasive and non-invasive infections produced by different *Candida* spp. Thus, research on probiotic lactobacilli has become relevant as biocontrol strategy. The aims of this study were i) to evaluate the antifungal activity of two human lactobacilli on different clinical isolates of *Candida* spp., ii) to determine the minimum inhibitory concentrations of the bacteriocins L23 and L60 on susceptible *Candida* strains, iii) to study the types of interactions between the bacteriocin-producing lactobacilli strains on the yeast growth. *Lactobacillus fermentum* L23 and *L. rhamnosus* L60 were selected for both, their relevant probiotic properties and the production of organic acids, bacteriocins (L23/L60) and, in the case of L60 strain, for the release of hydrogen peroxide in cell-free supernatants (CFSs). Pathogenic *Candida* species belong to the strain collection of our research group. The antimicrobial activity test of each *Lactobacillus* strains was performed by a cross streak-diffusion method using *Candida* spp. as indicator microorganisms. The CIMs of L23 and L60 were carried out by a well-diffusion method. To obtain these metabolites, each CFS of *Lactobacillus* strains was treated with NaOH 1 N to inhibit the organic acid action and, only in the case of L60 strain; it was also treated with peroxidase (NP-CFS) to prevent the H_2O_2 action. Thus, the NCFS of L23 and the NP-CFS of L60 contains the bacteriocins L23 and L60, respectively. To determine the interactions, a qualitative perpendicular-streaks-diffusion method was used. Two perpendicular streaks of lactobacilli describing a right angle were done on agar plates, incubated in optimal conditions. A suspension of *Candida* spp. (1.5×10^8 CFU mL^{-1}) was seeded in the free zone between the lactobacilli streaks. Interactions, synergistic indifferent, and antagonistic, were interpreted based on the shape of the inhibition zone. Seventy six percent of all *Candida* species tested (n=21), including different strains of *C. albicans*, *C. tropicalis* and *C. guilliermondi*, were inhibited by each *Lactobacillus* species. The antifungal activity of the L23 and L60 strains did not show statistically significant differences ($P < 0.05$). High inhibition values on the growth of *C. albicans* (89%), *C. tropicalis* (83%) and *C. guilliermondii* (75%) strains were found. The average sizes of the inhibition zones were 17.33 mm and 16.73 mm for L23 and L60 strains. The MIC range of bacteriocin L23 was 40-160 AU mL^{-1} for *C. albicans* strains, 40-80 AU mL-1 for *C. tropicalis* strains and 40 AU mL^{-1} for *C. guilliermondii*. While the MIC ranges of bacteriocin L60 were 80-160 AU mL–1 for *C. albicans* and *C. tropicalis* strains, and 80 AU mL^{-1} for *C. guilliermondii*. A 73% of synergism and a 27% of indifference between the lactobacilli was found. Antagonistic interactions were not observed. In conclusion, the combined use of these lactobacilli strains together with the antimicrobial potential of their bacteriocins could represent a valuable biological alternative for future anti-Candida agents.

Código de Resumen: BB-021

Sección: Bioremediación y Biocontrol

Modalidad: Poster

A Tn5 INSERTIONAL MUTATION IN THE *gltA* GENE FROM A NATIVE ISOLATE OF THE *Pseudomonas chlororaphis* SUBGROUP INDUCES A PHENOTYPIC CHANGE ASSOCIATED WITH PHENAZINE PRODUCTION

B.C. Agaras[1], L.G. Wall[1], C. Valverde[1].

[1] *LBMIBS, DCyT, Universidad Nacional de Quilmes.*

betina_agaras@yahoo.com.ar

Members of the *Pseudomonas* genus colonize the rhizosphere of different plant species and display plant-probiotic traits. Therefore, they are interesting candidates for biological agricultural inputs to stimulate plant development and/or promote crop health. We have generated a collection of 19 isolates of pseudomonads obtained from either bulk soil or the rhizosphere of major extensive crops from different plots under no-till management located in Argentina. Among this collection, the native isolate SPAN5 was obtained from a bulk soil sample of grasslands located at Pergamino, Buenos Aires. This isolate, related with the *P. chlororaphis* subgroup by its 16S rDNA, *rpoB* and *oprF* sequences, showed several PGPR activities *in vitro*: growth inhibition of 12 fungal pathogens in PDA plates; production of phospholipases, exoproteases and HCN; presence of genes related with antibiotic production (*prnD* for pyrrolnitrin, *phzF* for phenazines); inorganic phosphorous solubilization; and production of IAA and ACC deaminase. In particular, phenazines produced by SPAN5 confer it an orange colour in saturated cultures. We carried out Tn*5* mutagenesis in order to identify genes related to the biocontrol and direct plant growth promotion potential. One of the isolated mutants, SPAN5-135, that has lost the orange pigmentation and cannot grow in PDA plates, suffered transposon insertion in the *gltA* gene encoding a type II citrate synthase. This is the first report of the requirement of *gltA* for phenazine production in pseudomonads. Further characterization of the antifungal activity and PGPR traits of the colourless-SPAN5 Tn*5* mutant is underway.

Código de Resumen: BB-022

Sección: Bioremediación y Biocontrol

Modalidad: Poster

NEW BACTERIAL COMPOUNDS WITH TENSIOACTIVE PROPERTIES AND BIOTECHNOLOGICAL APPLICATIONS

M.L. Ferreira[1 2], M.R. Barrionuevo[1 2], S.A. Ramirez[1], D.L. Vullo[1].

[1] *Universidad Nacional de General Sarmiento-CONICET.* [2] *Ambos autores contribuyen por igual al trabajo.*

ferreiramarialaura@gmail.com

Pseudomonas veronii 2E is an indigenous bacterium from the highly polluted Reconquista River (Buenos Aires Metropolitan Area) with great versatility in responses to environmental stimuli. Previous reported studies proved its ability for the synthesis of tensioactive compounds. This research was focused on the relation between those compounds and swarming motility, biofilm formation, and cadmium removal. Biosurfactants from *P. aeruginosa* PA01 were isolated as reference compounds. The secretion of biosurfactants was tested for *P. veronii* 2E and *P. aeruginosa* PA01 in Kay´s medium (g/L: $(NH_4)_2HPO_4$ 1.5, KH_2PO_4 4, yeast extract 0.4, $MgSO_4.7H_2O$ 1.97) supplemented with glucose 5%(w/v) or sunflower oil 5%(v/v). Two compounds from *P. veronii* 2E (named Sc1 and Sc2) and one from *P. aeruginosa* PA01 (named Sc5) were extracted from culture supernatants. Compound Sc1 was detected in presence of glucose while compounds Sc2 and Sc5 in presence of sunflower oil. The purity of the extracts was evaluated by HPLC-UV chromatography. A non-swarmer mutant of *P. veronii* 2E (strain 2E01) was used to evaluate the effect of compounds on swarming motility. For that purpose, swarming agar plates were supplemented with different concentrations of the extract. The result of those experiments indicated that surfactant Sc2 was able to restore 2E01 swarming motility at a concentration of 50 μg/mL. Compound Sc5 did not restore swarming and a concentration higher than 150 μg/mL affected the agar consistence. Compound Sc1 showed little but still positive effect on swarming. Biofilm formation was studied in the same mutant strain. The compound Sc2 and Sc5 restored the adherence of 2E01 up to normal levels while Sc1 did not. In fact, Sc1 decreased the biofilm formation of wild type strain. The obtained results indicate that Sc2 obtained from *P. veronii* 2E could be a natural surfactant capable to restore the phenotype in mutant strains. Compared to Sc5 from *P. aeruginosa* PA01, a lower concentration of Sc2 was needed for equal responses. The microbial surfactant produced by *P. veronii* 2E was studied for the potential Cd(II) recovery adsorbed on diatomite. Compound Sc5 from *P. aeruginosa* PA01 was used as control. The highest desorption efficiency was obtained at pH 6.5, with an initial concentration of 0.093mmol Cd(II)/g diatomite. The results showed a 16% desorption for *P. veronii* 2E and 42% for *P. aeruginosa* PA01. Additionally, electrochemically monitored titrations of *P. veronii* 2E biosurfactant were studied at 25°C and a pH value of 6.5. This work evidenced the production of a compound by *P. aeruginosa* PA01 that enhanced swarming, biofilm attachment restoration and Cd(II) complexation as well. Two new compounds, extracted from *P. veronii* 2E cultures, behaved as positive factors in

swarming and biofilm formation. Further steps will involve more studies in Cd(II) recovery from wastes.

Código de Resumen: BB-023

Sección: Bioremediación y Biocontrol

Modalidad: Poster

NUCLEAR WASTEWATER WITH HIGH LOAD OF AMMONIUM AND URANIUM TREATED BY NITRIFICATION AND BIOSORTION

M. Venturini[1], M. Perez[1], C. Gustavo [1], P. Ramon [1].

[1]Comisión Nacional de Energía Atómica. [2]Universidad Nacional de Sam Martin. [3]Dioxitek S.A..

venturini@cae.cnea.gov.ar

In the nuclear industry, ammonium is used to get uranium concentrates (yellow cake) which are used as U_3O_8 to obtained a first step toward convert it into UO_2. For this reason the effluent of this process contain high quantities of ammonium and uranium, but in lowest levels. Therefore these high concentrations must be diminishing for complying local's environment law. Ammonium is a very soluble ion and then it is difficult to precipitate. In the present work we studied and applied the nitrification process (autotrophic aerobic oxidation) and biosortion of uranium. A key point in this process is the immobilization of the biomass, avoiding the washing and enhancing the performance in kinetic and effectiveness terms, this process and biomass generation is complex and slow because is inhibited by substrate (NH_4^+), product (NO_2^-), and the uranium that was diminished by biosortion. Taken this into account, we work with a hydrogel which were obtained by gamma irradiation for retaining biomass. To understand the nitrification process in the nuclear effluents, we maked assays of the kinetic inhibition (Monod) by product and substrate and toxic elements; and then continued to scale up the process. To make it, we correlationed variables pH, respirometric assay and carbonate addition. This proved to be effective for use in bioreactors semi continuous at laboratory scale of 5 liter at 100 days assay. The performance of process increase in terms kinetics rates to 350mg/l.day and 300ppm of uranium in the final effluents. The final levels of ammonium oxidation were at 90% and uranium declined significantly in the liquid effluent. The treatments was evaluated a correlative with external parameters (RO_2 and CO_3 Consumed). This results were a realistic variables to treated the effluent of the nuclear industry.

Código de Resumen: BB-024

Sección: Bioremediación y Biocontrol

Modalidad: Poster

EFFECT OF PHOTOACTIVATED TITANIUM DIOXIDE ON SURVIVAL OF *Pseudomonas aeruginosa* BIOFILMS

M. Pezzoni[1], P. Catalano [2], R. Pizarro[1], M. Bellino[2], C. Costa [1].

[1]Departamento de Radiobiología - Comisión Nacional de Energía Atómica . [2]Departamento de Micro y Nanotecnología - Comisión Nacional de Energía Atómica.

pezzoni@cnea.gov.ar

Photocatalytic oxidation has been studied as an alternative to traditional disinfection methods. Titanium dioxide (TiO_2) is one of the most popular photocatalysts employed because of its non toxic nature, chemical stability and low cost. When TiO_2 is photoexcited by ultraviolet A radiation (UVA, 315-400 nm) in presence of water and oxygen, reactive oxygen species such as hydrogen peroxide, hydroxyl radical and superoxide anion are generated, responsible for biocidal activity. The objective of this study was to compare the effect of photoactivated TiO2 in different forms: comercial powder (Degussa P25) and mesoporous thin films obtained by combining sol-gel and molecular self-assembly, on the survival of *Pseudomonas aeruginosa* biofilms. Mesoporous and non-mesoporous titania films were prepared by dip coating TiO_2 on standard microscope glasses. To mesoporus films, two surfactant templates were used: TiBrij-58, to form a pore size of 4 nm, and Pluronics-F127, to form a pore size of 10 nm. Films were calcined to 350ºC or 500º C to obtain amorphous or a higher proportion of cristaline fase (anatase), respectively. Control surfaces were glasses without TiO_2 and non-mesoporous TiO_2 films obtained by the same protocol but in the absence of surfactants. To analyze the biocidal effect of photoactivated TiO_2, two systems were investigated. Biofilms grown on glass slides without TiO_2 were submerged in demineralized water or containing powdered TiO_2 P25 (75% anatase) in suspension. On the other hand, biofilms grown on TiO_2 coated glasses were submerged in demineralized water. Both systems were either exposed to UVA at a fluence rate of 20 Wm^{-2} for 180 minutes (total dose 216 KJ m^{-2}) or maintained in the dark. Cell viability was evaluated by the ability of biofilm cells to form colonies on LB solid medium at 37ºC. After UVA exposure, TiO_2 showed a significant antibacterial activity in all assays. The presence of TiO_2 as a powder in the irradiation medium decreased survival in about 1 log compared to control slides. In presence of TiO_2 coated surfaces, the survival fraction decreased by about

2-3 logs compared to the control. The films calcined at 500ºC were about 1 log more efficient in disinfection than amorphous films. These results confirm the efficacy of photoactivated TiO2 coated surfaces compared to comerercial titania nanopowdered to control *P. aeruginosa* biofilms, and demonstrate that its anatase form is very useful in this regard. In addition, the efficiency per unit mass resulted four orders higher in the case of titania film structures.

Código de Resumen: BB-025

Sección: Bioremediación y Biocontrol

Modalidad: Poster

ARABIC GUM, A NEW STRATEGY FOR MICROBIOLOGICAL CONTROL IN THE DAIRY INDUSTRY

M.L. Boiero [1,1], L. Breser [2], V. Gonzalez Estevez [2], R. Bachetti[2], C. Morgante[2], C. Porporatto[2], M. Montenegro[2,1].

[1] *Universidad Tecnológica Nacional-Facultad Regional de Villa María..* [2] *CIT VM (CONICET-UNVM). Instituto A.P. de Ciencias Básicas y Aplicadas. Universidad Nacional de Villa.*

lauraboiero@hotmail.com

The nutritional quality loss of food, usually is caused by microbial growth during different stages of production. Particularly in raw milk, the initial microbial content will affect the quality, shelf life and safety of processed milk and its derivatives. Traditionally, one of the strategies most used for reducing the risk of microbial contamination of raw milk, is storage under refrigeration immediately after to milking. However, this selectively favor the development of psychrotrophic microorganisms that can produce heat stable extracellular enzymes, causing alterations in the chemical composition and nutritional value of milk. In the last decade, the demand for minimally processed, easily prepared, and ready-to-eat fresh food products has grown globally, prompting the development of new methodologies as alternatives to thermal treatment. A growing trend is the addition of preservatives of natural sources, as a safe and healthy alternative to synthetics preservatives. The Arabic Gum (AG) is an edible biopolymer obtained as exudates of trees of Acacia, which is being widely used as a stabilizer, a thickener, and an emulsifier. However, to date, no antimicrobial activities studies have been conducted against psychrotrophics microorganisms. The aim of this work was to evaluate the effect of the addition of AG over the psychrotrophics bacterial growth, and viability. Several bacteria isolated from raw milk (Enterobacter spp.) were cultivated with different concentration of AG (0, 10, 20, 75, 100, 200, 400 M) during a period of 7 days at 4ºC and analyzed each 24 h. The bacterial growth in different conditions was evaluated by CFU counts, viability assays was performed using the LIVE/DEAD BacLight Bacterial Viability Kit (FACS), and metabolic activity was determined by colorimetric assay using tetrazolium salt (MTT). We observed that the addition of 200 and 400 M AG controlled proliferation of bacterial growth in more than a 50%, respect to the control condition and these effects were dose-dependent. In another hand, AG did not show significantly effect on the bacterial viability evaluated by the incorporation of propidium iodure. However using the tetrazolium salt as MTT we could determine that 75, 100, 200 and 400 M of AG were able to inhibit the metabolic activity of psychrotrophics bacterial growth significantly in all the assayed times. These data shown that AG had an important effect in the initial proliferation over psychrotrophic bacterial milk, which was maintained during analyzed times. Is important to hightlight that, for dairy factory the initial times are determinants to conserve the milk quality up to industrial processing, improving nutritional value in the final product. In base of these results, we can suggest that AG provides an additional beneficial effect to their usually technological use in food industry.

Código de Resumen: BB-026

Sección: Bioremediación y Biocontrol

Modalidad: Poster

ARBUSCULAR MYCORRHIZAE FUNGI IN AGRICULTURAL SOILS CONTAMINATED WITH LEAD

A. Blanco[1], M.J. Salazar[1], A. Becerra[1], M.L. Pignata[1], J.H. Rodriguez[1].

[1] *Instituto Multidisciplinario de Biología Vegetal, Facultad de Ciencias Exactas, Físicas y Naturales,.*

beceale@gmail.com

Regarding to high toxicity of heavy metals, polluted agricultural soils affects not only crop growth and the quality of agricultural products but also poses serious threats to human health through contamination of the food chain. Among industrial practices that contribute to heavy metals pollution in soils we can mention the lead smelters and secondary lead smelters (recycling of Pb from Pb-containing products).

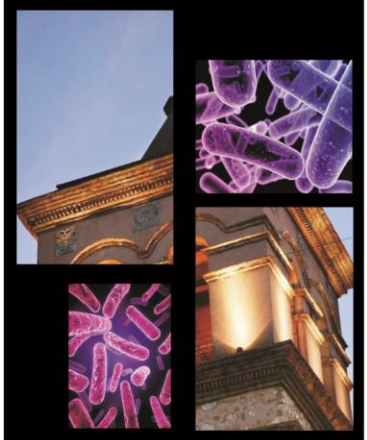
On the other hand, soil microorganisms play an important role in the mobilization and immobilization of metals, changing its availability to plants. In this context, the arbuscular mycorrhizal fungi (AMF) belonging to the phylum Glomeromycota, have been described in sites with heavy metals, indicating that exist a fungal inoculum adapted to these contaminated environments. AMF play a significant role in phytostabilization of toxic elements in contaminated soils by kidnapping and, in this way, helping

mycorrhizal plants to survive.

In the town of Bouwer, Córdoba, it worked years ago a recycling plant of lead batteries which was closed for failing to comply with emission standards of pollutants, leaving high levels of lead in soil. This area is characterized by intensive agriculture, mainly soybean (*Glycine max*) and other crops associated like *Sorghum bicolor*.

The purpose of this study were: I) to evaluate the Pb content in top soils and crops (soybean and sorghum) growing in the vicinity of a former smelter in Bouwer, Córdoba; II) to determine the AMF in soybean and sorghum crops that grow in Pb contaminated soils.

The concentrations of Pb in soybeans and sorghum and in top soils (bioavailable, mobilized and residual fraction) were investigated. Furthermore, AMF morphospecies were identificated. The results show that the concentrations of Pb in crops at all sites (controls and close to the smelter) were above the maximum permitted levels. Regarding to Pb in soil a pollution gradient in relation to the smelter distance was observed, which values above 1000 ppm near to the source. On the other hand, the study of AMF community showed a geometric distribution based on their rank-abundance diagram, being *Glomus brohultii* the dominant species. This distribution is typical of disturbed environments, being the species of Glomeromycota mentioned in association with heavy metals. However, no relationships were found between arbuscular morphospecies and Pb in soils. Taking into account these findings, future studies need to be performed in order to evaluate its relationship with the bioavailability of toxic metals in agricultural soils. The purpose not only is to assess the current state of crops in terms of food security but also evaluating possible soil remediation techniques.



MICROBIOLOGÍA AMBIENTAL Y DEL SUELO

Código de Resumen: MS-001

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

MICROBIOLOGICAL, ENZYMATIC AND GENOMIC CHARACTERIZATION OF A *Paenibacillus* sp. XYLANOLYTIC ISOLATE

S. Ghio[1], F.E. Piccini[2], M. Insani[2], D.H. Grasso[1], E. Campos[2].

[1] *Instituto de Suelos, CIRN, CNIA, INTA Castelar.* [2] *Instituto de Biotecnología, CICVyA, CNIA, INTA Castelar.*

silvighio@gmail.com

Paenibacillus sp. strains isolated from soil and lignocellulosic sources have been reported to produce extracellular enzymes useful for industrial applications. In second generation bioethanol production, enzymatic degradation of lignocellulosic biomass is considered the bottleneck, due to the recalcitrant nature of biomass, composed mainly by cellulose and hemicelluloses, mostly arabinoxylan. Therefore, improving its deconstruction is of key importance for full utilization of biomass. In this context, a cellulolytic and hemicellulolytic strain has been isolated from a previously characterized bacterial consortium obtained from a forest soil sample. By 16S rRNA sequencing and phylogenetic analysis it was classified as *Paenibacillus sp*, closely related to a cluster formed by *P. taichungensis* and *P. pabuli*. We named this strain *Paenibacillus sp* A59. It is a Gram positive, mesophilic and facultative anaerobe endospore-forming bacillus. Physiological and biochemical characterizations showed a high hydrolytic potential, indicated by good degrading activities of substrates such as xylan, cellulose, casein, citrus pectin and starch. It also showed tolerance to a wide range of pH and high salinity, being able to grow in culture from pH 5 to 10 and up to 7% NaCl. Scanning electron microscopy of bacteria growing on xylan in anaerobiosis showed high adherence to the substrate. In order to optimize culture media for enzymatic secretion, carboxymethyl cellulose, xylan from beechwood and sugarcane residue were used as sole carbon sources. The maximum xylanase activity was obtained when growing on minimal medium supplemented with xylan from beechwood, for 72 hours at 30°C. The optimal xylanase reaction condition was achieved at 50 °C, both pH 6 and pH 10, and the enzymatic extract was stable at 50 °C for 48 hours. Zymograms of cell free supernatants using xylan as substrate, revealed the presence of 30 and 70 kDa protein bands with xylanolytic activity. HPLC analysis of hydrolysis products released from xylan by cell-free supernatants showed three main peaks corresponding to xylotriose, xylobiose and xylose, indicating that this microorganism secretes the set of enzymes to obtain a complete degradation of xylan. A draft genome sequence was obtained by Illumina Miseq. In accordance to the observed enzymatic activities, genes coding for different enzymes were identified in the bacterial genome, in particular those involved in xylan hydrolysis, xylanases (of GH10 and GH11 families) and a beta xilosidase (GH43). These results showed the high xylanolytic potential of *Paenibacillus sp* A59 for industrial applications as well as for studying the xylanolytic bacterial system.

Código de Resumen: MS-002

Sección: Microbiología Ambiental y del Suelo

Modalidad: Oral

APPLICATION OF MALDI-TOF MASS SPECTROMETRY TO THE IDENTIFICATION OF ENDOPHYTIC BACTERIAL COMMUNITIES ASSOCIATED WITH PLANTS

F. Alvarez[1 2], J.L. López[1], A. Lagares[1].

[1] *Instituto de Biotecnología y Biología Molecular, Fac Cs. Exactas, Universidad Nacional de La Plata.* [2] *CEQUIBIEM, Facultad de Cs. Exactas, Dpto de Química Biológica, Universidad de Buenos Aires.*

alvarezflorencia.mail@gmail.com

In the last years, matrix-assisted laser-desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has been increasingly used for the identification of microorganisms. Mainly ribosomal and housekeeping proteins contribute to the signals in a typical whole-cell mass spectrum, which explains the species-specific conservation of peaks with coincident *m/z* ratios (even among samples grown under different cultivation conditions). The advantageous of the technique -such as simple handling, speed of processing, cost-effectiveness, and high-throughput capabilities, among others- have positioned the MS profiling of bacterial extracts as a highly convenient typing method over other more traditional approaches (i.e. biochemical typing, rDNA sequencing). In the present study we have expanded the application of MALDI-TOF MS analysis to the characterization of the bacterial diversity present in communities associated with plants, specifically bacteria that interact with varieties of *Medicago sativa* (alfalfa). Good quality spectra were collected from the main bacterial species present in alfalfa plants (seeds, roots, aerial part), which had been previously isolated in our laboratory and identified based on the partial

sequencing of their corresponding 16S rDNA. The collected spectra from the plant-associated bacteria included data for more than 25 different genera and were used to expand the MALDI Biotyper database (Bruker Daltonics). Interestingly, the MALDI-TOF-based analysis allowed in some cases the distinction between isolates of a same bacterial species. We are now using MS typing to characterize the relative abundance of bacterial species in different plant niches to characterize their specific microbiomes. Contrasting with classical population analyses based on PCR of bulk 16S rDNA and high-throughput sequencing, the new approach -based on a clone-by-clone analysis- also allows for the preservation of individual clones of interest for their further biological characterization.

Código de Resumen: MS-003

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

METAGENOMIC ANALYSIS OF THE NITROGEN-FIXING ENDOPHYTE COMMUNITIES IN TOMATO CROP: INORGANIC FERTILIZATION EFFECTS

E. Ramos[1], P. Calderoli[1], M.O. Aguilar[1].

[1] *Instituto de Biotecnología y Biología Molecular UNLP-CONICET.*

efrenramos1287@gmail.com

Bacterial endophytes are colonizing microorganisms inside plant tissues without causing negative effects to host or confering ecological threat in the plant (Quispel, 1992). The plant inside is a suitable environment for biological nitrogen-fixation, in which the diazotrophic endophyte communities can influence plant growth. In this work diazotrophic endophyte communities were studied in tomato Solanum lycopersicum analyzing nifH gene and the effects of inorganic fertilizer on the structure of the community. The field experiment was conducted in greenhouse at the experimental farm Ing. Agr. Julio Hirschhörn (La Plata) with tomato var. Elpida F1 hybrid. A group of plants was treated with inorganic fertilizer (N:150ppm, P:80ppm, K:80ppm) and other group without inorganic fertilization, four samples were taken since seedling stage until senescence. The explants were cut in small pieces and surface sterilized using sodium hypochlorite 5%, ethanol 70% and finally washed with sterile water. Subsequently, the metagenomic DNA was extracted by using the protocol described by Murray and Thompson et al. (Nucleic Acids Res,1980). DNA was used as template for amplification of nifH (360 pb) gene by PCR. After nifH DNA purification from gel, the nifH pool was cloned into the plasmid pK18 to generate a nifH library. The resulting 62 clones, 30 clones from the control and 32 clones from the treatment with fertilization, were sequenced. After applying Blast we found that our nifH database showed homology whit known nifH sequences that corresponded to the order Burkholderiales 7%, to Rhizobiales 15%, to Aeromonas 4%, and to Enterobacteriales 9%. Phylogenetic analysis was performed to characterize community profiles with Reference database (NCBI). Interestingly, we found that an important proportion (67%) corresponds to sequences that have no taxonomic assignment to known bacteria. The fingerprint technique RFLP applied to nifH pools showed variation in profile between first sample (pre-implantation) and samples from other stages of growth. Variation in RFLP profiles was also observed in DNA samples from treated and non treated tomato. We conclude that the tomato endophytic communities is represented by different taxa, however predominance of certain uncultivated microorganism was detected, yet without characterization. This finding opens the possibility of discovering new endophytic genotypes in nature, which may be associated with tomato and eventuality involved in biological nitrogen fixation.

Código de Resumen: MS-004

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

ISOLATION AND IDENTIFICATION OF *Escherichia coli* AND OTHER GENDERS OF *Enterobacteriaceae* GROUP, FORMING BIOFILMS, IN WATERS OF THE SAN JUAN RIVER, CAUCETE AREA IN THE SAN JUAN PROVINCE

A. Pastor[1 3], R. Aciar[2], D. Bustos [2 3], D. Bustos Crescentino[2 3], P. Varela[1 3].

[1] *Instituto de Biotecnología, FI- UNSJ.* [2] *Instituto de Ciencias Básicas, FFHA- UNSJ.* [3] *Departamento de Física y Química, FFHA- UNSJ .*

licbiodani@yahoo.com.ar

The presence of *Escherichia coli*, a member of the *Enterobacteriaceae* group in water or sediment from river beds is considered an indicator of fecal contamination, which is originated in poor treatment in the waste plants of urban effluents, or by direct feces dump. Diverse pathogenic microorganisms are able to persist, multiply and become integral constituents of microbial communities of biofilms attached to surfaces and embedded in a matrix of extracellular polymeric substances (EPS) excreted by themselves. Their heterogeneous arrangement results in differential physiological behavior of the population in time and space, allowing communities of cells interact with microorganisms, including pathogens and may increase their resistance to chemicals

(synthetic or natural origin) and higher infectivity. Pathogens belonging to different groups or families, that may have been integrated to the biofilm matrix could enter the human body by direct contact, or indirectly through water that has been contaminated from the system, creating a serious health risk. We aimed to isolate and identify bacteria of the genus *Escherichia coli* and other *Enterobacteriaceae* members forming biofilms present in waters of the San Juan River in the Caucete region. To this end, we filled two cells (C1 and C2) with 4 liters of water from the river, and horizontally submerged a rough acrylic plate. Cells were maintained at 34 °C for 5 days, with facilitated aeration, adding 10 mL of whole milk in C1 and of Nourishing Broth in C2, 2 twice in that period. The biofilms formed on each plate were removed with cotton, and subsequently resuspended in 250 mL of sterile phosphate buffer saline (PBS) upon, stirring in a shaker at 80 rpm for 2 minutes. The biofilm suspensions were diluted appropriately and plated on PCA plates for total mesophilic count. Because of the high population density, and the inability to isolate and identify all individual colonies, 25 isolates from the highest dilution were randomly selected in each of the 4-sampling times (M1 to M4), and plated on the following selective media: BEM, SS. To identify isolates, the following biochemical tests were perfomed: Gram stain, INVIC, TSI and LIA. Total counts of mesophiles (UFC / 100mL) for each of the four samples were: M1: C1: 1 x 108; C2: 1 × 1011; M2: C1: 1 x 106; C2: 2 x 1010; M3: C1: 5 x 106; C2: 1 x 1010; M4: C1: 5 x 1010; C2: 1 x 1011. Among the 100 randomly selected colonies, we detected: Escherichia coli: 13%; Shigella flexneri: 5%; *Salmonella enteritidis*: 32%. Considering the observed population of microorganisms, both in number and identified genders and, tha fact that when forming biofilms those microorganisms may be more resistant to external factors and have even greater pathogenicity that the isolated bacteria, and that we have seen people who fish and use the channel for recreational purposes, the ecological and sanitary importance of the data found is evident.

Código de Resumen: MS-005

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

EFFECT OF INTENSIVE USE OF SOILS IN SMALL HORTICULTURAL PRODUCTION UNITS OF MORENO DISTRICT, BUENOS AIRES METROPOLITAN AREA

L.J. Raiger Iustman[1 3], J. Di Schiena[2], S. Basack[2], D. Vullo[1 2].

[1]Dpto de Química Biológica. Facultad de Ciencias Exactas y Naturales- UBA. [2]Área Química, Instituto de Ciencias, Universidad Nacional de General Sarmiento. [3]IQUIBICEN-CONICET.

lri@qb.fcen.uba.ar

Small-scale agriculture around large cities (periurban agriculture) is performed by farmers that, in general, have not received adequate training in the use of agrochemicals and currently count with few economical resources. This kind of agriculture presents problems that differ from extensive production causing a strong environmental impact not only by the intensive use of the land, but also by the farming practices applied. The aim of this work is to study the effect of this horticultural practice by monitoring the bacteria community of a strawberry production soil (SS) and comparing with a non-productive soil (a grassland close to the productive plots but fallow for at least 20 years, RS), located in the same farm belonging to Cuartel V, Moreno, Buenos Aires Metropolitan Area. Both soils were analyzed after collecting in 2014 spring several samples of 5 cmof soil using a clean metal punch, obtaining a composite soil of each. The applied pesticides in SS as declared by the farmer were *Decis Forte* (Deltamethrin), *Vertimec* (Abamectin) and *Rovral 50 WP* (Iprodione). Chemical analysis was performed for an integral characterization of the samples. SS showed less conductivity, total carbon, organic matter, and inorganic P content than the reference soil RS. On the other hand, humidity and copper content was higher in the productive soil SS compared to RS. Finally, chlorpyrifos was also found in the strawberry production soil, probably consequence of a previous application on another crop of the same plot. To analyze bacterial diversity, total DNA of both composite samples was extracted using Power soil isolation kit (MoBio) and V1-V3 region of 16S rRNA gene was sequenced by 454 pirosequenciation (Chunlab-Korea). Results showed that bacterial abundance and diversity in SS was higher than in the RS (OTUs number 2527 and 1769 respectively, Shannonindex 7.268 and 6.845 respectively). Taxonomically, the class in the SS particularly increased was *b-Proteobacteria*, while in RS was *a-Proteobacteria*. Interestingly, another difference was observed: anaerobic bacteria like *Anaeronilales*, *Clostridiales, Desulfomonadales* were found in SS but not in the RS perhaps correlated to the addition of poultry manure, a current practice implemented by the local farmers. As conclusion, the horticultural soil SS showed several alterations in both composition and microbiota, perhaps due to the intensive use in conjunction with the addition of a wide spectrum of agrochemicals. SS became less aerobic, promoting the development of the anaerobic taxa found and, on the other hand, this polluted soil evidenced an increase on β-*Proteobacteria* population, which could be related to -regarding their metabolic diversity- the xenobiotic biodegradation.

Código de Resumen: MS-006

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

APPLICATION OF FLUORESCENT *IN SITU* HYBRIDIZATION IN THE EVALUATION OF THE BIOCIDES USED IN THE OIL INDUSTRY

C. Terada[1], M.T. Del Panno[1], M.R. Viera[2].

[1]CINDEFI (CCT CONICET La Plata, Facultad de Ciencias Exactas UNLP). [2]CIDEPINT (CCT CONICET La Plata, Facultad de Ingeniería UNLP).

marisa.rviera@gmail.com

Microbiologically influenced corrosion (MIC) and souring of oilfield reservoirs as result of the presence of sulfate-reducing bacteria (SRB) are of great concern in the oil industry. Considering the limitations of conventional culture-based methods for studying SRB, techniques involving the direct analysis of the microbial population from their genetic material are getting more attention. One of them is Fluorescent *in situ* Hybridization (FISH), which uses fluorescently labeled oligonucleotide probes that hybridizes specifically to its complementary 16S rRNA target sequence within the intact cell. Using FISH, the abundance of the detected microorganisms can be determined by counting the cells stained with a general DNA-binding dye and the cells hybridized with a specific probe. To control bacterial populations, biocides are commonly applied to injection waters and production facilities. The aim of this work was to evaluate the possibility of using FISH to help in the selection of an appropriate biocide for the water treatment plant of an oil secondary recovery plant (OSRP). Three commercial biocides based on THPS and quaternary ammonium salts were used in the assay in a concentration of 200mg/l. Three replicates of each biocide in each concentration were done. The following probes (5P end-labeled with Cy3) were used: Eub338; Non338 and SRB385. The fluorescence was detected with a Leica microscope, analyzing 15 to 20 images per sample. The inhibition effect of the biocides was tested in OSRP water filtered inoculated with a microbial culture in PostagteB medium, obtained from the same water. The inhibitory effect was determined by counting the cells hybridized with the Eub338 and SRB385 probes. The percentage of hybridized vs DAPI-stained cells, RS%, was calculated. The RS% values for the Eub338 probe obtained after 4h of incubation at60ºC(water treatment plant condition) in the presence of biocides B1 and B3 were higher than those obtained with the control (without biocide). This behavior suggested a metabolic activation, associated with a high content of RNA in the cells. It could be related with the mode of action of the chemicals or the application of a sub lethal dose. The RS% values obtained with B2 were no significantly different from those of the control, associated with no inhibitory effect. The RS% values obtained after 4h of incubation for SRB population showed that none of the tested biocides produced metabolic activation. B1 and B3 biocidal effects were not different from that observed in the control. Instead, the lower RS% values obtained with B2 were associated with a significant inhibitory effect on SRB population, showing that the SRB population was more sensitive to B2 effect under the tested condition. These results suggested that FISH could be helpful in the screening of biocides, being a responsive and suitable test to detected inhibitory concentrations.

Código de Resumen: MS-007

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF EFFLUENTS IN DAIRY FARMS OF RIO PRIMERO DEPARTMENT (CÓRDOBA, ARGENTINA)

L.F. Petruzzi[1], C. Merlo[1], C. Vázquez [1 2], B.X. Camiletti[1 3], M. Bruno[1], M.S. Salloum[1 3], E.I. Lucini[1].

[1]Microbiología Agrícola, Facultad de Ciencias Agropecuarias, UNC. [2]Instituto Multidisciplinario de Biología Vegetal, IMBIV-CONICET. [3]Centro de Investigaciones Agropecuarias, CIAP-INTA.

bcamiletti@gmail.com

The main problem of milk production is the generation of effluents containing excreta, organic matter (OM), nutrients, etc. If effluents are not adequately managed affect the quality of water, soils and human health. Therefore the utilization of the effluents for irrigation of crops and fodder after of its treatment with system of 2 or 3 lagoons is a good option to minimize effluents impact. There is little local information on the quality of livestock effluents and the impact of its discharge. The objective was to evaluate the effluents quality in order to determine the efficient of the treatments used. It is worked in three dairy farms of Río Primero Department, Cordoba. In the first (DF1) and second dairy farm (DF2) the water used is deposited in one lagoon that when is filled the content is discharged to the field. The third dairy farm (DF3) presented two lagoons: one for the water of the first wash (T3-FW) and other for the second wash (T3-SW). Two water samples were taken in each lagoon: one near the effluent input (I) and other in the farthest area (F). The samples were collected in sterile receptacles and stored refrigerated until analyses. In each sample the following parameters were determined: a) pH, b) total organic matter (TOM), c) mesophilic bacteria, d) total and fecal coliforms and e) *Escherichia coli*. The obtained data were compared with the allowable limits for the use of effluents for irrigations (Environmental Protection Agency of EEUU-EPA) and effluent discharge to surface water courses (Subsecretaría de Recursos Hídricos de Córdoba - SRH). The DF1 sample showed an alkaline pH, while the other samples presented pH near the neutrality, except for DF3-FL that showed an acid pH. The TOM was higher in the samples near the effluent input, whereas the lower values were obtained in DF2-F. All samples presented an elevated number of mesophilic bacteria, total and fecal coliforms and*E. coli.* No effluent met the allowed values (≤1000 fecal coliforms/100 mL) according EPA and SRH. The fecal coliforms exceeded the allowable limits, while pH values met the norms, except for T3-FW (I). Our results

show that the treatments settling lagoon is not an adequate system due to the great quantity of fecal and*E. coli*microorganisms founded which indicate that the discharge or utilization of this effluents for irrigation present a health risk for workers and consumers. The high TOM values indicate low microbial degradation, which could be due to the lagoons lack of aeration, generating anaerobic conditions that promote the development of anaerobic and fermenters microorganisms that produce bad smell, nutrient loss and slow degradation of OM. We suggest that dairy farms perform a treatment that include effluent aeration due to it will be promoted aerobic activity with high OM degradation and nutrient production. These effluents could be utilized for plant growth by coupling of plant-animal production, leaving to be waste to become resources

Código de Resumen: MS-008

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

BACTERIA WITH TOLERANCE AGAINST LITHIUM-CHLORIDE AND THEIR DIFFERENTIAL GROWTH BY SEM OBSERVATION

F.L. Martínez[1], V.B. Rajal[1 2], V.P. Irazusta[1].

[1]Instituto de Investigaciones para la Industria Química INIQUI-CONICET-UNSa. [2]Facultad de Ingeniería, Universidad Nacional de Salta.

tp2012pel@gmail.com

Fossil fuels, being non-renewable resources will eventually come to an end. This is the main reason why alternative energies are being studied. Clean energies are the most important and are thought as the future sources of energy for the planet. Lithium batteries have a special importance in this area because of their efficiency, since they can save relatively large amounts of energy in small sized and lightweight batteries. Trapping lithium compounds from the environment through microbial mechanisms would be really interesting because of the great uses it could have (soil decontamination after incorrect batteries disposal and obtaining lithium for battery production). Our investigation focuses on searching for microorganisms that are able to interact with lithium and grow in high-lithium concentration environments. After isolating bacteria from water and soil samples from El Salar del Hombre Muerto (Argentina), one of the most lithium-rich brines from South America, we selected the most tolerant to lithium chloride. Starting with 54 bacterial isolates, the following experiment was carried out: the bacteria were inoculated in 5 ml of minimum media (MM) with different lithium chloride concentrations and the tubes were incubated at 30°C and 200 rpm for 5 days. Growth was evaluated qualitatively by the development of turbidity, filamentous or pellets. Twenty-nine out of the 54 initial isolates grew at 30 g/l LiCl, and only 6 of them at 60 g/l LiCl. Seven strains were selected, 6 of which are rod shaped and only one is coccus. Gram staining allowed us to determine that 5 of them were found to be Gram negative bacteria and 2, Gram positive. Taxonomic identification is underway. These isolates were also observed by SEM. For that, they were grown in 50 ml of MM without salt and with 30 g/l LiCl in 250 ml Erlenmeyer flasks. After a week of incubation, the cells were harvested by centrifugation at 5400 rpm, fixed with formaldehyde and dehydrated by serial washes with increasing alcohol concentrations, until absolute alcohol. Then, the dehydrated samples were taken to LASEM (Laboratory of Scanning Electron Microscopy and Microanalysis) for the proper treatment for SEM observation (critical point drying and gold-covering). The cells grown in MM without LiCl showed a defined and normal shape. Interestingly, when grown in presence of LiCl, some cells were more round-shaped and in most cases an exopolysaccharide-like substance could be seen covering them. It is well documented in the literature that one of the mechanisms used by microorganisms against adverse conditions is the production of this kind of substances and the formation of biofilms, so it can be the case of our bacteria. We are looking forward in the future to establish the specific function and composition the secreted substance and the significance of changes in cell shape. Further investigation will be conducted to study the differential gene expression of the microorganisms for protein production in the presence and absence of lithium chloride.

Código de Resumen: MS-009

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

ANALYSIS OF *nifH* TRANSCRIPTS IN ARGENTINEAN SOILS: AN APPROACH TO EVALUATE BIOLOGICAL NITROGEN FIXATION

P. Calderoli[1], E. Ramos Cabrera[1], M. Collavino[2], M. Aguilar[1].

[1]Instituto de Biotecnología y Biología Molecular - CONICET CCT La Plata - UNLP. [2]Instituto de Botánica del Nordeste - CONICET- UNNE.

pcalderoli@yahoo.com.ar

Biologically available N is often a limiting nutrient in agricultural soil and other environments. About 4.32 x 10^5 tons of nitrogen fertilizers are used annually in the productive agricultural region of Argentina. In this context, we were interested in analyzing the active diazotrophic community in the Argentinean soils by assessing *nif*H-mRNAs transcripts using pyrosequencing technology. We sampled cropping fields located in Pergamino (Buenos Aires) subjected to the following no-till treatments: intensive crop rotation, nutrient replacement and minimal use of agrochemicals (good agricultural practices, GAP); soybean monoculture, low nutrient replacement and high agrochemical use (poor agricultural practices, PAP), and a grassland soil non disturbed by human activity used as a natural reference environment (NE). Each treatment was sampled at depths of 0-10 cm and 10-20 cm. A total of 112.493 reads were retrieved from the six pyrosequencing-derived datasets. Poor-quality reads (low-quality base-calling, frameshifts and chimeras errors) were removed and the final set of 28171 reads was clustered into 1661 OTUs defined at 98% amino acid sequence similarity. Finally, 437 OTUs with more than 3 sequences were selected and phylogenetic analysis was performed using reference *nif*H datasets (Zher et al., 2004) with the ARB software. The following *nif*H subclusters were represented in our datasets: 45% of 1A (*Anaeromyxobacter* y *Desulfuromonadales*), 29% of 1K (*Rhizobiales*), 17% of 1B (Cyanobacteria) and 8% of 3B (*Desulfovibrionales* y *Verrucomicrob*iales), while the remaining subclusters represented less than 1% of the total number of OTUS. The proportions of subclusters were clearly different in the soil depths analyzed. 1A OTUs (facultative anaerobic deltaproteobacteria) increased their relative proportion in the 20 cm whereas subcluster 1K (aerobic alphaproteobacteria) displays the opposite trend. On the other hand, subclusters 1B (cyanobacteria) and 3B (anaerobic bacteria) were represented at a significant proportion (>1%) only in the first 10 cm (1B 33%) and 20 cm (3B 15%), respectively. Most abundant OTUs from subclusters 1A, 1K, 1B and 3B were related to *Geobacte*r, *Bradyrhizobium*, *Nostoc* and *Anabaena*, and uncultured bacteria, respectively. Soil management practices also seem to affect the distribution of the active diazotrophs in both depths differentially. Subcluster 1K predominated (98%) in the upper layer and 1A (99%) at 20 cm depth in the soil under crop rotation (GAP). Under monoculture (PAP) treatment cyanobacteria (98%) predominated at 10 cm decreasing at 20 cm where the subcluster 1K (52%), 3B (45%) and 1A (3%) were found in greater proportion. By contrast, in the grassland soil (NE) the communities were found similar at both depths, with a high proportion of subclusters 1A (79-87%) and 1K (11%). We concluded that the structure of the active diazotrophic community displays features associated with the use, management and soil depth examined.

Código de Resumen: MS-010

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

MODULATION OF PLASMIDS CONJUGATIVE TRANSFER BY NOVEL HYPOTHETICAL PROTEINS

D. Dip[1 2], J. Nilsson[1 2], I.P. Salto[1 2], F.J. Albicoro[1 2], L. Cervantes[3], S. Brom[3], M. Pistorio[1 2], G.A. Torres Tejerizo [1 2].

[1] Instituto de Biotecnología y Biología Molecular. [2] Universidad Nacional de La Plata. [3] Centro dCiencias Genómicas. Universidad Nacional Autónoma de México. Cuernavaca, México..

albicoro@gmail.com

Rhizobia are gram-negative bacteria with ability to fix atmospheric N_2 in symbiotic association with the roots of legumes. The genomes of these bacteria are usually composed of a chromosome and various plasmids of sizes ranging between 40 and 2000 Kb. Plasmids have played a major role in bacterial evolution, mainly by their capacity to perform horizontal gene transfer. Plasmid pLPU83a, an accessory replicon from *Rhizobium* sp. LPU83, is able to transfer from its parental strain (*ca.* 10^{-5} transconjugants / donor cell), from *Ensifer meliloti* or from *Rhizobium etli*, but not from *Agrobacterium tumefaciens.* The mechanisms that regulate conjugative transfer (CT) of pLPU83a are not fully understood. In this work, we analysed structural and functional aspects of the CT of pLPU83a. Bioinformatics analyses were performed; this plasmid contains a complete set of transfer genes, featuring a particular organization, shared with only two other rhizobial plasmids. The elements required for CT are organized as a cluster of genes involved in formation of the mating pair (Mpf, Mating pair formation), genes involved in the processing of DNA (Dtr, DNA transfer and replication), and an *oriT* site, where transfer is initiated. The plasmid contains a TraR quorum-sensing (QS) transcriptional regulator, but lacks an acyl-homoserine lactone synthase gene. Furthermore, between the Dtr and the Mpf we found three genes encoding hypothetical proteins that could not be assigned to a known function. Similarly, hypothetical genes are often located in contiguous regions in bacterial genomes and, recently, Lopez-Fuentes *et al.* showed that the genes located among conjugation genes are involved in CT of a plasmid of *Rhizobium etli* CFN42, pRetCFN42a . In order to determine if the hypothetical-protein encoding genes localized in pLPU83a participate in CT, we constructed mutant derivatives and analyzed their phenotype. We obtained insertional mutations in three genes of pLPU83a (LPU83a_00145, LPU83a_00146 and LPU83a_00148) by homologous recombination and evaluated their conjugative transfer properties. The mutation of LPU83a_00145 did not modify CT (*ca.* 10^{-5} transconjugants / donor cell), the mutation of LPU83a_00146 enhanced CT (*ca.* 10^{-3} transconjugants / donor cell) while the mutation of LPU83a_00148 abolished CT (< 10^{-9} transconjugants / donor cell). Complementation of the mutants restored the conjugative transfer frequencies to the wild-type levels. The mode of participation of these genes in CT is still unknown. Elucidation of the mechanisms that differentially regulate plasmid CT will be helpful to understand the boundaries of plasmid exchange in bacteria.

Código de Resumen: MS-011

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

POPULATION DYNAMICS OF *Fusarium graminearum* SPECIES COMPLEX IN A FILD WITH SOYBEAN/WHEAT ROTATION

M.L. Chiotta[1], J.M. Palazzini[1], E. Alberione[2], G. Barros[1], S. Chulze[1].

[1] *Universidad Nacional de Río Cuarto.* [2] *INTA Marcos Juarez.*

gbarros@exa.unrc.edu.ar

Soybean crop in Argentina is one of the more relevant economic activities and it is commonly grown in rotation with wheat. Infection by *Fusarium graminearum* has been observed in wheat causing yield and quality losses. Recently, this species and others within the *F. graminearum* species complex (FGSC) were identified as soybean pathogens in South America, producing pod discoloration, seed decay and root rot beside trichothecene contamination. However, the dynamic of FGSC population in a mixed cropping system of soybean/wheat has not yet been evaluated. Thus, to determine whether the wheat crop is an important primary inoculum source of FGSCin the soybean agro-ecosystem, a study in a field with soybean/wheat rotation during 2012/13 and 2013/14 harvest season was carried out. The FGSC population was monitored evaluating wheat debris, implanted seed and different vegetative and reproductive developmental stages of the soybean. In the vegetative stage, root at V1 was analyzed while in the reproductive stage, pods and seeds at R6 (fully developed seed) and R8 (total maturity) were analyzed. The isolation in roots was performed on internal tissues and rhizoplane. Samples of wheat debris were collected during the V1, R2 (total flowering), R6 and R8 stages. FGSCspores in the air surrounding the soybean lot evaluated were collected during R6 stage in Petri plates containing Nash-Snyder selective medium. The plates were placed exposed to wind on the soil surface and others on the plant canopies. Contamination with deoxynivalenol (DON) was evaluated according to Barros et al. (2008). The results showed that the infection percentages of FGSC in debris varied from 4 to 21% during two years evaluated. In the internal tissues of roots were not observed infection and only during the second year in the rhizoplane were detected low levels (8%). Seed contamination was higher in R6 stage than R8 in two harvest seasons, 7 and 0% in 2012/13 and 26 and 4% in 2013/14 respectively. Regardless DON contamination, in 2012/13 harvest only one seed sample at R6 stage showed contamination at level of 0.3 ppm while in 2013/14 harvest a higher number of samples both R6 and R8 stages showed contamination with DON at levels ranging from 0.7 to 4.3 ppm. In air samples, the infection percentage was higher in samples placed on the soil surface ranged from 5 to 12%. The data demonstrate that FGSC inoculum is higher in debris than air surrounding the soybean. The declination in seed infection rates from their maximum development to maturity could be due to decrease of the water availability in seeds. Higher moisture levels recorded during the 2013/14 harvest could explain the higher levels of DON contamination in seeds. Therefore, environmental conditions predisposing could indicate a higher potential risk of DON contamination, mainly at R6 stage.

Código de Resumen: MS-012

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

MYCELIAL COMPATIBILITY OF *Fusarium graminearum* SPECIES COMPLEX ISOLATES FROM CROP RESIDUES IN A FIELD WITH WHEAT/SOYBEAN ROTATION

M. Bonacci[1], M.L. Chiotta[1], G. Barros[1].

[1] *Universidad Nacional de Río Cuarto.*

martin_bonacci@hotmail.com

Members of *Fusarium graminearum* species complex (FGSC) are economically important pathogens of cereals crops causing yield and quality losses in their production. In Argentina, soybean often is used in rotation with wheat in a reduced till or no-till system. Species within the FGSC can survive in crop residues left on the surface, increasing the inoculum density and providing an inoculum source for wheat infections in subsequent year. In filamentous fungi, the ability to distinguish self from non-self is essential for vegetative growth, sexual reproduction and defense against pathogen invasion. This vegetative non-self-recognition could be identified by micelial incompatibility assay. The aim of the present work was to differentiate genotypes in FGSC isolates that could be interacting in crop residues. Fifty isolates of FGSC isolated from crop residues in a field with wheat/soybean rotation were selected for this study. For test mycelial compatibility interactions, 1225 pairs representing all possible combinations of FGSC isolates were grown on V8-soybean medium and incubated at 25°C for 5-7 days. A compatible reaction was indicated by mycelial continuity between the interacting colonies without a zone line. An incompatible reaction was indicated by formation of a barrage zone (thick mycelial layer and/or a dark pigmented line between the paired isolates). All FGSC isolates were self-compatible,except for two isolates that were excluded from further analysis. Out of 1175 pairings between isolates, 104 were compatibles representing 8,9% of the pairings. These results showed a high level of diversity within the population of FGSC as it was suggested in previous studies using vegetative incompatibility (VCG

analysis) and amplified fragment length polymorphism (AFLP) markers. Mutually compatible isolates had very different patterns of compatibility with the other isolates. This suggests that barrage formation is determined by different, although possibility overlapping set of loci to those controlling vegetative compatibility.

Código de Resumen: MS-013

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

USE OF RECOMBINATION-BASED *IN VIVO* EXPRESSION TECHNOLOGY (RIVET) FOR THE IDENTIFICATION OF *Sinorhizobium meliloti* GENES INDUCED BY SUB-LETHAL CONCENTRATIONS OF HYDROGEN PEROXIDE

D. Escanciano[1], M.E. Salas[1], F. Alvarez[1], J.L. López[1], W.O. Draghi[1], A. Lagares[1], M. Lozano[1].

[1] *Instituto de Biotecnología y Biología Molecular, Fac. Cs Exactas, Universidad Nacional de La Plata.*

alvarezflorencia.mail@gmail.com

Sinorhizobium meliloti is a gram-negative proteobacterium able to establish nitrogen-fixing symbiosis with legumes of the genera *Medicago*, *Melilotus*, and *Trigonella*. This symbiotic interaction is a highly regulated process that involves a complex bidirectional molecular dialog which ends with the differentiation of the rhizobium into a nitrogen fixing bacteroid and the generation of a new plant organ, the nodule. Several of the signal molecules involved have been identified, and among them, a signal role for the hydrogen peroxide has been proposed. In this work we make use of a *Recomination-basedin vivoexpression technology* (RIVET) system modification created in our laboratory for the identification of genes induced during the growth of *Sinorhizobium meliloti* in complex media supplemented with sub-lethal concentrations of hydrogen peroxide. Using this approach we were able to identify that the *cyaD1* gene, which shows a typical type III adenylate cyclase/guanylate cyclase (AC/GC) structural organization (namely, an amino-terminal signal peptide, a CHASE2 extracellular domain, a set of three membrane-spanning domains, and a cytoplasmic catalytic domain), is differentially induced by hydrogen peroxide. This fact might indicate a possible role of the second messenger cAMP in the signaling path of hydrogen peroxide during the symbiotic interaction. Preliminary results obtained with a *cyaD1* mini-Tn5 insertional mutant indicate also a possible role of this protein in hydrogen peroxide tolerance.

Código de Resumen: MS-014

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF CLASS 1 INTEGRONS IN PLASMID-CARRYING BACTERIAL ISOLATES RECOVERED FROM A BIOPURIFICATION SYSTEM USED FOR PESTICIDE REMOVAL

M.P. Vieta[1], M.C. Martini[1], M.P. Quiroga[2], D. Centrón[2], A. Lagares[1], M.F. Del Papa[1].

[1] *IBBM - Instituto de Biotecnología y Biología Molecular, UNLP-CONICET, La Plata, Argentina..* [2] *Laboratorio de Investigaciones en Mecanismos de Resistencia a Antibióticos, IMPAM (UBA/CONICET).*

martini.mcarla@gmail.com

Biopurification systems (BPS) are complex soil-related and artificially-generated environments usually designed for the removal of toxic compounds from polluted wastewater sources. BPS receives high loads of pesticides and other toxic compounds, thus imposing a strong, constant and long-term selective pressure on the evolution and growth of the associated microbiome. Recently, we have isolated and characterized a collection of 35 plasmid-carrying bacterial isolates recovered from a BPS contaminated with more than 50 different pesticides. These cultivable bacteria were selected using a plasmid-screening based method, in order to study the role of plasmids in such environment. It is well known that mobile genetic elements (MGEs) play an essential role in the evolution and adaptation of bacteria as they encode accessory genes that increase bacterial fitness in a given environment. In order to go deeper in the analysis of MGEs, we decided to expand our study looking for the presence of type 1 integrons, which have been found in plasmid backbones. Integrons are assembly platforms that incorporate exogenous open reading frames harbouring *attC* sites by site-specific recombination and convert them to functional gene cassettes by ensuring their correct expression. In particular, class 1 integrons are widely distributed in the clinical setting as well as in natural environments. Their ability to acquire, exchange, and express antimicrobial resistance gene cassettes, makes them an important MGE to be studied. The aim of this work was to investigate the presence of class 1 integrons as well as their genetic content, in a plasmid-containing bacteria collection obtained from a model on-farm biofilter used for wastewater decontamination in intensive agricultural production. In order to evaluate the presence of class 1 integrons, we performed a PCR-based screening for detection of the integrase of type 1 (*intI1*) . Out of the screened isolates, 23% were positives for *intI1*, thus

suggesting the presence of class 1 integrons in the collection. In a second step, we investigated the presence of genes that frequently constitute the 3´conserved fragment in this type of integrons. The screening revealed that all isolates that contain the *intI1* gene posses also the *sul1* and *qacE* genes, wich confer resistance to sulfonamides and quaternary ammonium compounds, respectively. Besides, 4 of these isolates were positive for *qacEΔ1*, a *qacE* gene variant. In order to go deeper in the integron characterization, a second PCR-based screening was performed using primers that bind to the 5´and 3´conserved fragments, flanking the variable region. Out of the 8 integron-containing isolates, 3 of them gave a PCR fragment whose sequencing and analysis revealed the presence of a adenylyltransferase gene, which in all cases confers resistance to streptomycin. The analysis of MGE in soil bacteria is a critical issue for improving our current understanding of the mechanisms underlying microbial-genome dynamics.

Código de Resumen: MS-015

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

NODULATION STUDIES IN NATIVE LEGUMES FROM CENTRAL ARGENTINA

L. Bianco[1], R. Malpassi[1], J. Angelini[1].

[1] *Universidad Nacional de Río Cuarto.*

lbianco@ayv.unrc.edu.ar

Although several nodulation studies in native legumes have been carried out in different countries in the world, there are many native legume species in Argentina that might be able to nodulate and fix atmospheric nitrogen but they have not been studied in their natural ecosystem yet. The nodulation study and the characteristics of endosymbionts associated with native legumes will allow to conserve, manage, and use the species of interest. Such species and their symbionts represent a unique germplasm that can be taken to enhance progress in agriculture and then be used to mitigate and adapt to the effects of climate change. The aim of this study is to evaluate the nodulation status of native legumes in their natural ecosystem, the different phenotypes of nodulation, and the anatomy of their nodules. Sampling was conducted in Río Cuarto, Calamuchita, and Tercero Arriba Departments (Province of Córdoba). Nodules of *Acacia caven* (Molina)Molina var. *dehiscens* Burkart ex Ciald., *Rhynchosia senna* Gill. ex Hook. var. *senna,* and *Robinia pseudoacacia* L. were collected. The presence or absence of nodules was recorded. Also, the location, the phenotypic appearance, and color inside thereof were described for determining the presence of leghemoglobin. Transverse and longitudinal serial sections of nodules were made to make histological slides. Nodules are found in the lateral roots of all species studied and they are red inside. These species have nodules of different types. *Acacia caven* show no-branched indeterminate nodules, while *Robinia pseudoacacia* show branched indeterminate ones. Furthermore, *Rhynchosia senna* show spherical determinate nodules, that corresponds to the desmodiod type. In cross sections, all nodules show two zones: cortex and bacterial zone. The three species have periderm and vascular system in the cortex. In the cortical zone, *Acacia caven* exhibits some cells with tannins. In indeterminate nodules, a meristematic zone between cortex and bacterial zone is formed. The three species have the central area occupied by the infection zone, which is continuous and is formed by infected and uninfected cells. *Robinia pseudoacacia* cells of both cortex and bacterial zone show abundant starch. In this study the ability of nodulation of three native legumes in their natural environments were confirmed. Different nodulation phenotypes (morphology and structure) were observed in each species, what it could be related to specific characteristics of the genera.

Código de Resumen: MS-016

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

AGRICULTURAL MANAGEMENT INFLUENCE ON VINEYARDS SOIL QUALITY INDICATORS

E. Medina[1], H. Paroldi[1 3], A. Vega Avila[2 4], V. Pesce[1 2], N. Manrique[2], F. Balaguer[3], M. Toro[1], F. Vazquez[1].

[1] *Instituto de Biotecnología. FI. UNSJ.* [2] *Departamento de Agronomía. FI. INTA. UNSJ.* [3] *Departamento de Biología. FCEFYN. UNSJ.* [4] *INGEBI. CONICET.*

ferbalaguer@hotmail.com

Soil quality is defined as its ability to function as a living system. Soil microbiological properties (vg. enzyme activity) and physicochemical properties (vg. level of nutrients) have been used as soil quality indicators. These properties are very sensitive to changes triggered by agronomic management (tillage system, use of agrochemicals, addition of organic materials). The aim of this work was to assess impact of different agronomic management (zero, conventional and organic) on macronutrients and enzyme activities associatedtovineyard soils. Soil samples (0-10 cm depth) were randomly taken in April and November (2013) according to the physiological state of the vine in rows and in soils between rows of the three systems. Total nitrogen was determined using the Kjeldahl digestion technique. The availability of potassium (K) and phosphorus (P_2O_5) was measured by

atomic absorption spectrum. The enzyme activity values were determined by p-nitrofenol colorimetric technique, using as substrate paranitrophenyl-β-D-glucopyranoside (β-glucosidase activity) and p-nitrophenyl phosphate (acid phosphatase and alkaline phosphatase activity). Results showed that phosphorus and nitrogen values were significantly higher in soils of inter-rows associated to organic system in the first sample (p=0.0001), whereas potassium content was significantlyhigher in soils of inter-rows correspondingto zerotillagesampling in April and November (p=0.0001). β-glucosidase activity values were similar in the three systems studied (p=0.0235). Acid phosphatase activity was significantly higher insoilsof rows associated to zero tillage of the first sampling (p=0.0406). Values of alkaline phosphatase activity were significantly higher in soils of inter-rows associated to organic management of the first sample (p=0.0253). Macronutrients values and enzyme activities were lower in soil conventional management system. These results suggest that agricultural system where agrochemicals are used cause a decrease in microbiological indicators, affecting soil vineyard quality.

Código de Resumen: MS-017

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

TEMPORAL VARIATIONS OF MICROBIAL PHOSPHATASE ACTIVITY AND AVAILABLE PHOSPHORUS IN SOILS AMENDED WITH OLIVE MILL RESIDUES

H. Paroldi[1 3], E. Medina[1], A. Vega Avila[4 5], V. Pesce[1 4], F. Balaguer[3], M. Toro[1], F. Vazquez[1], P. Monetta[2].

[1]Instituto de Biotecnología. FI. UNSJ. [2]Estación Experimental Agropecuaria San Juan. INTA. [3]Departamento de Biología. FCEFYN. UNSJ. [4]Departamento de Agronomía. FI. INTA. UNSJ. [5]INGEBI. CONICET.

ferbalaguer@hotmail.com

Alperujo (oiled two-phase olive mill waste) can be used as soil amendment inolive groves. This practice has been widely evaluated in Mediterranean countries, however, there is still little consensus about the effects that it may produce on soils quality microbiological indicators. Soil degradation is closely related with the diminution of the available phosphorus and phosphatase activity, therefore their quantitation is an important indicator of soil quality. Soil phosphatases are mostly microbial exoenzymes involved in the biochemical process of phosphorusmineralization. These enzymes are responsible for the inorganic phosphorus release from organic and inorganic compounds. In phosphorus cycle they have an essential role in the phosphate solubilization process, increasing its availability for crops.The objective of this study was to evaluate temporal variations of phosphatase activity and available phosphorus in olive groves soils within two years of alperujo applications. The assays were performed in San Juan, Argentina, during 2013 and 2014 in an intensively managed olive orchard. Samples (0-15 cm depth) were taken twice a year (in winter and summer seasons). Treatments were: 1- superficial application of alperujo (50 kg pl^{-1}), 2-superficial application of alperujo (50 kg pl^{-1}) with subsequent incorporation to first 15 cm of soil. Controls were: a- Unremoved soil without amendment, b- Removed soil without amendment. The available phosphorus (P_2O_5) was measured by colorimetric assays. The enzymatic activity values were determined by p-nitrophenol colorimetric technique, using p-nitrophenylphosphateas substrate. Results of availablephosphorus and enzymatic activity were different among thedifferent treatments and controls. These differences were closely related with alperujo soil treatments. Analyzed data of the first year showed no significantdifferencesin the summer samples, while in the winter samples the alperujo incorporated treatment showed the highest values with significant differences for both variables, 127,67 +/- 26,73 ppm $P_2O_5$and 182,77 +/-27,8 gp-NF. In summer sample of the second year both treatments showed higher values than control treatments, while in the winter samplesalperujo incorporated treatment recorded the highest values. These results show that the addition of alperujo as amendment increase the available phosphorus and phosphatase activity.Both of them are important factors related to improving soil quality.

Código de Resumen: MS-018

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

AGGRESSIVENESS OF *Fusarium graminearum* SPECIES COMPLEX ISOLATES ON ROOT INFECTION OF SOYBEAN VARIETIES FROM DIFFERENT MATURITY GROUPS

M.L. Chiotta[1], M. Bonacci[1], S. Chulze[1], G. Barros[1].

[1]Universidad Nacional de Río Cuarto.

gbarros@exa.unrc.edu.ar

Members of *Fusarium graminearum* species complex (FGSC) are recognized as a primary pathogen of soybean in several countries in the American continents. The presence of species belonging to *Fg* complex in soybean may be attributable to the increase of the conservation tillage system in Argentina. The no-tillage cultivation must leave at least 30% of the crop residues on the soil surface and members of *Fg* complex has been shown to readily colonize crop debris left behind wheat, corn and soybean. The objective of this study was to compare the aggressiveness of crop residues isolates within the FGSC on root infection of soybean varieties from different maturity groups under controlled conditions. The evaluation of seedlings height and disease severity was performed in a rolled-towel assay described by Xue et al. (2007). Soybean seeds of three varieties from maturity group IV (planting late, medium and early-maturing varieties) were used in this study. Ten isolates characterized morphologically as strains belonging to the FGSC were included. These isolates were collected in a previous study during the 2012/2013 harvest season from an experimental field at EEA INTA Marcos Juarez, Córdoba. Ten days after inoculation, plants were removed from the growth unit and visually assessed for root-rot severity. Symptoms were rated using a 0-4 scale: 0, no visible disease symptoms; 1, lesion visible, but infection confined to the inoculation site, with normal seedling growth; 2, lesion size extended and the plant growth retarded; 3, infection of the entire root, and the plant growth halted; and 4, massive infection of the entire root resulting in plant death. In the rolled-towel assays the seedling height of the inoculated seedlings was reduced by all isolates in the three cultivars relative to the control seedlings. All isolates caused root rot and there were significant differences in disease severity among isolates were observed in both experiments. The mean disease severity averaged across all isolates ranged between 1 and 2 in a 0-4 rating scale where 0 = healthy seedling and 4 = dead seedling. Analysis of variance demonstrated significant differences for genotype–isolate interactions for disease severity. Based on contrast analysis, no significant differences were detected in the susceptibility of three of soybean varieties from different maturity groups.

Código de Resumen: MS-019

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

EFFECT OF RAPESEED (*Brassica napis* L) INOCULATION ON ROOT MYCORRHIZATION, PLAT GROWTH, AND NODULATION ON THE SUBSEQUENTE SOYBEAN (*Glicyne max*) CROP

L. Valetti[1], L. Iriarte[2], A. Fabra[1].

[1]Depto. Cs. Naturales. Fac. Cs. Exactas Fco-Qcas y Naturales. Universidad Nacional de Río Cuarto. [2]Chacra Experimental Integrada de Barrow. INTA.

lvaletti@exa.unrc.edu.ar

Mycorrhizae are mutualistic interactions between nearly all plants on earth and soil fungi (Basidiomycota, Ascomycota y Glomeromycota). Mycorrhizae increase the phosphorus absorption by roots making this nutrient available to the plants and the fungus benefits from a steady supply of organic nutrients (Bago et al., 2003). However, plants belonging to the *Brassicaceae* family, generally *do not* associate with mycorrhizal fungi (Tester et al., 1987). Little is known about the effect of rapeseed cultivation on mycorrhizal colonisation of root plants from subsequent crops. In Argentine, one of the crops frequently used in rotation with rapeseed is soybean. The aim of this study was to evaluate the effect of the inoculation of rapeseed seeds with plant growth promoting rhizobacteria (PGPR) on soybean plants growth, nodulation and arbuscular mycorrhiza (AM) root colonization. Soybeans seeds , inoculated with *Bradyrhizobium japonicum* E109, were sown in pots containing soil samples taken from plots where inoculated and uninoculated rapeseed have been growing. Soybean plants were growing under controlled environmental conditions, and harvested after two months. Plant growth, symbiotic behavior and the percentage of roots infected with AM were evaluated. Results indicated that there were no differences in plant growth and the number of nodules formed among the different treatments. However, the percentage of roots colonized by AM in plants growing in soil without rapeseed history was significantly higher than in plants growing with soil previously cultivated with rapeseed inoculated or uninoculated. The results of this study demonstrate that rapeseed cultivation has a negative effect on the soybean AM root colonization when this follows in crop rotation. However, the nodulation and growth of soybean plants is unaffected under the conditions used in this study.

Código de Resumen: MS-020

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

EFFECT OF PROTEOLYTIC ACTIVITY OF *Bacillus subtilis* ON BIOFILM FORMATION AND POLYHYDROXYALKANOATES ACCUMULATION

M.E. Galelli[1], G.C. Sarti[1], S.S. Miyazaki[1].

[1] *Área de Agroalimentos, Dpto Biología Aplicada y Alimentos, FAUBA.*

mgalelli@agro.uba.ar

Biofilms are formed by a matrix that creates a regulated environment for microbial communities that allows the passage of nutrients, gases and water and restricts the entrance of harmful substances. Biofilm formation is dependent on the environment in which the bacterial cells are in their planktonic form. During biofilm development enzyme systems are activated, including proteases. Another aspect of bacterial survival is the accumulation of polyhydroxyalkanoates (PHA). *Bacillus subtilis* (bacterium considered as generally recognized as safe (GRAS) by the Food and Drug Administration) is able to produce biofilms free of endotoxins and accumulate PHA. In this work it was studied the production of proteolytic enzymes in different culture media and their relationship with the formation of biofilms, spores and PHA synthesis. *B. subtilis* free of plasmids innoculum was one percent (v/v) of a 12h growth at 30°C in nutritive agar. The culture media were nutritive broth with glucose, mannitol or xylose. It was also studied the effect of different inorganic nitrogen sources. The biofilm formation was determined in static conditions at 48 h incubation. Protease activity was measured with azocaseine. The sporulation rate was determined as the number of viable cells present after a treatment at80°C for 20 min. The PHA was determined by fluorescence with Nile Blue and by crotonic acid formation. Data were analyzed by ANOVA test. The proteolytic activity of the liquid medium under the biofilm was dependent on the carbon source, being higher for xylose and mannitol than for glucose (87; 70 and 11 protease units, respectively). This activity was directly correlated with the production of biofilm (0.27; 0.20 and 0.13 mg biofilm/ml incubated) and spore formation in the biofilm (2,0E+07; 6,2E+04 and 6,0E+02 cfu/mg biofilm). Instead, the protease activity was inverse to PHA accumulation in the biofilm (48; 79 and 132 µg PHA/mg biofilm). Using mannitol as carbon source, the presence of inorganic nitrogen (ammonium and nitrate) decreased proteolytic activity in the static liquid media (12 and 15 units protease, respectively) and the biofilm formation (0.13 and 0.17 mg biofilm/ml incubated); nitrate but not ammonium increased PHA accumulation in the biofilm (115 and 78 µg PHA/mg biofilm). These results suggest that the proteolytic activity could have an important role in bacterial biofilm production, being directly related to spores formation and inversely with PHA accumulation. This might suggest that the *B. subtilis* in the biofilms from diverse culture media could favored the generation of bacterial structures in the biofilm with different capacities to survive in the environment, according to the spore formation and the PHA accumulation in the sessile cells.

Código de Resumen: MS-021

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

ISOLATION AND USE OF EXTREMOPHILES IN THE DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND IN SALINE WASTEWATER

M. Grosselli[1], J. Aparicio[2], L. Vilegas[1], P. González[1], C. Almeida[1].

[1] *Instituto de Química de San Luis, INQUISAL (UNSL-CONICET), Chacabuco 917. San Luis, C.P. D5700BWQ, A.* [2] *PROIMI (Planta Piloto de Procesos Industriales Microbiológicos), CCT-Tucumán. .*

lbvilleg@hotmail.com

Biochemical oxygen demand (BOD) is a useful parameter for assessing the biodegradability of dissolved organic matter in water. At the same time, this parameter is used to evaluate the efficiency with which certain processes remove biodegradable natural organic matter. However, the values of BOD in saline water are very low because NaCl destroy the seed. Therefore the incorporation of a suitable selection of bacteria (which is not always easy to obtain) is required. In this sense the extremophile bacteria, particularly halophilic bacteria or halophytes can contribute to the development of this technique. The objective of this work was to develop a BOD analysis protocol for the analysis of saline effluent. Within this context a halophilic microorganisms was isolated from Salinas del Bebedero, San Luis, Argentina. Salt sample was cultured in agar Dussault and Lachance. Subsequently, the isolated colonies were cultured in medium Dussault and Lachance broth for 7 days at 37ºC with constant stirring (180 rpm). Subsequently the nucleic acid extraction method was performed by Brosius et al., based on the lysis of the cells with glass beads, and 10% SDS. To amplify the variable region V3-V5, 16S rDNA primers described as F344-R915 (Stahl et al, 1991) were used: 344F: 5'- ACG GGG CGC YGCAGCAGG GA-3 'and 915R: 5'- GTG CTC CCC CGCCAATTC CT-3 '; *Halorubrum salsolis* DNA was used as control. The size of the reaction products was characterized on agarose gel 1% and evaluated on an image analyzer. The molecular weight was determined by comparison with molecular weight marker 100 bp DNA Ladder (Promega). The PCR fragments were sequenced by Macrogen (Korea), with Reaction Kit READY PRIMS a model ABI prisma373A sequencer (PE AppliedBiosystems). The sequencing results were analyzed by comparison with 16S rRNA genes databases (GenBank). The organism under study under study showed a 100% identity to gender *Haloarcula sp*. One milliliter of the strain (10^6 cell per mL) was used as seed in the BOD analysis protocol. Standard solution of glucose-glutamic acid (BOD = 20 mg L^{-1}) with different concentrations of NaCl (5%, 10%, 20%, 30% and 40%) were used. BOD bottles were incubated at 20 °C for 5 days. No significant differences were found among differents samples, since their organic matter content was similar (CV <2.1%). In conclusion, *Haloarcula sp* can be used in the determination of BOD in saline effluent.

Sección: Microbiología Ambiental y del Suelo

Modalidad: Poster

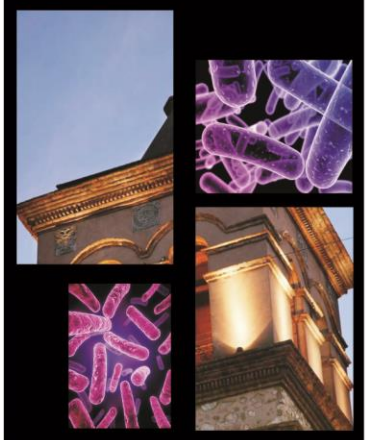
PHENANTHRENE DEGRADATION BY *Halobacterium piscisalsi*: DESIGN AND OPTIMIZATION OF A METHOD FOR BIOFILM CULTIVATION IN CONTINUOUS FLOW SYSTEM

L. Di Meglio[1], J.P. Busalmen [2], D. Nercessian[1].

[1] *IIB (UNMdP-CONICET).* [2] *Laboratorio de Bioelectroquímica (INTEMA-CONICET).*

ldimeglio@fi.mdp.edu.ar

Phenanthrene is a three ring polycyclic aromatic hydrocarbon (PAH) used as a PAH model to study aerobic degradation. PHAs are highly toxic because of their irritant, mutagenic and carcinogenic effects on living organisms. One important source of PHAs pollution is the produced water of oil production process, which is indeed generated in large amount. Bioremediation has become one of the most effective and economical strategy to remove these contaminants. This 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. Because of the high salinity of produced water, microorganisms conventionally employed in PHAs remediation cannot be effective in biological treatment of this wastewater, which took researchers to consider extremophilic microorganisms, as halophilic archaea. Firstly, we identified which of the microorganisms isolated from La Pampa saltern ponds (Argentina) were able to degrade phenanthrene in planktonic culture. These 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). *Halobacterium piscisalsi* was the microorganisms selected to perform biofilm assays due to its capacity to completely degrade phenanthrene and its higher growth rate than the other isolates. Biofilm assays were performed in a continuous flow growth chamber, using glass coverslips as substrate for the sessile population. With this system, we could study microbial activity *in situ* through direct microscopic observation. For the optimization and tune up of the biofilm system, different strategies were conducted in order to obtain phenanthrene derivatized cover slips. Time of cell adhesion (before starting the continuous flow) was adjusted to achieve cell attachment to the hydrophobic surface. Flow rate and temperature assay were also adjusted to avoid crystals dissolution. Results suggest that *H. piscisalsi* is able to develop biofilms on phenanthrene derivatized substrate and to grow using it as its sole carbon source. Phenanthrene crystals degradation through time was observed and the association between cell and phenanthrene crystals was determined by scanning electronic microscopy (SEM). As phenanthrene emits in the blue range when excited with UV, we are optimizing a quantification methodology to follow fluorescence along time. To achieve this, we are changing different parameters like microscopic magnification and UV exposition, aiming to develop a phenanthrene biodegradation system not described before.



BIOTECNOLOGÍA Y FERMENTACIONES

Código de Resumen: BF-001

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

EFFECTS OF GLOBAL REGULATORS ON THE PRODUCTION OF ORGANIC ACIDS AND SOLVENT TOLERANCE IN *Escherichia coli*

D.E. Egoburo[1], M.S. Godoy [1], M.J. Pettinari[1].

[1] *Universidad de Buenos Aires.*

diego_ezeth@hotmail.com

Transcriptional regulation in *Escherichia coli* comprises a network of specific and global regulators. Some of the latter control operons related to central metabolism affecting carbon flow and reducing power. Mutations in these genes may be advantageous for the production of compounds of biotechnological interest. Among different global regulators that affect central metabolism we studied ArcA, CreC, Cra and Rob. The majority of Cra targets are genes coding for the enzymes involved in central carbon metabolism. Rob is involved in solvent tolerance and affects some genes of glucose metabolism and TCA cycle. ArcA is known to be one of the main regulators that affects C metabolism in response to O_2 availability, while CreC is known to respond to both C source and aeration. To investigate the effect of deleting these regulators on the metabolism of *Escherichia coli*, cultures of mutants in each regulator were carried out in M9 mineral medium supplemented with glucose 5 g·liter^{-1} as the sole carbon source. Different conditions of oxygen availability were assayed: (I) low aeration (125 rpm and 1:2 flask-volume:medium-volume ratio) and (ii) anaerobiosis (sealed bottles full of media, with vertical rotation – 4rpm). Metabolic profiles showed that all mutations affected carbon distribution in low oxygen availability. Cultures grown in low aeration conditions showed a significant increase in the production of lactate in *arcA* mutants, both lactate and acetate for the *rob* mutant and an increase in succinate and acetate formation for the *cra* mutant strain. When the *cra* and *rob* mutants were grown in anaerobiosis, an increase in the production of all acids was seen for the *cra* mutant. Interestingly, a significant increase was observed in the production of succinic acid in this mutant, probably through increased carbon flow in the reductive pathway of TCA and inhibition of the *sdhCDAB* complex. However, no significant differences were seen for the *rob* mutant. MICs assays revealed that inactivation of *rob* did not increase ethanol sensitivity, probably due to the overlapping roles of *marA* and *soxS*. On the other hand, the *cra* mutant strain showed an increased sensitivity to ethanol compared to wild type strain, suggesting that it could be involved in alcohol tolerance. In conclusion, all global regulators seemed to affect carbon distribution and to behave differently in the aeration conditions tested. Results obtained with the *cra* mutant indicates that manipulation of this regulator could be potentially useful to increase the accumulation of some bioproducts (as seen in the *cra* mutant) or to enhance resistance to solvents (that could be achieved by the overexpression of the regulator). As for Rob, even though no effects were seen in anaerobiosis, more experiments must be done to evaluate its effect on central metabolism in other conditions or genetic backgrounds.

Código de Resumen: BF-002

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

CHARACTERIZATION OF THE CT DOMAIN OF SlpA FROM *Lactobacillus acidophilus* ATCC 4356 AND ITS USE AS AN ANCHOR TO DISPLAY HETEROLOGOUS PROTEINS ON THE SURFACES OF LACTIC ACID BACTERIA

P. Waehner[1], J. Fina Martin[1], L. Malone[1], M. Allievi[1], J. Tarsitano[1], M. Prado Acosta[1], S. Ruzal[1], M.M. Palomino[1].

[1] *Departamento de Química Biológica, FCEN-UBA, IQUIBICEN-CONICET.*

pwaehner@qb.fcen.uba.ar

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 SlpA 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 means of the C-terminal (CT) region of S-layer protein SlpA from *Lactobacillus acidophilus* ATCC 4356. To evaluate the potential application of the CT domain of SlpA as an anchoring protein, the green fluorescent protein (GFP) was fused to the N-terminus of two different CT regions, which differ in length and quantity of charged amino acids. The fused proteins were successfully produced in *Escherichia coli*. Subsequently, the purified GFP-CT

regions were added to various lactic acid bacteria (*L. casei, L. plantarum, L. acidophilus, L.brevis* and L. *helveticus*) cells *in vitro*, and the binding was viewed by fluorescence microscopy. Different growth conditions and cell pretreatments were evaluated. Cell grow to stationary phase showed higher GFP binding than exponentially did. In addition, when cells were depleted for their native S-layer, by extraction with LiCl or SDS pretreatment exhibited an increased binding of the GFP-CT. Furthermore, *L. acidophilus* cells grown in a culture containing NaCl exhibited increased binding to the GFP-CT. In order to evaluate which CT regions has a better binding and different conditions to improve the attachment to the *Lactobacillus* carrier, experiments of flow cytometry and fluorimetry are being performed to quantify the binding capacity. In conclusion, the CT of SlpA protein fused to a foreign protein like GFP 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 lactobacilli decorated are non- genetically modified organisms, therefore their GRAS status is not altered.

Código de Resumen: BF-003

Sección: Biotecnología y Fermentaciones

Modalidad: Oral

ANTILISTERIAL PEPTIDES FROM SPANISH DRY-CURED HAMS: PURIFICATION AND IDENTIFICATION

P. Castellano[1], L. Mora[2], E. Escudero[2], C. Melian[3,1], G. Vignolo[1], F. Toldrá[2].

[1]Centro de Referencia para Lactobacilos (CERELA), Chacabuco 145, T4000ILC, Tucumán, Argentina. [2]Instituto de Agroquímica y Tecnología de Alimentos(CSIC), Avda. Escardino 7, Valencia, España . [3]Universidad Nacional de Tucumán, Ayacucho 491, Tucumán, Argentina.

laconis_10@hotmail.com

The production process of Spanish dry-cured ham generally last from 7 to 24–36 months and consists of a stabilizing phase, which includes curing and resting stages at low temperature (wusually below 0.90). However, dry-cured ham is also commercially distributed as sliced ready-to-eat product and post-processing manipulation such as slicing and packaging, enable cross-contamination and serve as a vector for the spread of pathogenic bacteria, such as *Listeria monocytogenes*. Complex biochemical reactions occur in dry-cured hams process, proteolysis being among the main reaction responsible for the characteristic texture, flavor and final quality of this typical Spanish product. During ripening, muscle proteins are hydrolyzed by muscle enzymes with the release of small peptides and free amino acids. In this study, a peptidomic strategy has been used to identify and sequence those naturally generated peptides showing antilisterial activity derived from dry-cured ham protein degradation. Water soluble peptide extracts from dry-cured ham were purified by size exclusion chromatography and reversed phase high performance liquid chromatography and then, further identification of sequences was carried out by nano-liquid chromatography coupled to tandem mass spectrometry. A total of 105 peptide sequences were identified from active fractions. All identified peptides showed a mass between 502 and 2065 Da and amino acid sequences between 5 and 18 amino acids in length. Considering the low molecular mass and structural requirements for antilisterial inhibition, some of the identified peptides were synthesized and their CIM calculated. Eleven peptides were able to inhibit the growth of *L. monocytogenes*, being the pentapeptide RHGYM the most effective (MIC value: 6.25 mM). Results from this study show that Spanish dry-cured ham may represent a source of natural peptides with potential antimicrobial action.

Código de Resumen: BF-004

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

HYDROXILATION OF A TERPENE WITH A PHYTOPATHOGENIC FUNGUS *Fusarium verticillioides* AND DETERMINATION OF BIOLOGICAL ACTIVITIES

D. Bustos Crescentino[1], A. Pastor[1], P. Varela[1], A. Pacciaroni[2], D. Bustos[1], V. Sosa[2].

[1]Universidad Nacional de San Juan. [2]Universidad Nacional de Córdoba.

licbiodani@yahoo.com.ar

(-)-Ambroxide is a terpene like compound isolated from ambergris a prized scent which is a secretion of sperm whale. It is structurally related to a wide variety of compounds, in particular to the cytotoxic sclareol. The aim was to intend to transform (-)-Ambroxide by biocatalysis using the enzymes excreted by the filamentous fungus *Fusarium verticillioides*. Biotransformation was carried out according to a two standard protocol. In stage I potato glucose broth was inoculated with a refrigerated agar culture of the microorganism and grown 72 hours. Stage II was then started by inoculating the same amount of medium

containing the organic compound, with biomass from stage 1 and grown aerobically at 25ºC on a reciprocal shaker. The biotransformation was carried in preparative scale in the same culture medium 25 days. After that three compounds were obtained which were isolated, purified and analyzed by H^1 NMR and C^{13} NMR. These compounds were identified as 3β-hydroxyambroxide, 6β-hydroxyambroxide and 1β-hydroxyambroxide when compared with bibliographical data. One might assume that the enzymes produced by the plant pathogenic fungus *F. verticillioides* were presumably monooxygenases. These enzymes functionalized not reactive carbons C-1, C-3 and C-6 of subtrate (-)-Ambroxide. Biological activities of both the starting substrate as the metabolites obtained were determined. Antioxidant capacity assays were performed using the DPPH method and cytotoxicity by *Artemia persimilis* lethality. It was seen in the results obtained by bioassay lethality, that the cytotoxicity of metabolites obtained was lower than the starting substrate. It was also observed in the determination of antioxidant capacity that 1β-hydroxyambroxide compound was shown to have higher antioxidant capacity than the starting substrate, while for 3β-hydroxyambroxide and 6β-hydroxyambroxide the activity was lower than the starting compound. The biotransformation using whole cells phytopathogenic fungus in this case, are a simple, economical and clean method for modifying structurally organic compounds. Enzymatic potential of *Fusarium verticillioides* for biotransformation of (-)-ambroxide yielded hydroxylated derivative with β stereochemistry, a fact which shows that the oxidations were regio and stereoselective. Many naturally occurring antioxidants have one or more hydroxyl groups attached to aliphatic or aromatic carbons. Therefore, to obtain hydroxy compounds may be a potential new compounds substitutes of some of the synthetic antioxidants used in pharmaceutical and food industries source. Regarding cytotoxicity, all hydroxylated metabolites showed lower cytotoxicity than the starting substrate. This shows that the formation of hydroxy compounds, have proved to be detoxification products, because these types of compounds are generally more water soluble and therefore more susceptible to be eliminated by the organisms.

Código de Resumen: BF-005

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EXPRESSION OF *fol* GENES DURING FOLATE BIOSYNTHESIS BY *Streptococcus thermophilus* CRL803 IN CONTROLLED AND NON-CONTROLLED PH CONDITIONS

J.E. Laiño[1], M. Juarez del Valle[1], G. Savoy de Giori[1 2], J.G. LeBlanc[1].

[1] *Centro de Referencia para Lactobacilos (CERELA-CONICET).* [2] *Cat. Microbiología Superior Fac. Bioquímica, Química y Farmacia UNT.*

jonathan.laino@gmail.com

Folates play a key role in many metabolic pathways such as DNA and RNA synthesis. Folate deficiency is very frequent, reason for which many countries adopted mandatory fortification programs; However, high intakes of folic acid (the synthetic form of folate used in fortification) can mask vitamin B12 deficiency, and other secondary effects. These adverse health effects do not occurs with natural folates. S*treptococcus (S.) thermophilus* is able to synthetize folates *de novo*. However, there are no reports about expression of *fol* genes (*folE*, *folQ*, *folK*, *folP*, *folA* and *folC*) during folate synthesis. In this study, expression of folates biosynthesis genes by *S. thermophilus* CRL803 in low-folate culture medium was evaluated during batch fermentations with controlled and non-controlled pH. LAPTg without yeast extract (main source of folate) was used as culture medium. The temperature was maintained at 42ºC and the pH was kept automatically at 6.0 and 5.0 with 3 M NaOH. To perform gene expression analysis by qRT-PCR, samples were aseptically withdrawn at 4, 6, and 10 h of incubation from the fermentation vessel and immediately cooled on ice, using as a reference condition, the strain grown at uncontrolled pH. Optimal condition was selected based on highest folate concentration in shortest time of incubation. Cell populations (live, damaged, and death cells) during incubation (4, 6, and 10 h) were evaluated by flow cytometry (FACS). Folate production (µg/L), cell viability (log CFU/mL) and turbidity (OD_{580nm}) were evaluated for 12 h of incubation in both conditions. Folate levels were estimated by the microbiological assay using *Lactobacillus rhamnosus* NCIMB 10463 as the indicator strain. The results showed that folate production and yields were higher under constant pH condition compared to uncontrolled pH. The highest folate production was reached at 6 h of incubation at pH 6.0 (237±14 µg/L) compared to uncontrolled pH conditions (89±8 µg/L). Besides, at pH 6.0, highest values of viability (log CFU/mL 9.1±0.3) were observed, compared to uncontrolled pH (log CFU/mL 7.9±0.4). Based on these results, optimal condition for qRT-PCR was pH 6.0 at 42ºC. A significant increase in *de novo fol* genes expression (*folE*=3.7±0.2, *folQ*=2.7±0.1, *folK*=4.1±0.2 and *folP*=2.6±0.2 fold changes at pH 6.0*vs* free pH) was observed after 6 h of incubation, without significant modifications in *folA* and *folC* expression. Flow cytometry analysis revealed an increased sensibility to culture medium pH as is reflected by increase in damaged (15.4±2.4%) cell population at uncontrolled pH respect to pH 6.0 (4.1±0.7%). *S. thermophilus* CRL803 grown at pH 6.0 was able to increase almost 3 times the folate concentration by a highest biosynthesis of vitamin as was reflected by increase of *de novo fol* genes and lower percentage of damaged cells compared to uncontrolled pH.

Código de Resumen: BF-006

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EFFECT OF DIFFERENT NUTRIENTS ON RIBOFLAVIN PRODUCTION AND GENE EXPRESSION BY *Lactobacillus plantarum* CRL 725

M. Juarez del Valle[1], J.E. Laiño[1], G. Savoy de Giori[1 2], J.G. LeBlanc[1].

[1] *Centro de Referencia para Lactobacilos (CERELA-CONICET).* [2] *Cat. Microbiología Superior Fac. Bioquímica, Química y Farmacia UNT.*

jonathan.laino@gmail.com

Riboflavin (vitamin B2) participates in a wide variety of metabolic reactions that are essential for human life. In blood, this vitamin is found as the cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which act as electron carriers in oxidation–reduction reactions performing as coenzymes for flavoproteins such as, FMN- or FAD-dependent enzymes. The aim of this work was to evaluate the influence of temperature and certain components of a chemically defined medium on riboflavin production and gene expression by *L. plantarum* CRL 725. Chemically defined media (CDM) were prepared from concentrated individual stock solutions which were stored at -4°C after sterilized by filtration through cellulose acetate membranes (0.22um. *L. plantarum* CRL 725 was exposed to different carbohydrates (sucrose, lactose and glucose), which were added individually to CDM at a final concentration of 20 g/L, and different concentrations of sodium acetate, casamino acids, asparagine and guanosine. The incubation was carried out at 30 and 37ºC. Growth parameters (OD_{600} and cell viability), riboflavin production and the expression of vitamin B2 genes were evaluated. The total riboflavin concentration was determined using a microbiological assay and these results were confirmed by HPLC analysis. In respect to the influence of carbohydrates, *L. plantarum* CRL725 showed higher viability and growth rates with glucose (log 8.68, μ 0,651h^{-1}) or sucrose (log 8.73, μ 0.758h^{-1}) than with lactose (log 6.56 at 12 h). The riboflavin production in presence of sucrose was higher than that observed with glucose. The omission of casamino acids, affected both the cell growth in CDM and the vitamin production. The addition of guanosine at different concentrations had not effect on strain growth while at a concentration of 0.04 g/L it enhanced the riboflavin production reaching values of 2588,5 ± 250 ng/. Based on all of these results, the optimum conditions for growth and riboflavin production by*L. plantarum* CRL 725 were defined using CDM containing sucrose 2% (w/v) and guanosine 0.04 (g/L) and an incubation temperature of 30ºC. In optimized conditions, *L. plantarum* CRL 725 was able to increase vitamin B2 production two fold respect to reference condition and this was directly associated with significant increases in riboflavin biosynthesis gene expression such as *ribA*, *ribB* and *ribC* (2.62; 2.36;2.28 fold increases, respectively). In this study, optimized parameters were defined that allow increased riboflavin production in a CDM. These observations could be employed to increase production of riboflavin not only in microbial growth media, but also in different food matrices.

Código de Resumen: BF-007

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

BIOTRANSFORMATION OF (-)-ABROXIDE TO 3β-HYDROXIAMBROXIDE BY MICROBIAL COMMUNITIES

N.H. Paños[1], D.A. Bustos[1], A. Senese[1], G. Villafañe[1], D. Bustos[1].

[1] *Universidad Nacional de San Juan.*

idbustos@ffha.unsj.edu.ar

Various microorganisms, such as fungus *Alternaria alternata*, have been used for the transformation for instance from compounds of the family of bufadienolides. (-)-ambroxide is a natural, inexpensive and easily available terpene, attractive for biotransformations as it is structurally related to phytoalexins. Since phytoalexins have antioxidant capacity, compounds resulting from transformation of (-)-ambroxide may have this capability, which help in the prevention of various diseases such as cardiovascular or cancer. All metabolites obtained, one of the most common is the 3β-hydroxyambroxide, constituting the carbon-3 position of the ring A, apparently the most exposed to enzymatic oxidation by most microorganisms used. However the yield obtained in this compound is low, being at best 10% relative to the starting compound, having always been given the biotransformation capacity of particular microorganisms, individually analyzed and obtaining a low percentage of biotransformation after several weeks of incubation. To increase the likelihood of allowing the development of microorganisms with capacity to transform (-)-ambroxide efficiently, the strategy was to take advantage of the enormous genetic and metabolic diversity of microorganisms in nature working with microbial communities (not individual organisms) "selected" for his ability to develop under certain conditions and produce a specific transformation. They were designed and tested nutrient culture media which are simple and inexpensive by changing the pH and medium composition. For comparison was tested in parallel a culture medium conventionally used (Medium Sabouraud) sterilized and inoculated with*Aspergillus niger*. Samples for analysis at times 0, 1, 2, 4 and 7 days were taken. At each sampling time corresponding extraction with ethyl acetate was carried out and analyzed by Thin Layer Chromatography (TLC) and HPLC. It was obtained the target compound 3-β-hydroxyambroxido, in times of four to seven days incubation (instead of several weeks) by the action of microbial communities selected by culture conditions with pre-established economic sense (culture medium). This is the first report of obtaining 3β-hidroxiambroxido by

biotransformation, using a microbial community and in just four days of incubation

Código de Resumen: BF-008

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

QUANTIFICATION OF *Bacillus thuringiensis* DELTA ENDOTOXINS BY PROTEIN ELUTION FROM POLYACRYLAMIDE GELS

M. Mentel[1], M. Baigori[1 2], L. Pera[1].

[1] Laboratorio de Morfogénesis y Fermentaciones (PROIMI - CONICET). [2] Universidad Nacional de Tucumán.

isabelmentel@gmail.com

Bacillus thuringiensis (*Bt*) is a gram-positive and spore forming bacterium. It is characterized by the production of insecticidal crystal proteins (Cry) named delta-endotoxins, which exhibit larvicidal toxicity upon ingestion by susceptible insect larvae. Biopesticides derived from*Bt*are the most prominent biological agents for selective control of pest insects. They are composed of a mixture of spores, crystals and minor cell debris harvested from the culture media at the end of the fermentation process. The production of*Bt*as biological control currently focuses on the use of different supplements and substrates to find the best combinations to obtain an efficient product, using operational conditions that facilitate the reduction of the production costs at an industrial scale. This can be achieved through the use of low cost culture media such as residues from agro-industry so that production can become economically viable. Furthermore, several studies have reported the utilization of several media based on complex substrates, which are efficient for*Bt*bioinsecticides production. Our group already designed a production medium based on the use of agro-industrial residues. The aim of this work is to find a method for delta endotoxin quantification compatible with complex culture medium. The native isolate*Bt*RT was used throughout this study. The Cry concentration was determined by the method of dye elution in SDS-isopropanol using bovine serum albumin as a standard. Electrophoresis was conducted essentially as described by Laemmli. Gels were stained with Coomassie Brilliant Blue R250. The amount of protein in the Cry1Ac bands was determined measuring the optical density at 594nm of the elution solution. Thus, a very simple method for delta endotoxin quantification has been developed. This method allows us to quantify the Cry1Ac proteins produced in a complex medium without the interference of any undesired proteins. In addition, the highest Cry1Ac concentration was observed after three days of fermentation

Código de Resumen: BF-009

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

YEASTS AS BIOCATALYSTS IN THE STEREOSELECTIVE REDUCTION OF ACETOPHENONE

M. Decarlini[1], C. Manzoni[1], E. Medici[1], A. Vázquez[1], M. Aimar[2].

[1] Facultad de Ciencias Químicas, Universidad Católica de Córdoba. ARGENTINA.. [2] Cátedra de Química Aplicada, Dpto. de Química, FCEFyN. Universidad Nacional de Córdoba. ARGENTINA.

florencia.decarlini@gmail.com

The asymmetric reduction of prochiral ketones represents a pivotal transformation for the production of chiral alcohols because several of them are considered as key starting materials in obtaining pharmaceuticals. Nowadays, bio-reductions are an important methodology in organic synthesis for the production of chiral drugs and in this sense, certain microorganisms are considered an outstanding tool for the obtaining these chiral building blocks. Some authors have reported that species belonging to*Saccharomyces*and*Geotrichum*were used with this purpose. They have been isolated from fruits, vegetables and foods. We aimed to analyze the capacity of the yeasts, isolated from the different sources, to produce 1-phenylethanol enantiomerically pure using acetophenone as substrate.

1) Isolation of yeasts: 15 strains were isolated of samples of grapes, carrots, grapefruit, as well as ready-made products such as camembert cheese and craft beer were taken. Swab samples were performed in peptone 0.1% p/v with Tween 80 0.05%, and maltose broth in the case of beer, and then insulated in 3 medium: GPYA, PDA and SDA.

2) Identification of the strains: Coloration with lactophenol for the colonies that presented morphology corresponding to the sought yeast was prepared, and then they were identified with the key of Pitt and Hocking (1997).

3) Reaction with the acetophenone: 15 selected strains were tested. They were obtained after 3 days of growth at 30°C in GPY broth, and the yeast cells were separated from the culture broth by filtration. Yeast cells (≈ 2 g) were put in a 125 ml conical flask sterile container containing 80 ml of 0.1M KH_2PO_4 buffer (pH 7.0). The substrate (50 mg), dissolved in 1 ml of dimethyl

sulfoxide, was added and the incubation was made on an orbital shaker at 100 rpm at 30 ° C for 7 days. Samples were analyzed and identified by chiral GC-FID and GC-MS using standard references.

A total of 15 strains were isolated and identified. They belonged to the genera*Saccharomyces, Zygosaccharomyces, Debaryomyces*and*Geotrichum*. All the strains studied showed reduction from acetophenone to 1-phenylethanol. Only those ones owning *Geotrichum spp* showed selectivity for the *R* isomer, while the others exhibit selectivity for the*S* isomer. Two strains proved to be the most active:*Zygosaccharomyces* ZBC4 which presented a 23% reduction with 99 e.e.% (*S* isomer) and *Geotrichum* GZ1 which present a 99 % reduction with >99.9 e.e.% (*R* isomer). The excellent conversion and stereoselectivity achieved with *Geotrichum GZ1* (*Geotrichum spp* isolate from carrot) makes this microorganism a good candidate for conducting further studies on its ability to reduce other substituted acetophenones.

Código de Resumen: BF-010

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

MECHANISTIC STUDY OF THE STEREOSELECTIVITY REDUCTION OF ACETOPHENONE PROMOTED BY A STRAIN OF *Geotrichum spp*.

M. Decarlini[1], A. Vázquez[1], M. Aimar[2].

[1]Facultad de Ciencias Químicas, Universidad Católica de Córdoba. ARGENTINA.. [2]Cátedra de Química Aplicada, Dpto. de Química, FCEFyN. Universidad Nacional de Córdoba. ARGENTINA.

florencia.decarlini@gmail.com

Nowadays, biocatalytic stereoselective reduction of prochiral ketones is one of the most important methodologies in organic synthesis for the preparation of chiral alcohols. This reaction is important because enantiomerically pure alcohols are key precursor in the obtaining of pharmaceutical compounds. Recently, a screening on fifty six strains of bacteria and fungi was made for our team to identify microorganisms capable of performing the stereoselective reduction of acetophenone (AP) to 1-phenylethanol (1-PE). From this study a strain of*Geotrichum spp*. has been isolated and characterized, and this yeast has presented an excellent conversion (>99%) and stereoselectivity (>99.9 e.e.%) in the preparation of (*R*)-1-PE from AP. We aimed to study the mechanism by which a strain of*Geotrichum spp.*transforms stereoselectively AP to (*R*)-1-PE. To meet our objective, two studies were made: 1) Effect of different pH: The inoculum was developed in GPY broth (30 °C; 3 days) and the yeast cells were separated by filtration. Wet cells (≈2 g) were put in a 125 ml sterile conical flask containing 80 ml of 0.1M KH_2PO_4 buffer.The pH values tested were adjusted to: 5.0; 6.0; 6.5; 7.0; 7.5 and 8.0. AP (50 mg) dissolved in 1 ml of dimethyl sulfoxide was added. The incubation was made on an orbital shaker at 100 rpm (30°C, 48h). Samples were taken at 48h and analysed by chiral GC-FID.2) Kinetic study: The study was made in the same conditions described in (1) using phosphate buffer pH 7.0. Samples at one hour intervals were taken during 48h. The assay was made in triplicate and the samples were analysed by chiral GC-FID.

1) Based on pH studies, it was determined that the optimum working range was 6.5-7.5 pH. It was noteworthy that the e.e.% remained virtually constant (99,0- >99.9%) at the different pH studied. However, the greatest effect was observed on the percentage of conversion of AP which varied significantly. At pH 5,0 the reduction was 42.5%; at pH 6,0 was 45%; at the range between 6,5 to 7,5 was >99%, and at pH 8.0 decreased to 32%.

2) In the kinetic study can be observed that in the first 6h of reaction, the quantity of AP decreased and the two isomers (*R* and*S*) increased but in different proportions: the*S*isomer was present in greater quantity. From this time, another enzyme began to oxidize the*S*isomer to AP, so the AP concentration began to increase while a disminution in the S isomer could be observed. Meantime the R isomer continued increasing but in a major rate. From the 15h the *S*isomer disappeared, the amount of acetophenone decreased and the*R* isomer is the only product formed. In a period of 24h the reduction is completed and the e.e.% is maximum for the *R*isomer.

From pH studies it could be established that pH 7.0 is the better pH for this reaction. From the kinetic study it could be concluded that at least three enzymes are involved in the process of conversion of AP to (R)-1-PE.

Código de Resumen: BF-011

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

FACTORIAL DESIGN IN OPTIMIZATION OF PHB PRODUCTION

O. Yashchuk[1 2], D. Nygaard[1 2], E. Hermida[1 2].

[1] *Universidad Nacional de San Martin.* [2] *CONICET.*

oyashchuk@unsam.edu.ar

Poly-β-hydroxybutyric acid (PHB) is a natural, biodegradable polymer that a large number of bacteria accumulate as an energy reserve. They are produced when the carbon source is in excess while nutrients such as nitrogen or phosphorous are available in limited. In terms of morphological and mechanical properties, the PHB homopolymer is comparable to some of the more common petrochemical thermoplastics. However, the high price of commercial grade PHB limits its use to specialist niches. Spreading the use of PHB requires reduction in the production costs by improving fermentation strategies, using low-cost media, and applying easier downstream recovery methods.

This contribution presents an experimental design-based developed to optimize the medium for the production of PHB by *Cupriavidus necator*, American Type Culture Collection 17697, in 500-ml shake flasks (30±1ºC, 200 rpm).

The experimental design proposes an efficient way to obtain optimization of a process or product with a minimum number of experiments. The medium optimization is based on three stages of experimentation: screening, optimization and verification. Screening enabled to determine the relevance of five experimental conditions: concentration of the carbon source, concentration of the nitrogen source, culture pH, ionic strength and agitation intensity. A two-level fractional factorial design (5 factors, 16 combinations) was employed. Optimization experiments allowed to provide in-depth information about the factors with higher impact on performance during screening. Finally, verification experiments were used to validate the results under specific experimental conditions.

Biomass content was evaluated by gravimetry and turbidimetric measurements. Fructose and Ammonium concentration in the supernatant was determined by colorimetric methods. Crotonic acid formed from PHB during acid digestion was detected by absorbance at 234 nm.

Among the main results it is observed that the yield of PHB strongly depends on the culture growth and that its degradation begins at the onset of the stationary phase. Hence, timing the harvest is essential to prevent a loss of the produced PHB. When using ammonium sulfate and fructose, the optimal C/N ratio was 23,5 which allowed a PHB concentration of 2,84g/l. The sensitivity of the biomass and PHB yields to changes in C/N depends on the nitrogen source used. The characteristics of the optimal culture were: maximum specific fructose consumption rate of 0,49 g/lh; a maximum specific PHB production rate of 0,048 g/lh; a maximum product yield coefficient of 0,51 and the cell yield coefficient for fructose of 0,38.

Código de Resumen: BF-012

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

LACTIC ACID BACTERIA ISOLATED FROM AMARANTH SOURDOUGH: TECHNOLOGICAL AND FUNCTIONAL PROPERTIES

S.L. Carrizo[1], J.E. Laiño[1], G. Vignolo[1], J.G. LeBlanc[1], G. Rollan[1].

[1] *Centro de Referencia para Lactobacilos (CERELA-CONICET) .*

lcarrizo2402@gmail.com

The pseudocereal amaranth (*Amaranthus* sp.) is an ancient Andean crop, gluten free (GF), with high nutritional value, higher than that of most cereal grains, owing to its high protein content and balanced essential amino acid composition.. Moreover, amaranth grain protein is rich in lysine, which is usually deficient in cereal grains. Also, these grains contain different levels of vitamins and minerals, in addition to other beneficial compounds such as polyphenols, phytosterols and flavonoids. However, many of these compounds are altered or removed during milling, processing or cooking. Lactic acid bacteria (LAB) are widely used as starter cultures for the fermentation of different foods, thus improving the nutritional value, organoleptic characteristics, self- life and overall quality of fermented products. LAB are usually auxotrophic for several vitamins, although some strains have the ability to synthesize B vitamins, suggesting that the use of adequately selected strains could increase the concentration of these vitamins in fermented foods and their nutritional value. In previous work in our laboratory, LAB were isolated from spontaneously fermented dough prepared with amaranth flours from Northwestern Argentina (NOA). LAB were identified by biochemical and molecular methods. The aim of this study was to evaluate technological and functional properties: ropiness, capsular EPS (CPS), vitamin B2 (riboflavin) and B9 (folate) production and amylolytic activity of LAB isolated from amaranth sourdough. Fifteen strains belonging to the species *Lactobacillus* (L.) *plantarum, L. rhamnosus,* and *Enterococcus (E.) mundtii* were evaluated. Intra, extracellular and total concentrations of B2 and B9 were determined using microbiological methods. The CPS formation was determined by the India ink negative-staining technique. The production of ropiness was assessed by visual observation of the elevation of colonies by touching them with sterile toothpick and by their mucoid aspect. The results showed that of the total tested strains, 14 grew on the B2 free medium and the B2 production was variable, *L. plantarum* A2 M10 9 produced the highest concentration of this vitamin (162,7±19 ng/ml). All isolated strains grew in synthetic medium without B9 and shown to produce this vitamin in a range between 16 and 138 ng / ml. *L. plantarum* A2 M6 3 produced the highest concentration of intracellular (7,2±0,1 ng / ml). From all the tested strains, 73% produced visible ropiness. Six strains of *L.*

plantarum and one of *E. mundtii* produced CPS. Respect to amylolitic activity, 50% of the evaluated strains showed capacity to hydrolyze starch. The majority of *L. plantarum* strains isolated from amaranth sourdough demonstrated capacity to produce ropiness and amilolytic activity.

These results put in evidence the positive effects of autochthonous LAB isolated from amaranth sourdough for the development of healthy novel foods with higher quality and nutritional value.

Código de Resumen: BF-013

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

CreC, A NEW TOOL FOR METABOLIC MANIPULATIONS IN *Escherichia coli*

M.S. Godoy[1], P.J. María [1].

[1] *Universidad de Buenos Aires.*

msgodoy@qb.fcen.uba.ar

Succinic acid is used as a specialty chemical in the agricultural, food, and pharmaceutical industries. Although succinic acid is currently produced from petroleum derived maleic anhydride, considerable interest in the fermentative production of succinate from sugars has emerged. Many genetic strategies have been performed to enhance succinate production in *Escherichia coli*, a natural but poor producer of this compound. The pleiotropic regulation over celullar functions carried out by two-component systems (TCSs) makes them interesting targets for genetic manipulations to achieve this purpose. CreC is a TCS responsive to the carbon source present in the media, whose absence was seen to enhance succinate production. In this work, additional strategies were tested to increase its production in *creC* mutants. Two plasmids carrying carboxylating enzymes were introduced in the parental and *creC* strains: pEcPpc, that overexpresses the carboxylating enzyme phosphoenolpiruvate caboxilase (Ppc) from *E. coli,* and pSBF2, that overexpresses the formate dehydrogenase from *Candida boidini* (Fdh). In both plasmids the genes are under the control of the *lacZ* promoter, and can be induced by IPTG. Two different concentrations of IPTG (0,1mM and 1M) were used to get a better estimation of the relative weight of the conversion catalyzed by Ppc and Fdh on succinate production. With the lowest concentration of IPTG, the *creC* mutant produced 3 times more succinate than the parental strain. These concentrations were only slightly higher than those observed in the absence of plasmids for both strains. However, when IPTG was supplied in a higher dose (1 mM), succinate production was triggered, with marked increases in all cases. The mutant strain overexpressing both plasmids produced more than four times what it had produced with 0,1 mM IPTG, and 40% more than the wild type in the same condition. In order to eliminate side products and increase NADH availability, the ethanol pathway was deleted and *ackA* was also eliminated to conserve carbon atoms in the form of acetyl-CoA, a substrate for succinate formation via the glyoxylate pathway. The double mutant *creC*, *adhE* and the triple mutant *creC*, *adhE*, *ackA* were cotransformed with pEcPpc and pSBF2, and succinate was measured in cultures of these strains grown in the same conditions previously described ($NaHCO_3$ 100mM, IPTG 0.1mM and 1mM). In contrast to what was expected, these strains did not present significantly higher amounts of succinate when compared to the simple mutant *creC* harboring both plasmids. In all cases, a very marked increase was observed with higher amounts of IPTG, indicating that in the *creC* background, overexpression of Ppc and Fdh had an important effect on succinate production, while the mutations in *adhE* and *ackA* did not. These results indicate that CreC appears as a good candidate for genetic manipulation in order to improve a reduced compound of commercial interest, such as succinate.

Código de Resumen: BF-014

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

LACTIC ACID BACTERIA FROM ARTISANAL TANNERIES: ISOLATION AND EVALUATION OF ACIDIFYING ABILITY

A .C. Deza[1], C.L. Gerez[1], G.I. Martos [1], M. Nuñez[1], M. Fiori[2], G. Font[1].

[1] *Centro de Referencia para Lactobacilos (CERELA-CONICET).Tucumán, Argentina. MINCYT-FONCYT.* [2] *Instituto Nacional de Tecnología Industrial. Centro Regional INTI Tucumán. Tucumán, Argentina.*

clugerez@cerela.org.ar

The tanning process allows to transform animal skin in stable and non-putrescible products namely leather. This process consists in various steps such as washing skins, liming, depilation, lime removal by washing, purging and tanning. During purging, the skins are immersed in a cereal mix which is let to ferment for 12-24 h at room temperature (18ºC-37°C). After this period, the pH decreased to 4.5-5.0, a condition that is required for the final steps. Under these homemade working conditions, the cereal fermentation is quite variable and affects the quality of the leather. The objective of this study was to isolate lactic acid

bacteria (LAB) from the fermented cereal mixture in artisanal tanneries and to evaluate *in vitro* the acidifying activity of the strains. This is the first step for the formulation of a starter culture for tanneries to normalize the process. Samples of fermented cereals were taken from a tannery located in San Pedro de Colalao, Tucumán, Argentina, and colonies were isolated in MRS agar medium. The primary identification of the isolates included Gram stain, microscopic observations and catalase reaction. Gram positive, catalase negative strains (23) were cultured in a CERELA medium formulated for the production of lactic ferment and incubated at 18°C and 37°C for 24 h. At intervals, pH and titratable acidity (TTA) were determined. In most samples, a prevalence of cocci (95%) respect to bacilli was observed. The total isolated strains (56), 23 strains were Gram (+) and catalase (negative) which were selected. At 37 °C, most strains (21) acidified the culture medium within the first 8-h reaching a final pH $\leq$ 5.0 and a TTA $\geq$100ºD. At 18 °C, all strains showed a lower growth; however, the decrease in pH was 4.5-5.0 and the acidity developed (100-140ºD) after 24 hours of fermentation were similar to values obtained at 37°C. The best acid-producer strains were identified as *Enterococcus faecium* CRL 1943 and *Leuconostoc citreum* CRL 1945by phenotypic and genotypic techniques. Currently, studies are being conducted to formulate a lactic inoculant on the basis of these strains and their metabolites for artisanal tanning.

Código de Resumen: BF-015

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EFFECT OF STIRRING ON GROWTH AND TOLERANCE TO HEAT STRESS OF THE PROBIOTIC *Lactobacillus rhamnosus* CRL1505

A.C. Deza[1], C.L. Gerez[1], G. Font[1].

[1] *Centro de Referencia para Lactobacilos (CERELA-CONICET). Tucumán, Argentina. MINCYT-FONCYT.*

clugerez@cerela.org.ar

The market for functional foods, particularly those that incorporate probiotic bacteria, is constantly expanding, a fact that involves new technological challenges. The development of probiotic dietary supplements in powder requires feasible techniques at industrial scale such as spray drying. However, the process involves thermal stress to the cell with loss of viability and or metabolic activity. Previous studies showed that the fermentation conditions strongly affect robustness of lactic starter cultures. The aim of this study was to evaluate the effect to stirring on growth, acidification and tolerance to heat stress of the probiotic *Lactobacillus rhamnosus* CRL1505. Bach cultures were performed in CERELA medium (Under patenting process, pH 6.3) without pH control, under (150 or 400 rpm) or without stirring (control) at 37ºC. An active culture (16-h old) was inoculated (1%, w/v) and fermentation proceeded for 24h. Samples were aseptically withdrawn at 0, 4, 6, 8, 10, 12 and 24h. Growth (OD_{620nm} and plate-dilution method), pH, lactose consumption (HPLC) and organic acid produced (HPLC) were evaluated. The heat stress tolerance (60ºC/ 5 min.) of cells harvested at a late stationary phase was also determined. Slight differences in growth between stirred ($1.8\ 10^9$ uf/ml) and static ($8.4\ 10^8$ uf/ml) cultures were observed, after 24 h. Lactic acid production increased more rapidly under aerated conditions than in static cultures. Acetic acid and ethanol formation was detected only in agitated cultures (9-16 mM), The cells grown with agitation were significantly more resistant than cells grown in static condition to heat stress. After 5 minutes at 60ºC, viable counts for stirred cultures were 3.2-fold higher than for static cultures regardless the stirring rate. These results are encouraged for drying probiotic cultures by spray.

Código de Resumen: BF-016

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

MACROLIDE MEGOSAMINYLATION IN BACTERIAL SYSTEMS

B.A. Bercovich[2 1], E. Porta [3 1], G. Labadie[3 1], H. Gramajo[2 1], E. Rodríguez[2 1].

[1] *Fac. Cs. Bioquímicas y Farmacéuticas, UNR.* [2] *IBR-CONICET.* [3] *IQUIR-CONICET.*

bercovich@ibr-conicet.gov.ar

Glycosyltransferases from polyketide gene cluster determinate the glycosylation patterns of macrolides which define the bioactivities of these molecules. Previously we have demonstrated substrate flexibility of the UTP-dependent glycosyltransferase pair MegDI-MegDVI from megalomycin gene cluster toward both the TDP-zugar and macrolide substrates. Thus, a new megosaminyl-azitromycin derivative with improved antimalaria and antibiotic activity were produced by bioconversion experiments in *E. coli*. In order to study structural contribution for antibacterial and antimalarial activity of this compound, new derivatives were produced. Modifications into desosamine residue were introduced by synthetic chemistry and megosamine residue was introduced by bioconversion experiments generating two new megosaminil-azitromycin derivatives. The structures of the compounds were confirmed by mass spectrometry. Scaling up of this process will allow validating its structure and the biological activity will be analyzed in order to test the effect of structural modifications on activities. In addition, due to low

efficiency of bioconversion experiments in *E. coli*, we have developed a new glycosylation system using *S. lividans*. For this end, a *S. lividans* Δ*mgt* strain was developed toghether with an integrative plasmid carrying the megosamine operon. This pathway include five enzimes that convert glu-1-P into TDP-megosamine which is further transfere to the macrolide by glycosyltransferase pair MegDI-MegDVI. To optimize the system, we carried out metabolic engineering of endogenous pathways that consume the common glu-1-P intermediate in *S. lividans*. Single and double mutations in *pgm* and *manB* genes were performed and test improving carbon flux distribution toward TDP-megosamine when using galactose as carbon source. This hypothesis is being tested by analyzing NDP-sugar pool and bioconversion efficiency.

Código de Resumen: BF-017

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ISOLATION OF CELLULOLYTIC AND HEMICELLULOLYTIC BACTERIA FROM AN ARGENTINEAN TERMITE

F.F. Felice[1], E.B. Ben Guerrero[1], R.S. Salvador[2], E.C. Campos[1], C.G. Godoy[3], J.A. Arneodo[2], P.T. Talia[1].

[1]Instituto de Biotecnología, CICVyA, CNIA, INTA Castelar, Argentina. . [2]Instituto de Microbiología y Zoología Agrícola, CICVyA, CNIA, INTA Castelar, Argentina.. [3]Biología de los Invertebrados. Facultad de Ciencias Exactas y Naturales y Agrimensura. Universidad N.

facufelice@hotmail.com

In the last few years, the production of lignocellulosic bioethanol has been gaining attention in many science and engineering disciplines. This is mainly due to the climate change, the need to reduce emissions of greenhouse gases and the variability of petroleum cost. Many invertebrates have the capability to degrade cellulose. In higher termites, lignocellulose digestion is performed by endogenous and bacterial endosymbiont enzymes present in the digestive tract. In this context, our group has been working to isolate microorganisms that degrade cellulose efficiently. In this study, cellulolytic bacteria found in Argentinean termite guts were isolated and characterized. The objectives of this study are to isolate and evaluate bacteria with cellulolytic activity present in Argentinean termite guts. Workers *Nasutitermes aquilinus* (Isoptera) were sterilized superficially with 70% ethanol. Then, the insect guts were extracted and the rest of the body was discarded. Serial dilutions of termite guts were incubated with minimal media (MM) and a piece of filter paper (Whatman N°1; 2.5 cm x 1.0 cm). The filter papers were used as the only carbon source for 10 days at 37°C, in order to promote growth of microorganisms with the ability to use cellulose. A total of 100 µL of each liquid culture was streaked out on agar plates containing MM supplemented with 1% carboxymethylcelullose (CMC) and 0.05% trypan blue (TB), 1% xylan and 0.005% TB and 0.001% 4-Methylumbelliferyl β- D -glucopyranoside (MUG). After 24 h of incubation at 37°C and successive re-streaking, 10 bacterial isolates were obtained. Positive colonies were determined by a clear zone around the colony on all media containing the TB. The cellulolytic and hemicellulolytic activities of each isolate were evaluated with dinitrosalicilic acid assays (DNA) and paranitrofenyl-β-D-glucopyranoside (pNPβG). In order to identify bacterial genera, we extracted total DNA from each positive colony and then amplified, cloned and sequenced the partial 16S rRNA gene. *Stenotrophomonas*, *Cellulomonas*, *Afipia*, *Cohnella*, *Staphylococcus* and *Paenibacillus* were identified by sequence analysis of the 16S rRNA. The highest endoglucanase and xylanase activities were observed in *Cellulomonas* (Endoglucanase: 3.127 ± 0.100 UI/g; Xylanase: 1.419 ± 0.031 UI/g) and *Cohnella* (Endoglucanase: 3.042 ± 0.371 UI/g; Xylanase: 1.559 ± 0.017 UI/g). The evaluation of bacterial isolates obtained from *N. aquilinus* gut extracts showed their high cellulolytic enzyme production and their ability to degrade cellulose and hemicellulose. Therefore, these bacteria could be a useful tool to degrade lignocellulosic biomass into simple components for bioethanol production.

Código de Resumen: BF-018

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

TEMPERATURE EFFECT ON YEAST ECOLOGY DURING PREFERMENTATIVE COLD SOAK IN MALBEC WINES

M. Mestre[1 3], Y. Maturano[1 3], R. Martínez Beguerí[1 2], B. Kuchen[1 3], V. Pesce[1 3], M. Toro[1 2], M. Combina[4 3], F. Vazquez[1 2].

[1]Instituto de Biotecnología-FI-UNSJ. [2]Departamento de Agronomía-FI-UNSJ. [3]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). [4]Centro de Estudios Enológicos-EEA-Mendoza-INTA.

ramb3489@gmail.com

Prefermentative cold soak (CS) consists of the contact of fermentation solids (skins, seeds and occasionally stems) with the must in a non-alcoholic and low temperature environment (typically in the range of 0–15 °C) during variable time period (from 1-15 days) prior to the onset of alcoholic fermentation. Particularly the use of low temperatures results critical to the quantitative and qualitative microbiological composition of the must during the prefermentative phase. Yeasts present during this stage can affect directly to final product, therefore is relevant to analyze. The main objective was to study the temperature impact on the biodiversity and dominance of yeasts species during prefermentative cold soak. Grape musts of Malbec from Lujan de Cuyo-Mendoza were used. Stainless steel 100L tanks with 80L of must were used. Three different treatments were assayed: T1) CS 12±1°C, T2) CS 8±1 °C and T3) CS 2,5±1 °C. CS period was carried out during 7 days. Samples were taken in initial must and during CS (Day: 2, 5 and 7). A representative percentage of each different colony was isolated, purified and identified by sequencing the D1/D2 domain of the 26S ribosomal gene. Classical ecology indexes were used to obtain the richness (total numbers of species), biodiversity (Shannon-Wiener index) and dominance (Simpson index) of the yeasts species found during CS treatments assayed. As a result, 9 species belonging to 5 genera were found on grape juice and throughout CS. In the initial grape juice, specify richness was 5 species (*Hansenispora uvarum, Candida zemplinina, Pichia occidentalis, P. kudriadzevii and Saccharomyces cerevisiae*). Seven yeast species were isolated and identified during the 3 CS treatments analyzed, but only 4 of them were present throughout the maceration stage (*H. uvarum, C. zemplinina, P. occidentalis* and *S. cerevisiae*). Furthermore, in spite of that all treatments presented the same number of species (7), genotypic distribution was different in each treatment throughout the CS and across treatments. Considering the evolution of diversity and concentration of Dominance indexes, at the beginning of CS, all treatments registered the highest microbial diversity (1,7: T3; 1,3: T1 and T2) and the lowest index of dominance (0,21, 0,33 and 0,37 for T3, T1 and T2, respectively). CS treatments carried out at 2,5 ± 1 °C showed the highest Shannon indexes. Although differences were observed in the results obtained with the different maceration temperatures, it can be appreciated that *H. uvarum, C. zemplinina* and *S. cerevisiae* were the dominant species throughout cold soak period. Results obtained in this study suggest that the temperature is a factor responsible for the domain from one species over another one during maceration period. Furthermore, the temperature can exerts selection pressure on yeast ecosystem.

Código de Resumen: BF-019

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

CHARACTERIZATION OF *Anthonomus grandis* BOHEMAN (COLEOPERA: CURCULIONIDAE) LARVAE GUT CELLULASE AND HEMICELLULASE ACTIVITY ACROSS DIFFERENT DIETS

E.B. Ben Guerrero[1], R.S. Salvador[2], F.F. Felice[1], E.C. Campos [1], P.T. Talia[1].

[1] *Instituto de Biotecnología, CICVyA, CNIA, INTA Castelar, Argentina. .* [2] *Instituto de Microbiología y Zoología Agrícola, CICVyA, CNIA, INTA Castelar, Argentina..*

facufelice@hotmail.com

The use of lignocellulosic biomass for bioethanol production as an alternative to fossil fuels has gained great interest, mainly due to the high cost of oil and reduced oil reserves. The availability of environmentally-friendly and sustainable biofuels is crucial. Many insects can use lignocellulosic substrates as their main food source and are highly efficient at degrading cellulose to glucose with the help of endogenous and endosymbiont cellulases. The aim of this study was the detection and characterization of (hemi) cellulolytic activities in the gut of *Anthonomus grandis Boheman* with different diets. *A. grandis* larvae were raised on three different artificial diets: cotton-based (CB), maize milling waste (MMW) and *Pennisetum purpureum* (PP) at 28°C, 70% relative humidity, and with a 12-h ligth/darkness photoperiod. After 10 days, third-instar larvae were surface sterilized with 70% ethanol and dissected under a binocular microscope. By the dinitrosalicylic acid (DNSA) assay, the (hemi) cellulolytic activities were evaluated and characterized from *A. grandis* gut extracts reared on the three diets using. The effects of temperature and pH on enzyme activity of gut extracts from each diet were estimated at three temperatures (30, 50 and 80°C) and five pH values (4-8). Gut extracts from all three diets exhibited β-1,4 endoglucanase, xylanase and β-glucosidase activities. These activities were significantly affected by pH conditions and reaction temperatures across different diets. We observed that the larvae reared on a CB artificial diet showed significantly higher activity than larvae reared on the PP and the MMW diets. The endoglucanase activity of gut extracts on the three diets exhibited two optimal pH peaks at 5 and 7. The highest endoglucanase activitiy at pH 5 was 3.5±0.05 UI/g for CB, 2.72±0.09 UI/g for PP and 1.62±0.06 for PP artificial diet. The optimal pH for xylanase activities was quite different in two of the three diets. The gut contents of CB (2.01±0.26) and PP(1.62±0.26 UI/g) artificial diet exhibited the highest xylanase activity at pH 6 and the gut of MMW artificial diet showed the highest activity at pH 5 (0.55±0.18 UI/g). The optimal pH for the β-glucosidase activity was found at pH 6 for gut contents of MMZ (0.89±0.02 UI/g) and CB (1.75±0.04) artificial diet. The gut content of PP artificial diet showed the highest activity at pH 5 (1.48±0.05 UI/g). The higher activities of endoglucanase, xylanase and β-glucosidase enzymes, under optimum pH, were detected at 50°C. These values of pH and temperature are similar to the conditions used in industrial processes for the enzymatic hydrolysis of cellulose. This is the first report characterization of hydrolytic enzymes in the gut of *Anthonomus grandis* with different diets. Our data suggest a potential utility of these enzymes for the production of bioethanol.

Código de Resumen: BF-020

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ANTARCTIC PENINSULA ENVIRONS AS A SOURCE FOR NEW ANTIMICROBIALS

M.E. Danilovich[1], L.A. Sánchez[1], F. Acosta[2], V.G. Arnau[1], O.D. Delgado[2].

[1]PROIMI,CCT, Tucuman- CONICET. [2]CITCA,Facultad de Cs. Exactas y Nat. UNCA.

marianita.danilovich@gmail.com

Antarctica is the biggest pristine area on Earth and represents one of the most valuable environments due to its microbial diversity. It is indispensable to emphasize the study of pristine natural extreme-ecosystems since numerous biologically active compounds including antibiotics, pesticides, hormones, growth factors, antioxidants, biosurfactants and enzymes have been isolated from microorganisms belonging to them. In the medicinal area, research and development of novel compounds with antimicrobial activities has become in a priority due to the increased drug resistance in common bacterial pathogens along with the emergence of new pathogenicity. To deal with this situation, a value-increasing strategy is on the spotlight: Bioprospecting. This process tends to direct search for metabolites with biotechnological importance from microbial isolates in natural environments with specific characteristics. Based on this premise, thirteen cold-adapted isolates from soil and water samples surrounding Antarctic Peninsula were studied for enzyme and antimicrobial production. The ability to produce biosurfactants was also tested. The enzymatic activities most frequently found among the isolates were as follow: lipase (46%), cellulase (23%), amylase (7%) and gelatinase (13%). Biosurfactant production was detected in 46% of the isolates. Three isolates were able to inhibit the growth of common food-borne pathogen bacteria and also some phytopatogens. The isolates 2D, 5D and 6D were closely related to *Halomonas titanicae* (99.8, 98.9, 96.7% respectively) by 16S rRNA gene sequencing. The profile of sugar was studied by the API 50 CHB system and other physiological characteristics by API 20NE systems and API 50CH. Growth rate at different temperatures, pHs and NaCl tolerance were determined. The significant influence of culture media and incubation temperature on antimicrobial production were evaluated, being LB-SW medium and 25°C the optimal conditions for antimicrobial production. Besides to marked tolerance to enzymatic treatment and negative net charge at pH 8.0 during electrophoresis, the antimicrobial compounds showed wide inhibition spectrum against both, G-positive and G-negative pathogenic and phyto-pathogenic bacteria. The antagonist compounds were produced during stationary phase of growth and concentrated from cell-free supernatant by using SPE-C18 cartridges. The significance of this work lies in valuing pristine environments because of their importance as new sources of bioactive compounds and their possible role in agricultural or pharmaceutical biotechnological industries.

Código de Resumen: BF-021

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

FIRST INSIGHTS INTO THE *Pseudomanas yamanorum* 8H1^T ANTIMICROBIAL ACTIVITY AND ITS BIOTECHNOLOGICAL POTENTIAL

V.G. Arnau[1], M.E. Danilovich[1], L.A. Sánchez[1], J.I. Fariña[1], O.D. Delgado[2].

[1]PROIMI, CCT- Tucuman-CONICET. [2]CITCA, Facultad de Cs. Exactas y Nat. UNCA. .

marianita.danilovich@gmail.com

The increasing resistance of pathogens to common antibiotics currently used has encouraged the search for novel antimicrobials. On the other hand, environmental health concern has also led to the search of natural biocontrol agents for replacing or decreasing the use of agrochemicals. Microorganisms represent a valuable and frequently unexplored natural source of bioactive metabolites. In this scenario, bioprospecting programs involving pristine environments constitute an interesting alternative for discovering and developing novel bioactive compounds. *Pseudomonas* spp. exhibits a high metabolic diversity and many species have been reported as biocontrol agents, being able to produce a wide spectrum of useful biomolecules such as antibiotics, siderophores, volatiles, biosurfactants, among others. The aim of this work was to provide first insights about the antagonistic activity from *P. yamanorum* 8H1^T, a strain isolated from Isla de los Estados, Ushuaia. Antimicrobial activity production (expressed as AU mL^{-1}) was evaluated in different culture media: SNB, LB, King's B, R2A, CSM, M9 and M63 by using the critical dilution method. Effect of temperature and pH on antimicrobial production, siderophore activity and biosurfactant production was simultaneously evaluated. Treatments with catalase, lipase, a-amylase and different proteases aided at elucidating the biochemical nature of the antimicrobial produced. Antimicrobial net charge was determined by inhibition-zone movement after electrophoresis on agarose gel. Purification of the compound was attempted by reversed-phase SPE, followed by RP-HPLC coupled to a PDA detector. *P. yamanorum* 8H1^T antimicrobial activity was assessed against both, enteropathogenic and phytopathogenic microorganisms, showing a broad inhibitory spectrum against bacteria relevant for

health, as well as for the agricultural and food industry; although inhibition of phytopathogenic fungi was not observed. Maximum inhibitory activity was obtained in CSM medium at 20°C, showing that bacterial antagonism was neither related to siderophore- nor biosurfactant-like compounds, although both activities were detected for living-cell and cell-free supernatants. The antimicrobial showed similar properties, such as positive net charge, resistance to proteases and the UV-Vis spectrum, to those of pseudobactin, a well-known siderophore produced by species of *P. fluorescens*,. However, the non-siderophore nature of the produced antimicrobial makes difficult to relate it with pseudobactin. Further studies including HPLC-MS/MS and NMR are expected to help at elucidating the *P. yamanorum* 8H1^T antimicrobial structure and its potential novelty.

Código de Resumen: BF-022

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

IMPACT OF MANGANESE ON THE PRODUCTION OF A BIOMASS ASSOCIATED β-D-GLUCOSIDASE ACTIVITY USING A THERMOPHILIC *Bacillus licheniformis* STRAIN

M.B. Abdulhamid[1], M.D. Baigorí[1 2], L. Pera[1].

[1] *PROIMI-CONICET.* [2] *Universidad Nacional de Tucumán.*

beluabdul@hotmail.com

The β-Glucosidase (EC 3.2.1.21) constitutes a group of well-studied hydrolases. This enzyme catalyzes the hydrolysis of arylglucosides, alkylglucosides, cellobiose, and cellooligosaccharides. The interest in this biocatalyst centers on its roles in the enzymatic hydrolysis of cellulose. The rate of cellulose hydrolysis can be improved by supplementing commercial cellulases with immobilized β-D-glucosidase, which usually has high stability and can be recovered and reused. In addition, for industrial saccharification of cellulosic materials, β-glucosidases from thermophilic bacteria are also of particular interest due to their increased stability. In this work, we study the influence of manganese on the production of a biomass associate β-D-glucosidase activity using a thermophilic *Bacillus licheniformis* strain. Assays were performed at 45 °C in 500 ml Erlenmeyer containing 200 ml of LB medium supplemented with 0 - 1.0 mM $MnCl_2$. The β-D-glucosidase activity was also determined at 45 °C using 3.6 mM p-nitrophenyl-β -D-glucopyranoside (Sigma) as substrate. Cells were harvested by centrifugation and washed twice with 100 mM Tris-HCl buffer (pH 7). The pellet was resuspended in the same buffer, and it was directly used as the β-D-glucosidase source. The mixture was shaken at 1000 rpm. Then, the absorbance of the supernatant was measured at 405 nm and the enzyme activity calculated and related to the biomass dry weight. One unit of the enzyme was defined as the amount needed to release 1 µmol *p*-nitrophenol per min. Thus, dose-response experiments showed that in the presence of 0.3 mM $MnCl_2$ the enzyme production was increased by about 20%. Under this culture condition, a specific activity value of 19.99 U per mg of dry weight was obtaining after 4 h of cultivation. Finally, these results could be of relevance to the bioethanol industry where lignocellulosic material is used as feedstock for fermentation and, which should be treated enzymatically. The use of naturally bound enzymes is an important immobilization technique. This type of biocatalyst system is potentially cost-effective because the biomass can be directly utilized in the treatment.

Código de Resumen: BF-023

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

Aspergillus terreus STRAIN IMPROVEMENT FOR ENHANCED LOVASTATIN PRODUCTION

J.D. Babot [1], F.C. Caro[1], O.D. Delgado[2 3], J.I. Fariña[1 3].

[1] *PROIMI CONICET.* [2] *CITCA CONICET.* [3] *FACEN UNCA.*

florcecaro@gmail.com

Cholesterol plays a vital role in body metabolism and membrane transport, and acts as precursor for the synthesis of several key biomolecules. Nevertheless, changes in cholesterol level lead to cardiovascular disorders, like atherosclerosis and hypercholesterolemia, which are currently the main causes of death. This is why controlling cholesterol by inhibition of its biosynthesis is a promising approach. Cholesterol is synthetized from acetyl-CoA through a complex pathway, where the rate-limiting step is the conversion of HMG-CoA to mevalonate, catalyzed by HMG-CoA reductase. This key enzyme is selectively and competitively inhibited by lovastatin, a fungal secondary metabolite used as a hypocholesterolemic which can therefore reduce the risk of cardiovascular diseases. Lovastatin production is normally carried out using selected *Aspergillus terreus* strains, however industrial process yields may be improved by strain manipulation. Accordingly, the aim of this work was to develop a lovastatin-hyperproducing *A. terreus* strain. To this end, 10^7-spores/mL suspensions of lovastatin-producer *A. terreus* MEC were exposed to UV radiation for different times ranging from 5 to 15 min. Spores were kept in the dark for 30 min, plated onto PDA plates and incubated at 25°C for 48 h. Isolated colonies were transferred to an optimized lovastatin production medium (SQop) containing cheese-whey as substrate and incubated at 25°C for 14 days. Lovastatin was extracted from fungal colonies by using ethyl acetate and converted to its β-hydroxyacid form by alkaline hydrolysis. Organic extracts were preliminary

analyzed by TLC, and spot intensities were quantified with ImageJ software. The amount of lovastatin was quantified by RP-HPLC. In a second stage, selected lovastatin hyperproducing mutants were subjected to another mutation cycle and further evaluated. The first obtained 164 putative mutants were comparatively analyzed against the wild-type (WT) *A. terreus* MEC strain and, according to TLC results, 28 mutants produced 20% or less than it, whilst 20 out of the 164 produced 20% (or higher) more lovastatin than WT. These results, as confronted to the HPLC analyses, confirmed 6 mutant strains with 20%-lower production than WT strain, while only one showed a hyperproducing phenotype. This latter mutant, named C10´-27, produced 168% more (2.35 g/L) lovastatin than WT strain. After a second mutation cycle of *A. terreus* C10´-27, 157 putative mutants were analyzed. Lovastatin production, as witnessed by RP-HPLC, increased by 20% or higher than the one for C10´-27 for 5 of the obtained mutants. The highest lovastatin titer was achieved by mutant *A. terreus* S12,5´-9 with a 40% increase over the already improved production of *A. terreus* C10´-27. These results pave the way to a more efficient lovastatin production by using the selected mutant and may additionally open new perspectives for reducing its production costs.

Código de Resumen: BF-024

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

FIBRINOLYTIC ENZYMES PRODUCTION BY *Bionectria* sp. LY 4.1: THE RELEVANCE OF INOCULUM HOMOGENIZATION AND PH-CONTROLLED CONDITIONS AT FERMENTER SCALE

F.C. Caro [1], J.D. Babot[1], O.D. Delgado [2 3], J.I. Fariña[1 3].

[1] *PROIMI CONICET.* [2] *CITCA CONICET..* [3] *FACEN UNCA.*

florcecaro@gmail.com

Fibrin accumulation in blood vessels usually results in thrombosis, which can thereafter lead to myocardial infarction and other cardiovascular diseases. Fibrin is the primary protein component of blood clots and is physiologically formed from fibrinogen by the catalytic action of thrombin. During fibrinolysis, the insoluble fibrin fiber is hydrolyzed into fibrin degradation products by plasmin. Nowadays, fibrinolytic enzymes of microbial origin have attracted more attention than typical thrombolytic agents used for thrombolytic therapy. This choice is based on the high price and the undesirable side effects of the latter. The aim of this work was to study the production of fibrinolytic enzymes by *Bionectria* sp. LY 4.1, a wild fungus isolated from Las Yungas rainforest and already described in our group. In this case, we focused on the fermentation process upstream optimization in order to increase fibrinolytic enzymes production at fermenter scale. At first, inoculation process was standardized by hand-blender-aided homogenization of mycelial suspensions and evaluating the influence of power input and the number of pulses. The production of fibrinolytic enzymes was also preliminary evaluated at different initial cultivation pHs, from 4 to 8, at shake-flask scale and by using an optimized production medium based on glucose, soy peptone, NaCl and $MgSO_4$. Subsequently, batch cultures were carried out with a 1-L working volume fermenter either at uncontrolled pH or by controlling culture broth pH (with 1 N NaOH) at the optimal value obtained in previous assays, and results were comparatively assessed. Fibrinolytic activity was determined by the fibrin plate test and by using a plasmin standard curve. Inoculum standardization showed that, at 48 h of fermentation, inoculum homogenized with a higher number of pulses allowed to obtain an increased fibrinolytic activity (495 U plasmin/ml) as compared to the process started with a slightly homogenized inoculum (170 U plasmin/ml). The screening for optimum cultivation pH in shake-flasks assays revealed that a 20% higher production was obtained at pH=8 and accordingly, this value was selected for testing at fermenter scale. Batch fermentations were comparatively run under free pH (firstly set in culture medium at 6.6 and left uncontrolled afterwards) and with automatic control at pH=8. Further operative conditions were set as follows: agitation, 200 rpm; temperature, 25°C and airflow rate, 1.5 vvm. Fibrinolytic enzymes titers reached 1888 U plasmin/ml at pH-free conditions *vs*. 2437 U plasmin/ml under controlled pH. These findings provide first clues into the possibilities for the upstream fermentation process optimization through the automatic pH controlling strategy. Following studies will be focused on the use of a different pH controlling agent and further operative conditions. The elucidation of these optimal parameters will then be useful for the subsequent scaling-up.

Código de Resumen: BF-025

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EVALUATION OF THE INFLUENCE OF CARBON SOURCES ON CELLULASES AND XYLANASES PRODUCTION BY *Microbacterium* sp. AR462-2

E. Di Marco[1], N.I. Perotti [2 1], M.A. Martínez[1 2].

[1] *Planta Piloto de Procesos Industriales Microbiológicos - PROIMI - CONICET.* [2] *Facultad de Ciencias Exactas y Tecnología - Universidad Nacional de Tucumán - FACET - UNT.*

edimarco_unt@hotmail.com

The lignocellulose is one of the main constituents of vegetable biomass. Besides being a renewable and abundant source of energy, it has a high potencial for bioconversion to value-added bioproducts. Among its components, the hemicellulose portions (mainly xylans) represent around 25% to 40% of their composition after cellulose that constitute the main component which represents between 50% and 70% of the total. In adition to the lignin, both the cellulose and hemicellulose, make its structure to be resistant to the enzymatic degradation. Thus, it is necessary the cooperative and synergistic effect of various enzymes, including cellulases and xylanases, to reach higher yields for its degadacion. In the present study *Microbacterium* sp. AR462-2, a cellulolytic and xylanolytic actinobacteria isolated from intestines of wood beetle larvae, was characterized. The effect of various carbon sources was evaluated on cellulases and xylanases production into Omeliansky mineral culture medium. The substrates assayed as sole carbon source were xylan, lactose, glucose, sucrose, fructose, maltose, carboxymethylcellulose (CMC) and filter paper. This strain was taxonomically identified as a member of *Microbacterium* spp. according to the sequence analysis of the 16S ribosomal gene. Microscopic observation of AR462-2 strain showed small yellow rods (RAL 1014). Physiological studies showed that this strain did not produce melanic nor soluble pigment. Growth was observed between 5.0 and 8.0 pH units and showed halotolerance and lysozyme resistance. It was able to grow in presence of various carbon sources. *M.* sp. AR462-2 secreted cellulases when glucose 1% (w/v) was used as a sole carbon source which activity measured was 0.1 U/ml. Also, this strain showed evidence of mild degradation of filter paper in liquid mineral medium. Xylanolytic activities were detected in all culture medias, but the highest xylanase production was recorded when lactose 1% (w/v) was added individually in Omeliansky´s medium. The highest xylanase activity measured was 1.03 U/ml within 7 days using lactose, while in presence of CMC was 1.14 U/ml but after 14 days of culture. Further assays were performed in order to improve both growth and production, by adding casein peptone. Production rates were similar although growth was significatively shortened to 3 days. These results suggest that lactose could be used for enzyme production by *Microbacterium* sp. AR462-2 as an inexpensive carbon source, since lactic whey is an abundant industrial by-product.

Código de Resumen: BF-026

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EVALUATION OF THE INTERACTION AMONG YEAST β-GLUCAN AND POLYPHENOLS DERIVED FROM CINNAMIC AND BENZOIC ACIDS

G.F. Villalba[1], M.S. López Alzogaray[2], M.A. Nazareno[1].

[1] Centro de Investigaciones y Transferencia de Santiago del Estero (CITSE). [2] Universidad Nacional de Santiago del Estero (UNSE).

gfv_3091@hotmail.com

The β-(1-3)-D-glucan is a homo-polysaccharide of D-glucose with insoluble alkali-acid characteristics. It is widely used to activate the immune system, for its antiviral and antimicrobial properties. Considering its use by the food industry, the FDA (Food and Drug Administration-USA) has been recognized as GRAS. One of the most important sources of β-glucan is the cell wall of yeast, especially of *Saccharomyces cerevisiae.* Phenolic compounds are secondary plant metabolites occurring in fruits and vegetables. They are involved in the defence system against invading pathogens (including *Escherichia coli* and *Campylobacter jejuni*). Many of these phenolic compounds have valuable chemical and biological activities, as antioxidants, metal chelators as well as antimicrobial and antiviral agents. This study focuses on obtaining biomaterials with metal complexing and antioxidants properties based on secondary interactions between β-glucan from *S. cerevisiae* and a series of phenolic compounds (caffeic, ferulic, and gallic acids). The aim of this work is to develop antioxidant solid filters to remove free radicals and metal ions to be used in environmental remediation and food and pharmaceutical industry. YPD growth medium was supplemented with EDTA and adjusted to pH 4.00 with tartaric acid. Cells were incubated at 30 °C with shaking at 200 rpm for 24 h. The number of viable cells was analysed using the McFarland turbidity standards. The cell wall was obtained by cell autolysis at 50 °C for five days. The alkalis-acid soluble components of the cell wall were removed by alkali treatment (incubation in NaOH at 90 °C) and acidic treatment (incubation in H_3PO_4 at room temperature). Mannoproteins removal was performed by autoclaving at McIlvane pH 7.00 buffer for 90 min. The β-glucan extracted was analysed by IR and NMR. The interactions were assessed by monitoring the UV-Vis spectral changes for different ratios of polyphenol/glucan mass at pH 5 to 8 and its stability for one hour. The presence of $1{,}5x10^{12}$ viable cells/mL was observed. Amounts of 0.31 g of glucan per liter of culture were obtained. The yield obtained is low considering the physical size of the microorganism, as well as the energetic conditions used during extraction. The quantification of viable cells was null after autolysis. The purity of the material was analysed by IR and NMR spectroscopy, and results were satisfactory. For interactions between β-glucan and polyphenols the UV-Vis spectral changes indicated the existence of interaction between β-glucan and caffeic acid as well as between β-glucan and gallic acid. The evaluation of changes in the antiradical activity and metal complexing abilities when the polyphenols were combined with β-glucan was carried out. Further studies of high biological ongoing interest are in progress concerning the chemical modification of β-glucan to enhance the interaction with polyphenols.

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

STABILITY ASSESSMENT AND IMPROVEMENT OF ENZYMATIC ACTIVITY OF THE ENDOGLUCANASES FROM *Bacillus* sp. AR03

J.H. Pisa[1], A.P. Manfredi[1 2], N.I. Perotti[2 1], M.A. Martínez[1 2].

[1] Planta Piloto de Procesos Industriales Microbiológicos, PROIMI-CONICET. . [2] Facultad de Ciencias Exactas y Tecnología, FACET-UNT.

horacio_pisa@hotmail.com

The lignocellulosic biomass is well known like a promising source to biorefinery due to its abundant and its renewable feature. Cellulose, the major compound of this material, needs the cooperative action of at least three types of enzymes to be degraded: exoglucanases, endoglucanases and β-glucosidases. Microorganisms and their enzymes are biotechnical tools that nature has designed to utilize biomass that is present in the habitat around them. In this sense, Bacteria are extensively considered as a source of novel cellulases because of their diversity and due to their higher growth rate and their extensive repertoire of glycoside hydrolases. The aim of the present work was to produce and to characterize endoglucanases from *Bacillus* sp. AR03, isolated from sugarcane bagasse liquor, to further generate lignocellulosic hydrolysates. The isolate AR03 was grown in a peptone broth amended with carboxymethyl cellulose 1% (w/v) and sucrose 1%(w/v) at 30 °C and 200 rpm. After 48 h, the culture supernatant was recovered by centrifugation and the endoglucanase activity was estimated by measuring reducing sugar released from CMC by the dinitrosalicylic acid (DNS) method. Zymograms of the culture supernatant were carried out by native PAGE. The effects of temperature, pH, cations and others additives such as EDTA, PEG, SDS and Tween 80 were assayed to assess their influence on the activity and stability of the endoglucanases produced. The enzyme production reached 3 IU/mL in the crude extract (culture supernatant) and the optimal endoglucanase activity was registered at 60 °C and pH 6.0. The evaluated enzymatic extracts showed that the enzyme activity was completely retained after pretreatments at temperatures £ to 40 °C, although it did not show thermal stability after preheating at 60 °C for one hour. Endoglucanases from AR03 isolate maintained approximately 80% of the total activity within a wide range of pH (3.0 to 10.0). The native PAGE revealed at least three bands with endoglucanase activity, having apparent molecular masses of 286, 208 and 157 kDa. Even when most of the effectors assayed did not affect significantly the enzymatic activity, the addition of Mn^{2+} and Co^{2+} (5 mM) to the enzymatic reaction mixture produced a noteworthy improvement of the endoglucanase activity from the crude extracts. The endoglucanase activity was upgraded as much as 150% and 80% when salts containing Mn^{2+} and Co^{2+} were added, respectively. Those increments were confirmed by means of HPLC measurements since it has been reported interference between some divalent cations and the DNS reagent. The results regarding the broad range of pH stability and the strong improvement of enzymatic activity by the presence of manganese are the most relevant features of the endoglucanases from *Bacillus* sp. AR03 to be considered as promising for further studies and for biotechnological applications.

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

BIOETHANOL AND BIODIESEL PRODUCTION WITHIN A CYANOBACTERIA AND MICROALGAE BIOREFINERY

M.E. Sanz Smachetti[1 2], C.D. Coronel[1 2], L. Sanchez Rizza[1 2], M. Do Nascimento [1 2], A. Arruebarrena Di Palma[2], G.L. Salerno[1 2], L. Curatti[1 2].

[1] Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET). [2] Fundación para Investigaciones Biológicas Aplicadas.

lcuratti@fiba.org.ar

Oleaginous microalgae have great potential as a feedstock for biodiesel and other biofuels. However, the current cost of producing biofuels from microalgae biomass is still high to envision massive and profitable commercialization in the near future. One of the drawbacks of implementing large-scale cultivation of these organisms is the unsustainable requirement of N-fertilizers. It is presumed, however, that co-production of higher value by-products in the frame of a biorefinery would increase the profitability of producing biofuels and co-products from microalgae. Recently, we showed the efficient conversion of N_2-fixing cyanobacterial biomass into oleaginous microalgae biomass. We further modeled an integrated bioprocess that would require no N-fertilizer other than air and would yield 7.000 - 10.000-l microalgae oil . ha^{-1}. $year^{-1}$ in raceway ponds placed in southeastern Buenos Aires. This estimated yield would be 2- to 20-fold higher than that reported for current oleaginous plant feedstocks heavily fertilized with conventional N-fertilizers. In addition to oil, this process would roughly produce from 2 kg of *Nostoc* biomass about 1 kg of *Nostoc* residues (mostly carbohydrates) and 1.0 kg of carbohydrates and 1 kg protein from

Chlorella sorokiniana biomass. In this study, we obtained data of *Nostoc* productivity under Mar del Plata city environmental conditions in autumn in 5-l air bubbled photobiorreactors that further support the previous productivity model and found conditions of phosphorous deficiency for optimizing the carbohydrate to protein ratio of the biomass for a biorefinery. Both cyanobacterial and microalga carbohydrates could be hydrolyzed into soluble sugars in diluted acid (0.3 % H_2SO_4) at 100 °C for 1h with efficiencies higher than 85 %. These conditions are mild in comparison with those normally used for saccharification of lignocelulosic biomass for second generation bioethanol, supporting the convenience of using cyanobacteria/microalgae biomass as a suitable alternative. We further used saccharified cyanobacterial biomass as a feedstock to produce bioethanol by fermentation with the Baker's yeast *Saccharomyces cerevisiae*. We observed that yeasts fermented the syrup into ethanol at 11 % of the theoretical maximum conversion in the absence of yeast growth, while control experiments with hydrolyzed dextrose reached 28 % of the theoretical maximum at 20 h. Current work in our laboratory is focused towards improving, at a laboratory scale, the most critical steps of a conceptual bioprocess for the biorefinery of cyanobacteria/microalgae/yeast biomass for the co-production of biodiesel, bioethanol and protein to be used as biofuel and feed, respectively.

Código de Resumen: BF-029

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SYNERGISTIC EFFECT OF XYLANASES PRODUCED IN CO-CULTURE OF *Bacillus* sp. AR03 AND *Paenibacillus* sp. AR247

J.S. Hero[1], J.H. Pisa[1], N.I. Perotti[2 1], C.M. Romero[1 3], M.A. Martínez[1 2].

[1]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET). [2]Facultad de Cs. Exactas y Tecnología, UNT. [3]Facultad de Bioquímica, Química, Farmacia y Biotecnología, UNT.

horacio_pisa@hotmail.com

In nature, the plant biomass is degraded by a process that requires the cooperative action of multiple microorganisms capable of producing a variety of enzymes to attack the complex structure of lignocelluloses. This work assessed the production and the enzymatic activity over the main hemicellulolytic fraction of plant biomass, xylan, in monoculture and co-culture systems of bacteria isolated from regional niches associated with sugar cane bagasse. The enzyme activity was estimated by measuring reducing sugars released using the dinitrosalicylic acid method. All cultivation assays were performed at 200 rpm and 30 °C in a diluted peptone broth supplemented with 1% CMC (w/v). The viability and the growth of both isolates were estimated by the number of colony forming units, fact that was possible since both isolates exhibited different colony morphology. The specific xylanolytic activity of the co-culture of *Bacillus* sp. AR03 and *Paenibacillus* sp. AR247 was of 7.03 ± 0.46 IU/mg and 8.36 ± 0.49 IU/mg at 48 h and 96 h of cultivation, respectively. In contrast, each isolate assayed simultaneously under identical conditions, produced significantly lower xylanase activities, even when both isolates grew similarly in both, individual and co-cultures, reaching approximately 10^{11} CFU/ml in all cases. These values were of 4.18 ± 0.24 IU/mg and 4.55 ± 0.29 IU/mg of xylanolytic activity at 48 h and 96 h, respectively, for *Bacillus* sp. AR03, while *Paenibacillus* sp. AR247 reached values of 0.59 ± 0.09 IU/mg and 0.40 ± 0.03 IU/mg at the same periods of cultivation. When mixtures (1:1) of the cell-free supernatant of individual cultures were assayed, it was observed that the enzymatic activity reached a maximum of 4.16 ± 0.39 IU/mg after 48 h of cultivation. This value was close to that obtained by the sum of the enzymatic activity of individual cultures, which was 4.77 IU/mg, for the same cultivation time. The obtained results were consistent with the observation of a synergistic effect on the degradation of xylan in the co-culture evaluated, with an estimated degree of synergism of 1.69 at 96 h. This synergy, which has been described for enzyme mixtures on industrial substrates, was observed here during the co-cultivation of *Bacillus* sp. AR03 and *Paenibacillus* sp. AR247. This system displayed a higher xylanolytic activity with respect to the individual cultivation of each isolate and a different zymographic pattern along the cultivation period. The obtained results of the xylanolytic activity for individual strains and the co-culture might indicate that the observed effect could not depend on an only addition of enzyme activities so that we may suggest the existence of a synergistic cooperation during the growth in the co-cultivation of the microorganism evaluated.

Código de Resumen: BF-030

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

BIOACCUMULATION OF LITHIUM BY *Bacillus pumilus* ISOLATED FROM HIGH ALTITUDE ANDEAN LAKES

V. Curia[1], C. Belfiore[1], D. Kurth[1], M.E. Farias[1].

[1] *Planta Piloto de Procesos Industriales y Microbiologicos (PROIMI-CONICET).*

dgkurt@gmail.com

Lithium (Li) is a metal that has several industrial applications and due its large storage capacity, low weight and long life, Li ion rechargeable batteries are preferred for electronic devices. In recent years there was an exponential increase in demand for this mineral. It is widely distributed in nature, the largest reserves of Li (over 85%) are in the so-called "triangle of lithium" the Salar de Atacama in Chile, Salar de Uyuni in Bolivia and Salar del Hombre Muerto in Argentina, being Argentina the third world producer of Li. The recovery of Li from salar brines involves solar evaporation of the brine in several stages until its content reaches 6%, evaporating large quantities of water. The extremophile bacteria are an important asset to use in bioremediation and bioleaching, however at the moment there are few reports of the bacterial ability to biosorb Li. In this regard, the aim of the present work was to evaluate the ability of bacteria isolated from Andean lakes (HAAL), to grow in presence of Li and the faculty to recover this metal by biosorption, as that could be used in non-metalliferous mining. *B. pumilus* Act108 is a Gram-positive bacterial strain, isolated from Laguna Negra (Catamarca, Argentina). The first assays for determination of Li tolerance were carried out in plates containing LB agar medium supplemented with LiCl 0.7 and 1.1 M. Plates without metals were included as control. After incubation at 30° C for 48 hours, the strain showed good growth in either case. Cell growth on liquid cultures was monitored by optical density at 600 nm using a spectrophotometer Ultrospec 10. Cells were resuspended at an initial OD_{600}: 0.05 in fresh LB and LB + 0.7M Li. The strain was incubated at 160 rpm and 30° C for 24 hours, samples were taken periodically and OD was determined. The growth curve shows the ability to adaptation of the cells to grow in presence of Li, since they showed a behavior similar to control cells. The accumulation of Li by *B. pumilus* was examined in several conditions of pH, time, volume and molarity of the Li solution. Based on the results of all the tested conditions, the highest accumulation was obtained with the following method: 70 mg dry wt of microorganisms were resuspended in 45 ml of solution (pH 7.5) containing 0.1 M Li and the suspension was shaken for 3 h at room temperature. The supernatant was recovered by centrifugation. The amount of metal ions accumulated by the cells was determined by measuring the metal content in the supernatant using an inductively coupled plasma optical emission spectrometry (ICP-OES), the assay was performed in duplicate. The amount of Li accumulated on average was 39 mg/g dry wet cells (5619 µM/ g dry wet cells), representing 8,6 % of Li available. The results show that a high Li accumulating ability was exhibited by *B. pumilus*. Compared with results obtained by other authors our strain shows an increased capacity to bind Li.

Código de Resumen: BF-031

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

INFLUENCE OF THE ADDITION OF MALTODEXTRIN AND β-CYCLODEXTRIN DURING THE BIOSYNTHESIS OF SILVER NANOPARTICLES AND ITS ANTIBACTERIAL ACTIVITY

M.A. Quinteros[1], P.R. Dalmasso[2], V. Aiassa [3], A. Zoppi[3], M.R. Longhi [3], P.L. Paez[4].

[1] *IMBiV-CONICET-FCQ-UNC.* [2] *CITSE-CONICET.* [3] *UNITEFA-CONICET-FCQ-UNC.* [4] *Dpto. Farmacia. FCQ-UNC.*

mquinteros@fcq.unc.edu.ar

At present, the composition and morphology associated to the layer of stabilization around the silver nanoparticles is of great interest to understand its stability. The dextrins have the ability to form complexes with the majority of the drugs, improving its solubility properties and stability to the light and heat. That is why the dextrins have a great potential to be used in the pharmaceutical industry. In the present study, we analyze the influence of added β-cyclodextrin (BCD) and maltodextrin (MD) to the medium of biosynthesis of silver nanoparticles, in addition to observing the changes on the stability, polydispersity and antimicrobial activity. MD is a polysaccharide encapsulation with high activity and BCD is a ciclycal member of MD family. The silver nanoparticles (AgNps) were biosynthesized from the reduction of silver ions by the supernatant of cells of Pseudomonas aeruginosa. At supernatant of P. aeruginosa, 15 mL of silver nitrate 10 mM and different concentrations of MD and BCD was added. The biosynthesized nanoparticles were characterized by UV–Vis spectroscopy, Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and Infrared Radiation (IR). The standard macrodilution method was used to evaluate the antibacterial activity of the AgNps biosynthesized. The technique of UV-vis showed the appearance of a surface plasmon peak around 400 nm indicating the formation of pseudo-spherical nanoparticles. An increase in the intensity of the absorption maximum indicated the formation of complexes with the dextrins. This was corroborated by the images obtained by TEM and IR, which confirmed the interaction of AgNps with dextrins due to the presence of the corresponding functional groups. The AgNps were exposed to the light and it was observed that those AgNps with a dextrins layer suffered an oxidation less than those without dextrins. Results from the DLS showed no major differences between the different types of nanoparticles. An average diameter between 100-120 nm and a Z potential of -30 was obtained which is correlated with a high stability. It showed that antibacterial activity of all AgNps biosynthesized with dextrins against the following reference strains *Staphylococcus aureus* 29213, *Escherichia coli* 25922 and *P. aeruginosa* 27853 not change respect to the AgNPs alone. Faced with the growing demand for sustainable and environmentally friendly protocols, and the use of biodegradable and non-toxic precursors to prepare nanomaterials, we demonstrate the potential benefits to be gained by improving unfavorable physicochemical properties by the AgNps using dextrins as stabilizing agents.

Código de Resumen: BF-032

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EXPLORING THE MULTIPLE BIOTECHNOLOGICAL POTENTIAL OF HALOPHILIC MICROORGANISMS ISOLATED FROM TWO ARGENTINEAN SALTERNS

D. Nercessian[1], L.G. Di Meglio[1], R.E. De Castro[1], R. Paggi[1].

[1] *Instituto de Investigaciones Biológicas-Universidad Nacional de Mar del Plata-CONICET.*

dnercess@mdp.edu.ar

The biotechnological microbial potential of two salterns from La Pampa, Argentina has been explored. Salitral Negro and Colorada Grande are neutral hypersaline basins commercially exploded for NaCl extraction. Several microorganisms were isolated from water samples and screened for hydrolytic activities and bioactive molecules. Pure isolates were identified by sequencing a PCR-generated fragment of the 16S rRNA gene. Seven representatives of *Archaea* and two from *Bacteria* were obtained and screened for the presence of lipolytic and cellulolytic activities, as they are interesting enzymes for industrial application. Microorganisms were grown on SW agar medium supplemented with olive oil, Tween 20 and Tween 80 as source of triacylglycerols and esters of lauric or oleic acids. Tweens also allow testing the enzyme preference for saturated or un-saturated fatty acids. *Har. argentinensis, Har. japonica* and *Salicola* sp. degraded all the substrates while *Har. vallismortis* only hydrolysed olive oil and the un-saturated fatty acids contained in Tween 80. This observation suggests that the activities detected in *Har. argentinensis, Har. japonica, Har. vallismortis* and *Salicola* sp. may be attributed to different enzymes (lipase and esterase) or by an extracellular lipase exhibiting both activities. The activities detected in *Hbt. piscisalsi* and *Hrr. tebenquichense* only with both Tweens may correspond to an extracellular esterase since they failed to degrade olive oil. The assay for cellulolytic activity suggested that *Salicola* sp., *Har. vallismortis* and *Hbt. piscisalsi* degraded CMC (carboxymethyl cellulose). The occurrence of biosurfactants was tested by emulsifying assays using cell-free culture media of the isolates. Olive oil and xylenes were used as substrate and SDS and Triton X-100 as positive controls. *Har. japonica*, *Har. vallismortis*, *Hbt. piscisalsi* and *Salicola* sp. produced surfactants that emulsified both aromatic compounds and long chain hydrocarbons whereas those synthesized by *S. ruber, Har. argentinensis, Hbt. salinarum* and *Hrr. tebenquichense* or *Halobacterium* sp. specifically emulsified long chain hydrocarbons or aromatic compounds respectively. To search for antimicrobial compounds each microorganism was tested against each other and growth inhibition was analyzed by the presence of halos on agar plates. Bacterial isolates were resistant to the archaeal isolates and vice versa, indicating that no inter-domain interaction existed. *Har. argentinensis* and *Har. japonica* were the most effective producers inhibiting growth of most archaeal isolates whereas *Hrr. tebenquichense* was the most sensitive strain. These results show the potential of halophilic microorganisms inhabiting Argentinian salterns, reinforcing the idea of screening extreme environments as a source of potentially novel and useful molecules.

Código de Resumen: BF-033

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ANTIOXIDANT CAPACITY OF SOYBEAN PASTE FERMENTED WITH *Lactobacillus paracasei* subsp. *paracasei* CRL 207

A. Rodríguez de Olmos[1], M.S. Garro[1].

[1] *CERELA-CONICET.*

arodriguez@cerela.org.ar

The generation of reactive oxygen species (ROS) such as hydroxyl, peroxyl or superoxide radicals as well as other oxidant species is inevitable in aerobic metabolism of the human body. When ROS production in the body exceeds its natural defense mechanism, oxidative stress takes place leading to the damage of tissues and to cell death. The regular consumption of antioxidant-rich-foods may help to reduce the deleterious action of ROS and free radicals, and to balance the oxidative stress related to aging process and serious illnesses. Soybeans are important bioactive compounds source such as isoflavones and proteins. The isoflavones in the soybean are mainly found as glucosides but also as its corresponding aglycones. The mainly antioxidant properties are ascribed to the latter. On the other hand, in recent years, the antioxidant peptides have drawn the attention of researchers due to their low molecular weights, good absorption profiles, and strong biological activities. In this sense, use the soybean as source of antioxidant compounds is an alternative to obtain a novel product to human feeding with enhanced nutritional and functional properties. In previous work we studied the capacity of *L. paracasei* susp. *paracasei* CRL 207 to hydrolyze the soy protein and increase the isoflavones aglycones in soy pastes. The aim of this work was analyze the antioxidant activity in fermented pastes using *L. paracasei* subp. *paracasei* CRL 207. The paste from soy flour at 65% of moisture adding glucose 2% was inoculated with *L. paracasei* subp. *paracasei* CRL 207 and incubated 37ºC for 24h. The protein and isoflavones from fermented soy paste and unfermented (control) were extracted and their antioxidant activity of them

was analyzed. The antiradical and antioxidant activities were determined spectrophotometrically using the free radicals DPPH, ABTS and β-carotene-linoleic acid system. For isoflavones and proteins extracts both activities were increased at 24h of fermentation respect to the control. In the case of proteins an improvement of antiradical activity was observed regarding the isoflavones. In this work, the enhancement of the antioxidant and antiradical activities of a soy paste by fermentation with *L. paracasei* subp*. paracasei* CRL 207 was demonstrated. The consumption of natural and healthy foods is nowadays the major interest of consumers. Soybean products are very important foods due to their numerous nutritional benefits and, their global availability.

Código de Resumen: BF-034

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EVALUATION OF THE BACTERIOCINS ACTIVITY AGAINST PATHOGENS ASSOCIATED WITH GASTROENTERITIS ISOLATED IN THE CHILDREN´S HOSPITAL OF SAN MIGUEL DE TUCUMAN

S. Navarro [1], M. Chalón[1], G. Merletti[2], E. Barrionuevo[2], J. Assa[2], C. Minahk[1], A. Bellomio[1].

[1] *Universidad Nacional de Tucuman-Instituto Superior de Investigaciones Biológicas,CCT.* [2] *Hospital del Niño Jesus.*

silvitica6@gmail.com

Bacteriocins of Gram (-) bacteria are active on phylogenetically related to the producer strain and similarly, those bacteriocins of Gram (+) bacteria are active on Gram (+) bacteria. *Objective*: To study the *in vitro* antibiotic effect of different bacteriocins on clinical isolates in the Children's Hospital of Tucumán to select those that present greater antibiotic potency for the construction of chimeric peptides. The bacteriocins were obtained from the bacteria producing supernatants. To evaluate the antimicrobial activity, a modification of the Kirby-Bauer method was performed. A standardized amount of bacteria (0.5 McFarland) was spread uniformly on the surface of a Müller-Hinton agar plate to obtain a bacterial lawn. Then 10 µl of a solution of each bacteriocin were spotted on plates. The plates were incubated for 18-24 hours at 37°C. After the incubation different sensitivity degrees were determined according to the diameter and turbidity of the halos. Antimicrobial activity units (AU/ml) were calculated as the reciprocal of the highest dilution showing growth inhibition zone against *Escherichia coli* MC4100. The antibiotic effect of the microcins (Mcc) E492, H47 and V were assayed against Gram (-) bacterial pathogens. From the results we observed that strains of *Shigella sonneii* were sensitive to the three microcins. However, the different serotypes of *Shigella flexneri* showed different degrees of sensitivity. *Salmonella* Typhimurium and Enteritidis showed clear inhibition halos to MccV and MccE492. Adding EDTA (5 mM) to the culture medium increased the inhibitory effect of antibiotics. Some resistant bacteria became sensitive. Most cases of bacterial gastroenteritis in Tucuman are associated with the Gram (-) bacteria *Shigella flexneri* (70%), *Shigella sonneii* (16 %), *Shigella boydii* (1%), and *Salmonella spp*. (5%). Furthermore, 92% of the *S. flexneri* isolates were resistant to ampicillin and 92% of isolates of *S. sonneii* proved resistant to trimethoprim-sulfamethoxazole. The microcins, particularly MccE492, have an antibiotic effect on most of the bacteria causing gastroenteritis in children of Tucuman. Therefore it would be appropriate for use in the development of antimicrobial hybrid peptides in tandem with bacteriocins of gram (+) to obtain hybrids antimicrobial peptides with broad antimicrobial spectrum.

Código de Resumen: BF-035

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM DIFFERENT VEGETABLES

L.V. Rivero[1], M.J. Rodríguez Vaquero[1], M.A. Torres Soporsky[1], F.M. Saguir[1].

[1] *Universidad Nacional de Tucumán.*

lucianadvrivero@hotmail.com

Consumer demand today is for fresh and minimally processed (MP) vegetables with high level of safety. Lactic acid bacteria (LAB) can inhibit the pathogen microorganisms growth in contaminated vegetables by the formation of antimicrobial substances like organic acids and/or bacteriocins. The aim of this study was evaluate comparatively the antimicrobial activity of LAB isolated from different vegetables cultivated in our region (pepper, lettuce and eggplant) against pathogens mainly found in contaminated vegetables such as *Escherichia coli* ATCC 25922, *Listeria monocytogenes*, *Salmonella typhimurium*, *Enterococcus faecalis* and *Escherichia coli* 700 and their action mode. Antagonistic activities of LAB were screened using the drop test for which an active culture was spotted onto the surface of MRS agar and incubated for 48 h at 30°C to allow colonies to develop. Then, cells of the strains to be tested for sensitivity (indicator) were inoculated (5 $\log_{10}$ cfu/ml) into 7 ml of soft BHI and poured over the plate on which LAB were grown. Plates were incubated at 37°C for 48 h. The effect of the antibacterial activity on sensitive cells was investigated in cell-free culture supernatants (SN) obtained at the end of exponential growth of each LAB strain. SN were treated

as separate fractions (F), FA (neutralized pH SN), FB (neutralized pH SN treated with catalase 0.1mg/ml, 37°C,1 h) and FC (neutralized pH SN treated with trypsin 1 mg/ml, 37°C, 1 h). The growth of the sensible microorganisms in control medium and added with each SN fraction was measured by optical density at 560nm in a microplate reader. Only 3 strains of the total of 16 LAB tested (SLGR4, SLM7 from lettuce) and (SB1 from eggplant) showed no antimicrobial activity. The remaining strains showed moderate (halo diameters £ 20) and good (halo diameters > 20 mm) inhibitory activity profiles when screened by the drop test depending on LAB and indicator strain tested. In general *S. typhimurium*, *L. monocytogenes* and *E. coli* ATCC 25922 were the most sensitive to the inhibitory action with inhibition halos up to 20 mm for 100% of the positive LAB strains, while only one strain from lettuce showed a good inhibitory level against *E. faecalis*. When the FA was added to control medium there was no inhibition of sensible bacteria growths except for the JP11 strain from pepper. In this case *E. coli* and *S. typhimurim* grew lower than 27% and similar to control in the FA or FB and FC presence respectively. Thus, the LAB isolated from lettuce (4), eggplant (7) and pepper (2) showed, in general good antimicrobial activity which would be associated to the organic acids production and in the JP11 strain also to a bacteriocin like substance, supporting their potential as biocontrol agents of MP vegetables.

Código de Resumen: BF-036

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SOYBEAN SOURDOUGH: A STRATEGY TO ENHANCE THE TECHNOLOGICAL AND FUNCTIONAL QUALITY OF BREAD I

A. Bustos[1 2], B. Nacchio[2], G. Font de Váldez[3], L. Iturriaga[1 4], M.P. Taranto[3].

[1] Centro de Investigaciones y Transferencia de Santiago del Estero (CITSE CONICET). Argentina. [2] Universidad de San Pablo T. Tucumán, Argentina. [3] Centro de Referencia para Lactobacilos (CERELA-CONICET). Tucumán, Argentina. [4] Universidad Nacional de Santiago del Estero. Argentina.

yanina_bioq04@hotmail.com

Consumption of soybean flour has received special attention in recent years due to their nutritional profile and their beneficial effects on human health. In fact, soybean flour is an excellent source of high quality protein (with high biological value and essential amino acid pattern), fiber, lecithin, vitamins, minerals and isoflavone, that can be beneficial in the prevention of cardiovascular disease or even cancers. The addition of soybean sourdough is an alternative to improve the quality, flavor and increase the shelf-life of bread. The aim of this work was to characterize the soybean sourdough fermented with a selected acid lactic bacterium for its successful application in functional bread. The sourdough was prepared from soybean flour to get a yield of 200 and was tested in terms of acidification properties, antioxidant activity and rheological and textural behavior. Unfermented soybean dough and wheat dough were used as control. Results showed that after 24 h of fermentation, the sourdough pH descended to 4.4. The sourdoughs antioxidant activity, quantified using DPPH method, was 30 % higher than the unfermented soybean dough actitivity. The samples showed clear differences in terms of the fundamental rheological properties. The value of the viscous (G''), elastic (G') and complex modulus (G*) increased proportional to the angular frequency, behavior accentuated at higher frequencies. Moreover, G ' was higher than G' in all cases. G* also showed differences between the samples: unfermented soybean dough showed the highest values, followed by soybean sourdough while unfermented wheat dough had significantly lower value. Raw dough texture was analyzed replacing 20, 30 or 40 % (w/w) of wheat flour by soybean sourdough (samples) or soybean flour (control). A significant increase in the hardness was observed with the increase in the substitution level for all of the samples. However, the hardness of the sourdoughs systems was lower compared with the respective unfermented doughs, showing in the case of 20 and 30 % a behavior similar to the wheat dough included as reference. These results indicate that the use of soybean sourdough is a promising alternative to obtain functional bread.

Código de Resumen: BF-037

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

SOYBEAN SOURDOUGH: A STRATEGY TO ENHANCE THE TECHNOLOGICAL AND FUNCTIONAL QUALITY OF BREAD II

A. Bustos[1 2], B. Nacchio[2], G. Font de Váldez[3], L. Iturriaga[1 4], M. Taranto[3].

[1] Centro de Investigaciones y Transferencia de Santiago del Estero (CITSE CONICET). Argentina. [2] Universidad de San Pablo T. Tucumán, Argentina. [3] Centro de Referencia para Lactobacilos (CERELA-CONICET). Tucumán, Argentina. [4] Universidad Nacional de Santiago del Estero. Argentina.

yanina_bioq04@hotmail.com

Consumption of soybean flour has received special attention in recent years due to their nutritional profile and their beneficial effects on human health. In fact, soybean flour is an excellent source of high quality protein (with high biological value and essential amino acid pattern), fiber, lecithin, vitamins, minerals and isoflavones that can be beneficial in the prevention of cardiovascular disease or even cancers. The addition of soybean sourdough is an alternative to improve the quality, flavor and increase the shelf-life of bread. The aim of this work was evaluate the effect of soybean sourdough addition in the technological quality of bakery products. The sourdough was prepared from soybean flour to get a yield of 200. Breads were prepared following the standard formulation replacing 20, 30 or 40 % (w/w) of the components with the sourdough (samples bread) or soybean flour (control bread). Technological parameters (specific volume, crumb texture, colour) as well as antioxidant activity and consumer acceptance were determined in the products. In all of the breads, the specific volume decreased significantly with increasing ofthe percentage of soybean flour in the formulation. Only, when the sourdough was added at 20% (w/w) the specific loaf volume increased respect to the control. Crust colour showed decreases in lightness (L*) as well as a significant increase in redness (a*) and yellowness (b*) with each level of addition. Sourdoughs breads at 20% (w/w) of substitution showed a slightly increase in L*, a significant decrease of a* and b* values respect to the control. Regarding to the texture, in control breads a significant increase in hardness and chewiness of the crumb with each addition percentage was observed, while the resilience decreased with the increasing of the soybean flour. With the addition of 20 and 30 % of sourdough a significant decreases in hardness and chewiness and an increased on the crumb resilience was observed. Regarding to the antioxidant activity, breads sourdough showed higher inhibition of DPPH compared withthe control breads. he results about organoleptic characteristics revealed an overall acceptability of sourdough bread at 20% (w/w) substitution level. In conclusion, the addition of a 20% (w/w) soybean sourdough allows to obtain breads with higher nutritional and technological quality and acceptable consumer attitude.

Código de Resumen: BF-038

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

AUTOCHTONUS *Streptococcus thermophilus* UNSE321:GROWTH AND ACIDIFICATION CAPACITY IN MRS BROTH AND IN RECONSTITUTED GOAT MILK POWDER

G.N. Alléndez[1], M.A. Vidal [2], J.M. Cerezo[2], S. López Alzogaray[1].

[1]Center of Research and Transfer of Santiago del Estero (CITSE). [2]National University of Santiago del Estero (UNSE).

s.lopezalzogaray27@gmail.com

Streptococcus (*S*.) *thermophilus* has ‘‘Generally Recognized as Safe’’ status and is extensively used for the manufacture of several important fermented dairy foods, including yoghurt and some cheese varieties. In previous works, *S*. *thermophilus* UNSE321 was isolated from Argentinean artisanal goat cheeses. The aims of this study were to obtain the *S*. *thermophilus* UNSE321 growth curve (at 42°C) developing in MRS broth and in 10% (w/v) sterile reconstituted goat milk powder (RGMP) and to evaluate the growth rate constant (r) and the generation time (t_D) in both media. The growth development in MRS broth was measured by a spectrophotometer (460 nm), in RGMP by means of pH and titrable acidity (g of lactic acid/litre). An inoculum of 2% (v/v) of culture (at 1.10^6 cfu⁄ml, in MRS broth) was added in both media. The enumeration of microbial cells was made by the pour-plate method, in MRS agar, at 42°C for 48 h in semianaerobic conditions. The consumption of total sugars in MRS broth was determined by colorimetric method. In RGMP, lactose consumption was determined by metabolic counting from initial lactose concentration (Funke Gerber Lactostar). From the growth curve, in exponential phase, the best development was observed in MRS broth (r, 0.0083 min^{-1} and t_D, 36.3 min) in relation to RGMP (r, 0.0064 min^{-1} and t_D, 47 min). In previous works, similar values to r (0.0060 min^{-1}) and t_D (47 min) in sterile fluid goat milk were found. In MRS broth, during the lag phase (~125 min.) it was consumed 11% of total sugars and the acidification rate (absolute value) was 0.0016 ΔpH/min, during the exponential phase it was consumed 83% of total sugars and the acidification rate (absolute value) was 0.0077 ΔpH/min. In RGMP, during the lag phase (~110 min.) it was consumed 3.38% of initial lactose and the acidification rate (absolute value) was 0.0018 ΔpH/min, during the exponential phase the acidification rate (absolute value) was 0.0022 ΔpH/min. Thus, the inocula for goat cheese production can be conveniently spread in RGMP, reaching the multiplication rate and the level of acidification required in cheese technology.

Código de Resumen: BF-039

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EFFICACY OF LEMON ESSENTIAL OIL AND SODIUM HYPOCHLORITE OR SIMULTANEOUS WASHINGS IN KILLING NATURAL MICROBIOTA ON CHERRY TOMATOES

F.M. Saguir[1], M.A. Torres Soporsky[1], S.A. Sajur[1], L.D. Rivero[1].

[1] *Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán. Ayacucho 471. Tucumán .*

lucianadvrivero@hotmail.com

Vegetables such as tomato are possible vehicles of pathogenic microorganisms and outbreaks of food-borne illnesses have been linked to their consumption. Chlorine water (50–200 mg/l) is widely used to sanitize fruits and vegetables as well as fresh-cut produces. However, there is an increasing interest in developing alternative sanitizers for washing due to its limited efficacy, the possible carcinogenic chlorinated compounds formation as well as the occurrence of taste and odor defect in treated products. Essential oils, which are considered GRAS substances have been reported to have antimicrobial, antioxidative and food preservative properties. In a previous work demonstrated that lemon essential oil (LEO) added at 100 ppm in tomato puree removed *Escherichia coli* without adversely affect the organoleptic properties The aim of this study was therefore to evaluate the effectiveness of aqueous solutions of LEO (100 ppm) and sodium hypochlorite 5% (NaCLO) when applied individually and simultaneously as potential antimicrobial surface treatments to eliminate the natural microbiota on tomatoes. Cherry tomatoes were rinsed with sterile distilled water to determine the initial population, and then intact portions were cut into pieces (2 g) using sterile knife and used in experiments. Washing treatments were performed by immersing portions (10 g) in 50 ml of each treatment solution (LEO or NaCLO for 5, 10 and 15 min or LEO:NaCLO in proportions 50:50, 25:75, 75:25 for 5 min) in sterile glass flask with continuous agitation at 120 rpm (22±2ºC). At the end of contact time, the respective treatment solution was drained off and the treated samples were rinsed with sterile peptone water by shaking for 2 min. Serially diluted samples were spread-plated (0.1 ml) in duplicates over plate count agar (PCA); MRS agar with 1.3 µg/ml of Pimaricin, pH 6.5 (MRS-P) and Mc Conkey agar pH 7.1 (MAC) for enumeration of aerobic mesophilic bacteria, lactic acid bacteria (LAB) and Gram negative non-LAB respectively. Treatments were realized by duplicate with respective controls. Tomato surfaces contained average population levels determined on PCA, MRS-P and MAC of 5.58±0.3, 3.69±0.2 and 3.61±0.2 cfu/g respectively. Treatments with LEO alone resulted in $\log_{10}$ reductions up to 3.0 cfu/g after 10 or 15 min washing while treatment with NaClO in complete reductions. However in this last case decolorization of tomatoes was observed. Washing treatments with EOL or NaClO alone for 5 min did not lead to complete log reduction, however the Gram negative counts on MAC decreased by 75 and 100%, respectively. For this washing time simultaneous treatments with ELO:NaClO in proportions 50:50 or 75:25 completely reduced microbial populations but not 25:75 respectively, thereby enhancing NaClO significantly ELO efficacy. In conclusion the washing treatment with EOL(75):NaClO(25) may be an important component of overall contamination reduction process in whole and fresh-cut vegetables.

Código de Resumen: BF-040

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

ENZYMATIC AND PHYSICOCHEMICAL PRETREATMENTS OF WASTE COOKING OIL FOR USE IN THE BIODIESEL PRODUCTION

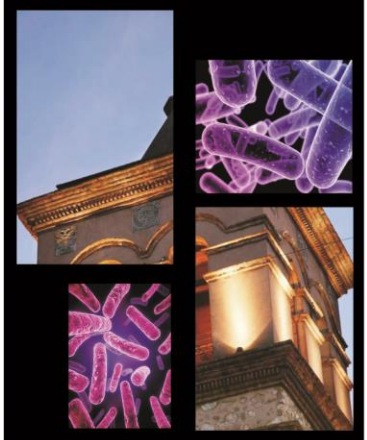
H.N. Salvatierra[1], M.D. Baigorí[1 2], L.M. Pera[1].

[1] *Laboratorio de Morfogénesis y Fermentaciones (PROIMI - CONICET), San Miguel de Tucumán.* [2] *Universidad Nacional de Tucumán.*

salvatierranati@hotmail.com

The waste cooking oil is a liquid residue that is periodically poured drains, causing water pollution. In this respect, its use for the production of biodiesel is of great industrial interest. However, the presence of water, provided by the food during cooking, and exposure to heat, results in an increase of viscosity and free fatty acids. Therefore, a combination of enzymatic (esterification by microbial lipases) and physical (decantation, filtration and heat) pretreatments allows that such oil meets the required specifications. The waste cooking oil was collected from a restaurant in the city of San Miguel de Tucumán. After heating the oil at 60 ° C for 1 h, an acidity value of 3.28 ± 0.16 % (w/w) was obtained by titration with 0.1N NaOH. This raw material was subjected to enzymatic and physical pretreatments according to a *Plackett-Burman* experimental design. Lipases from *Brevibacillus agri* E12, *Aspergillus niger* MYA 135 and *Yarrowia lipolytica* as well as several alcohols were analyzed. The influence of additional heat treatments was also studied. Among the examined variables, the constitutive mycelium-bound lipase from *A. niger*, the ethanol and the butanol significantly decreased the degree of acidity of the oil. Through a set of validation experiments, the waste cooking oil, heated at 60 ° C for 1 h and pretreated with the constitutive mycelium of *A. niger*, the butanol and the ethanol, showed an acidity value of 1.97% ± 0 (w/w). Thus, as a result of the assayed pretreatment steps the concentration of free fatty acids was significantly decreased by a lipase catalyzed esterification. In addition, the quality of fit of the polynomial model equation was expressed by the coefficient of determination (R2), which equaled 99.08 % indicating that 99.08 % of the variety in the response could be explained by the model. The value of the adjusted determination coefficient (Adj R2 = 98.23 %) was also very high showing the significance of the model. Finally, further experimentation will be needed to

estimate the optimum level of each variable and their interactions.



BIODIVERSIDAD

Código de Resumen: BD-001

Sección: Biodiversidad

Modalidad: Oral

ANALYSING BACTERIAL COMMUNITIES FROM MICROBIAL MATS AND SEDIMENTS LOCATED IN THE ATACAMA DESERT

A.B. Fernández[1], M.C. Rasuk[1], D. Kurth[2], M. Contreras[2], F. Novoa[3], D. Poire[1], M.E. Farías[1].

[1]Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas, PROIMI-CONICET, Argentina. [2]Centro de Ecología Aplicada, Santiago, Chile. [3]Universidad Nacional de la Plata. [4]Centro de Investigaciones Geológicas, UNLP-CONICET, Argentina.

anabfg82@gmail.com

The Atacama Desert has more than 100 basins with interior drainage and most of them contain salt flats. These ecosystems have extreme environmental conditions that allow the development of unique microbial communities. The objetctive of this was to study the bacterial diversity using independent culture tools of microbial mats and sediments from salt flats in the Atacama Desert. Some physicochemical conditions of the water surrounding these samples were analysed to discover if any physicochemical characteristic could be influencing in its taxonomic composition. Five samples were collected, three of them were microbial mats and two were sediments. The mat samples were taken from Laguna Llamara (samples named LL1 and LL2) and Laguna Cejar (Cej). Sediments were taken from Laguna Jachucoposa (Cop) and Laguna Pujsa (Puj) where microbial mats are not present. Total metagenomic DNA extraction was performed on each sample and the V4 hypervariable region of the bacterial 16S rRNA gene was amplified by pyrosequencing using the Ribosomal Database Project (RDP)-suggested universal primers. Diversity of the microbial community was assessed using the QIIME software package. Lakes that harbor microbial mats have a higher salinity and a lower dissolved oxygen concentration and proportion of organic matter and total phosphorous than lakes where mats are absent. All the samples have important concentrations of arsenic, with an extremely high amount in Puj. *Proteobacteria* and/or *Bacteroidetes* are the major phyla represented in all samples. Also, other phyla as *Spirochaetes, Chloroflexi* or *Verrucomicrobia* are found. However, cyanobacterial sequences are only observed in LL2 and Puj. On the other hand, we have found a higher diversity in sediment than in mat samples. The sediments samples contain phyla not observed in mat samples. 16S rRNA gene sequences classified within *Actinobacteria* and *Gracilibacteria* are only found in Puj and related to *Tenericutes*, *Gemmatimonadetes* and *Acidobacteria* are only observed in Cop. Finally, an important fraction of the sequences could not be classified at phylum level. The high diversity found in sediment samples may be explained by the physicochemical conditions in the environment. For example, they have a lower conductivity than mat samples. It is known hypersaline environments have a low diversity, where halophilic microorganisms are able to survive to these extreme conditions because they have specific strategies to balance the osmotic pressure. Besides, we found a low proportion or absence of *Cyanobacteria* in the ecosystems studied, suggesting the possibility that other groups may be playing an essential role as primary producers in these extreme environments. Additionally, the large proportion of 16S rRNA gene sequences that could not be affiliated to any known bacterial phyla suggesting that in these ecosystems there are potential novel representatives of bacterial phyla not yet described.

Código de Resumen: BD-002

Sección: Biodiversidad

Modalidad: Oral

EXTREME-HALOPHILES: THEIR ROLE IN THE ARSENIC BIOGEOCHEMICAL CYCLE

M.C. Rasuk[1], O.F. Ordoñez[1], M. Soria[1], M.E. Farías[1].

[1]Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas, PROIMI-CONICET, Argentina.

cecirasuk@gmail.com

Biofilms, mats and microbialites dwell under extreme environmental conditions (high salinity, extreme aridity, pH and arsenic concentration) in the Argentinean Puna and the Atacama Desert. Microbial communities inhabiting those ecosystems are poorly known. Arsenic metabolism is proposed to be an ancient mechanism in microbial life. Besides, some bacteria and archaea are not only able to use detoxification processes to grow under high arsenic concentration, but also, some of them are able to exploit arsenic as a bioenergetic substrate in either anaerobic arsenate respiration or chemolithotrophic growth on arsenite. Only four aioAB coding for arsenite oxidase and two *arrA* coding for arsenate reductase sequences from haloarchaea were previously deposited in the NCBI Database, but have not been reported in the literature. The *arrA* arsenate reductases are reliable indicators of anaerobic As (V) respiration and catalyze the electron transfer to the As (V) terminal acceptor in dissimilatory arsenatereducing prokaryotes (DARPs). In this work, we are presenting our first steps in the study of the arsenic biogeochemical cycle in these ecosystems. Thus, the aim of this study was to isolate and to study the arsenic metabolism genes of the isolated extreme halophile microorganisms as well as to test the growth in minimal medium using different carbon sources. Mats and microbialites samples were taken from the water's edge of Laguna Tebenquiche, Laguna Brava (Salar de Atacama, Chile)

during December 2012 and from gaylusite crystals (Laguna Diamante) in August 2014. Samples were enriched and plated in WS medium supplemented with arsenic (AsIII 0.5mM and AsV 20mM). Arsenite oxidase (aioB) and Arsenate reductase (arrA) primers specific for haloarchaea were designed using PrimerProspector software. Selected primers were *aioB*-1190F (5'-GCTCMTSACCGGCAGCGTCG-3'), aioB-1507R (5'-YGATCTCGTCGATGTCGGCG-3'), arrA-417F (5'CCCGAGTTCGAGCCSATCTC-3') and arrA-614R (5'GCRCAGATCGMGCTGTGGGA-3'). In order to identify the isolates we used Archaea-specific primers for 16S rDNA gene amplification: 344F (5´- ACG GGG YGC AGC AGG CGC GA-3´) and 915R (5´- GTG CTC CCC CGC CAA TTC CT -3´). Fragments of 577 bp, 317pb and 197pb were obtained from 16S rDNA, aioB and arrA genes respectively. Universal primers 27F and 1492R were used to amplify 16S rDNA in bacterial isolates. 25 isolates belonging to Archaea and Bacteria Domain were obtained; they are related to the Phylum Euryarchaeota, Firmicutes and Proteobacteria. AioB and arrA genes were found in most of the isolates and DNA from the samples (mats, microbialites and biofilm). The best carbon source tested was pyruvate and acetate, being pyruvate better in all cases. Promising results were obtained in the search of organisms able to use arsenic in their bioenergetic metabolism. More studies are underway to try to better understand these very interesting systems.

Código de Resumen: BD-003

Sección: Biodiversidad

Modalidad: Poster

IDENTIFICATION OF BACTERIA ASSOCIATED WITH TWO ENTOMOPATHOGENIC NEMATODE SPECIES OF THE GENUS S*teinernema* (RHABDITIDA: STEINERNEMATIDAE)

J.C. Rondan Dueñas [1], A. Muñoz[1], A. Belaus[1], P.S. Vélez[1], E. Del Valle[2], M.E. Doucet[3], P. Lax[3].

[1]Centro de Excelencia de Procesos y Productos (CEPROCOR), Córdoba. [2]Facultad de Ciencias Agrarias, UNL, Santa Fe. [3]IDEA (CONICET-UNC) y Centro de Zoología Aplicada, FCEFyN-UNC. Córdoba.

jrondan@ceprocor.uncor.edu

Entomopathogenic nematodes of the genus *Steinernema* are obligate parasites of insects and are used as biological control agents of insect pests. Third-stage juveniles (J3) carry symbiotic bacteria of the genus *Xenorhabdus* in their intestine; at the moment of infection, these bacteria are released to the insect hemocoel, where they multiply and become the food source for the nematode. Although this nematode-bacteria relationship is specific, other bacteria related with these parasites have occasionally been identified. The aim of this work was to isolate the bacteria associated with J3 of native isolates of *S. rarum* and *S. diaprepesi*, using two treatments (nematodes subjected or not to disinfection of the cuticle using NaClO). In both treatments, J3 were macerated; the supernatant was used to develop a culture with brain-heart agar medium. The colonies were isolated and total DNA was extracted for further identification based on the 16S rRNA gene. Disinfection of J3 allowed isolation of symbiotic bacteria *X. szentirmaii* and *X. doucetiae* from *S. rarum* and *S. diaprepesi*, respectively. Two strains of *Serratia* sp. were extracted from the nematodes that were not subjected to external disinfection. The results document the unusual foretic association between *Serratia* sp. and the two *Steinernema* species.

Código de Resumen: BD-004

Sección: Biodiversidad

Modalidad: Poster

A POLYPHASIC APPROACH FOR THE TAXONOMIC DESCRIPTION OF TWO NATIVE BLOOM-FORMING CYANOBACTERIA

A. Aguilera[1], R.O. Echenique [2], E. Berrendero Gómez[3], J. Kastovsky[3], G.L. Salerno [1].

[1]INBIOTEC-CONICET y CIB-FIBA, Argentina. [2]Department of Phycology, Faculty of Natural Science (UNLP),CIC-BA, Argentina. [3]Department of Botany, Faculty of Science University of South Bohemia, Czech Republic.

anabella.aguilera@gmail.com

The combination of genotypic and phenotypic methods for determining taxonomic positions (known as the "polyphasic approach"), is currently the choice for resolving taxonomic uncertainties. *Raphidiopsis mediterranea* and *Cylindrospermopsis raciborskii*, both planktonic, freshwater bloom-forming cyanobacteria, are of great concern because they can produce cyanotoxins. Although these species are morphologically similar, the presence or absence of heterocysts (in *C. raciborskii* or *R. mediterranea*, respectively) is the only character that has been used to distinguish between them. Importantly, this has led to misidentifications and to question the validity of the genus *Raphidiopsis*. In this work, studies of morphological variation in nature were combined with ecophysiological and molecular analyses to elucidate the taxonomic classification of two strains of *R. mediterranea* native from shallow lakes of the Buenos Aires province. The strains, studied in field for two years, were isolated,

grown in MLA medium, and analyzed for their morphological traits by light and transmission electron microscopy. Molecular analyses were based on the sequences of *16S rRNA* genes and 16S-23S internally transcribed spacers (ITS). On the basis of the original species description, both strains were classified as *R. mediterranea.* No heterocysts were observed during the whole sampling period and no differentiation to these cells were detected when the strains were grown in culture medium lacking nitrogen compounds. Also, the *nifH* gene, involved in nitrogen fixation, could not be amplified by PCR methodology. The *16S rRNA* and ITS sequences were 98-99% identical to those of *C. racibosrkii* strains. Phylogenetic analysis showed that both sequences grouped with *Raphidiopsis* and *C. raciborskii* sequences belonging to strains from American countries and Senegal, and separated from other clusters grouping sequences of strains from other continents. Based on these findings, we propose the unification of *Raphidiopsis* and *Cylindrospermopsis* under a single genus named *Raphidiopsis*, in accordance with the principle of priority. These results indicate that newly isolated strains must be classified on the basis of the polyphasic approach. Also, when required, previously classified organisms can be reclassified. Thus, current techniques allow microbiologists to evaluate the microbial diversity and decipher the natural phylogenetic relationships among taxa.

Código de Resumen: BD-005

Sección: Biodiversidad

Modalidad: Poster

FLAMINGOS PAMPEANOS AS RESERVOIRS OF MULTIDRUG-RESISTANT BACTERIA

M. Soria[1], D. Kurth[1], J. Dib[1 2], V. Fernández Zenoff[1 2].

[1]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET). Av. Belgrano y Pasaje Caser. [2]Instituto de Microbiología, Fac. de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán.

soria_mns@hotmail.com

Migratory birds can carry a wide range of viral, bacterial, fungal and protozoan zoonotic agents and some are pathogens that may be transmitted to humans. It has been postulated the role of flamingos as dispersers of antibiotic-resistant microorganisms. The aim of this work was to study the intestinal microbiota of these vertebrates and evaluate the resistance of the isolated bacteria to different antimicrobials. Bacteria from flamingo feces from Bella Vista and La Badenia lakes (33°42´34°18´S and 61°25´-62°32´O) were isolated by plating in R2A and R2A supplemented with four different antibiotics (ampicillin [Amp], 100 μg ml-1; chloramphenicol [Cm], 170 μg ml-1; erythromycin [Ery], 50 μg ml-1 and tetracycline [Tet], 50 μg ml-1). In Bella Vista lake, a count of 1.9×10^3 CFU μg-1 from flamingo feces was obtained; where 43% of the isolates were resistant to Amp, 17% to Tet and 5% to Cm and Ery. In Badenia lake, 2.2×10^3 CFU g-1 were determinated, with 78% of resistance to Tet, 55% to Cm, 14% to Ery, and 5% to Amp. 68 bacteria were isolated and they were grouped according to their RAPD profile. The identification of the isolates was performed by the sequence of the amplification products of 16S rRNA gene. This study contributes to characterize the antibiotic resistant pattern of bacteria isolated from pampean flamingo's feces and highlights the role of flamingos as reservoirs, vectors and/or dispensers of antibiotic-resistant bacteria.

Código de Resumen: BD-006

Sección: Biodiversidad

Modalidad: Poster

BACTERIAL AND ARCHAEAL DIVERSITY IN HYPERSALINE MICROBIAL MAT: CONTROLS ON MICROBIAL CARBONATES MICROTEXTURES AND BIOGEOCHEMICAL SIGNATURES

F. Boidi[1 2], F. Gomez[1], C. Mlewski[1], M.E. Farías[2].

[1]CICTERRA- Centro de Investigaciones en Ciencias de la Tierra, CONICET. [2]PROIMI- Planta Piloto de Procesos Industriales Microbiológicos, CONICET.

flajboidi@gmail.com

Microbial mats are laminated microbial communities typically developed in extreme environments. Their stratified appearance is due to vertical segregation of microorganisms in response to mm-scale gradients of light and redox potential. Laguna Negra, in the Puna region of Argentina is a high-altitude hypersaline lake under extreme environmental conditions (high UV-radiation, extreme temperatures and salinity), which harbors an extensive microbialitic system. It consists of carbonate microbialites, morphologically diverse microbial mats and mineral precipitation within them. Here we studied the microbial mat that prevails in the system, developing under shallow water (up to 10 cm). We recognized an orange-pink top layer, followed by purple and green layers in the undermat and underlain by a dark-colored horizon at the bottom. We explored bacterial and archaeal diversity in each strata using 16S rDNA pyrosequencing and related this information with carbonate microtextures and isotopic record reported there. The top layer revealed Bacteroidetes, Proteobacteria, Verrucomicrobia and DeinococcusThermus as prominent phyla. Cyanobacteria is four times more abundant in the upper layer than deeper in the mat, even though is not one

of the most abundant phylum. Diatoms are common as seen under de SEM and optical microscopy. Members of these groups have photosynthetic lifestyle and resist high UV radiation, which is expected near the surface and given the high UV light influx reported in the area. The second layer presented anoxigenic phototrophs: abundant Chloroflexi, mainly the Chloroflexia class, and the highest proportion of Gammaproteobacteria, enclosing sulfur oxidizing bacteria. Third green layer contained the highest proportion of sulfur reducing Deltaproteobacteria. Certain groups increased their presence deeper in the mat, at the dark bottom, such as Firmicutes and Archaea (mostly Euryarchaeota). The order Halanaerobiales prevailed within Firmicutes, comprising fermentative halophilic anaerobes. Euryarchaeota includes methanogenic extremophiles, likely to occur in the anoxic strata of the mat. Previous work showed that Ca-carbonate precipitation (typically calcite) is associated with exopolymeric substances segregated by microorganisms. In addition, aragonite has been observed closely related to cyanobacteria and diatoms suggesting some microbial control. C-O isotopes (within carbonates and organic matter) and S-isotopes (carbonate associated sulfates and pyrite) suggest a complex combination of physicochemical (evaporation, degasification) and biological processes (photosinthesis, methanogenesis, sulfate reduction, sulfur oxidation) controlling the isotope signature. The 16S rDNA data indicate the presence of organisms with these metabolisms. Further studies (e.g. metagenomics, culturing) will allow us to unravel the specific role of physicochemical and microbiological controls within the carbonates and its biogeochemical signatures.

Código de Resumen: BD-007

Sección: Biodiversidad

Modalidad: Poster

DETECTION OF METALLO-β-LACTAMASES IN *Pseudomonas aeruginosa* RECOVERED FROM CYSTIC FIBROSIS PATIENTS

D.S. Casco[1], P.F. Martina[1 2], E. Pegels[1], E. Valdez [1], M. Quiroga[1].

[1]Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Argentina. [2]Grupo de Investigación en Genética Aplicada (GIGA), IBS-UNaM-CONICET.

pfmartina@hotmail.com

Pseudomonas aeruginosa is one of the most important nosocomial pathogens, as well as one of main cause of respiratory chronic infection on patient with cystic fibrosis. Its high level of intrinsic antibiotic resistance, coupled with his extraordinary ability to develop additional resistance by chromosomal mutations make this pathogen one of the most difficult to treat. Faced with β-lactam antibiotics, especially carbapenems, common resistance mechanism is impermeability, and there are other mechanisms of clinical importance as the production of enzymes capable of hydrolyzing, called carbapenemases. These mechanisms often coexist, coupled with the presence of chromosomal β-lactamase AMP-C. The aim of this study was to evaluate the usefulness of the modified Hodge and EDTA-disk synergy test for the screening of metallo-b-lactamase-producing strains from imipenem-resistant clinical isolates of P. aeruginosa recovered from the sputum of CF patients with chronic respiratory infections. Thirty-two isolates recovered from the sputum of 32 patients with CF during 2013. Samples were seeded in chocolate agar, blood agar, agar Mac Conkey agar and mannitol salt agar, and incubated in stove at 35 ± 2 °C for five days. The isolated microorganisms were characterized according to standard biochemical tests. Sensitivity to various β-lactam antibiotics was determined and no β-lactams by diffusion assay solid medium and minimal inhibitory concentration (MIC) to Imipenem was determined according to CLSI recommendations. Twenty-one per cent of isolates were resistant to Imipenem. The MIC range of Imipenem for the isolates was between 32 and 256 mg/L. Among a total 8 Imipenemresistant isolates screened by the modified Hodge test and EDTA-disk synergy, we found similar results. To check, specific PCR for NDM genes yielded 2 positive isolates. The remaining six isolates had negative results for molecular detection of the carbapenemases tested (blaVIM, blaNDM, blaIMP, blaKPC and blaOXA genes).

Código de Resumen: BD-008

Sección: Biodiversidad

Modalidad: Poster

CONTINUOUS EMERGENCE OF NEW CA-MRSA CLONES FROM SUCCESSFUL MSSA LINEAGES

A.L. Egea[1], P. Gagetti [2], D. Barcudi [1], D. Faccone[2], J.L. Bocco[1], A. Corso[2], C. Sola[1].

[1]CIBICI CONICET. Fac. de Cs. Químicas, UNC. [2]INEI-ANLIS, CABA .

aegea@fcq.unc.edu.ar

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasing problem worldwide in recent decades causing both hospitalacquired (HA-MRSA) and community-acquired (CA-MRSA) infections. In Argentina CA-MRSA international clones belonging to major lineages coexist in different proportions, such as Sequence Type (ST)5-SCCmec type IVa Clonal Complex 5 (CC5), ST30-IV (CC30), ST97-IV (CC97), ST72-IV (CC8), ST8-IV (USA 300) (CC8) and ST1-IV (CC1).

We aimed assess the genetic relationship between CA-MRSA epidemic clones and MSSA strains identified in Argentina to investigate the possible local origin or its spread from other countries of these international CA-MRSA clones A total of 132 MSSA clinical isolates collected during Nov-2009 from 66 hospitals (20 provinces and Bs. As. City) were analyzed by *S. aureus* Protein A (spa) typing, Panton Valentin Leukocidin (PVL), Pulsed Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST). Molecular analysis by spa typing; showed 65 different spa types (t) grouped into 12 CC according MLST (%): CC5 (21), CC30 (16), CC1 (15), CC121 (10), CC8 (8), CC45 (8) CC12 (1), CC97 (8), CC25 (4) CC101 (2), CC15 (1), CC88 (1) distributed throughout the country. The proportion of isolates belonging to CC5 (t311 and t002) and CC30: (t012, t021 and t018) differed significantly between North and South of Argentina: 36% and 6% vs. 12% and 32 %, respectively. Based Upon Repeat Pattern (BURP) analysis showed that MRSA (previously analyzed) and MSSA isolates belonging to CC5 shared the same spa type (t311 and t002), suggesting that these were closely related. Contrary, no common spa type was shared between MSSA and MRSA belonging the CC30 (ST30). The remaining CCs also showed a closely genetic relation between the MSSA and MRSA by PFGE type, spa type and Sequence Type , sharing the spa type and the ST, such us: ST72 and t148 (CC8), ST1 and t127 (CC1), ST88 and t186 (CC88), ST97 and t359 (CC97) and ST121 and t159. We also detect MRSA and MSSA belonging to ST8 with spa t008, genetic characteristics of the major epidemic CAMRSA clone spread in EEUU: USA300. However as many as four different SCCmec (sub) types in five MRSA isolates with spa t008, indicating independent acquisitions in the MSSA ancestor. These results support the hypothesis about the local and continuous emergence of new CA-MRSA clones from successful MSSA lineages in addition to the dissemination of them is an important process of increasing resistance to betalactam antibiotics. However, only the whole genome sequence of these strains will clarify this hypothesis.

Código de Resumen: BD-009

Sección: Biodiversidad

Modalidad: Poster

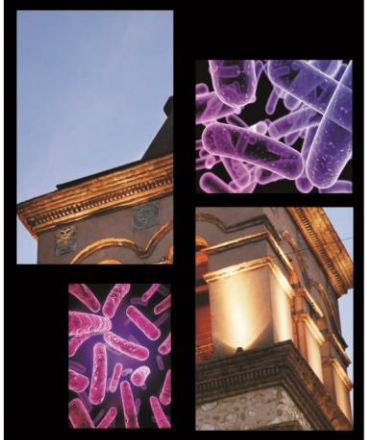
PROKARYOTIC DIVERSITY IN ECOSYSTEMS ASSOCIATED TO MINERALS FROM THE HYPERSALINE LAKE TEBENQUICHE IN THE ATACAMA DESERT

A.B. Fernández[1], M.C. Rasuk[1], M. Contreras[2], F. Novoa[2], D. Poiré[3], P.T. Visscher[4], A. Ventosa[5], M.E. Farías[1].

[1]PROIMI-CONICET, Argentina. [2]Centro de Ecología Aplicada, Chile. [3]UNLP-CONICET, Argentina. [4]University of Connecticut, USA. [5]University of Sevilla, Spain.

anabfg82@gmail.com

The Salar de Atacama is located in the Chilean central Andes and it is a huge evaporitic system with a large number of saline water bodies in its interior. Lake Tebenquiche is one of the largest and prokaryotic microorganisms inhabiting this lake are subjected to severe conditions as high solar radiation due to a lower barometric pressure at high altitude, extreme daily temperature fluctuations, intense changes in salinity caused by net evaporation and high arsenic concentrations in the water due to volcanic events. Therefore, we decided toanalyse the prokaryotic diversity of microbial mats, microbialites and one evaporite by pyrosequencing of the V4 hypervariable region of the 16S rRNA gene. In addition, the totalmetagenomic DNA of a microbial mat was sequenced to studythe genetic and metabolic diversity for understanding the microbial processes associated to minerals in a system at high altitude. Five different samples were collectedfrom lake Tebenquiche: two microbial mats, TebMa1 and TebMa2; two microbialites, TebMi1 and TebMi2; and one evaporite, TebEv1. The total metagenomic DNA of each sample was extracted and pyrosequenced the V4 hypervariable region of the prokaryotic 16S rRNA gene. The prokaryotic 16S rRNAamplicons were analysed using the QIIME software package.The totalmetagenomic DNA from microbial mat, TebMa1, was sequenced using paired-end Hi-Seq 1500 Illumina Technology and the raw reads obtained were filtered, assembled into contigs and annotated. Euryarchaeota is one of the most abundant phyla in all samples studied, especially in TebEv1 with 97 % of 16S rRNA sequences. Most of the euryarchaeal OTUs are classified within the class Halobacteriaor anaerobic and methanogenicarchaea. Specific genes as indicators of particular biogeochemical cycles were searched in the assembled contigs of TebMa1.Nitrogenase gene sequences are found in a high amount and these sequences were aligned with a range of 70%-89% identity to known nitrogenase sequences.Phosphate is mainly obtained by two mechanisms when there is a reduced availability of phosphorous: polyphosphate metabolism and phosphate recycling. Cytoplasmic arsenate reduction and arsenite oxidationare clearly present in the arsenic-rich habitat TebMa1. The high conductivity measured in TebMa2 and TebEv1 must be promoting the growth of members belonging to the class Halobacteria due to the dominance of this taxon in both samples.In TebMa1, we suggest could be carried out an active biological nitrogen fixation by bacteria and archaea and due to the low percentage identity to the closest relative an important part could be novel diazotrophic microorganisms.This ecosystem is rich in arsenic and its inhabitants use arsenic resistance strategies as cytoplasmic arsenate reduction and arsenite oxidation but a possible mechanism employed by these microorganisms could be through quelation of this metalloid using polyphosphates.



MICROBIOLOGÍA MOLECULAR

Código de Resumen: MM-001

Sección: Microbiología Molecular

Modalidad: Oral

NEW INSIGHTS FOR GROWTH AT LOW TEMPERATURES REVEALED BY RNA-SEQ IN THE ANTARCTIC BACTERIUM *Pseudomonas extremaustralis*

P.M. Tribelli[1 2], E.C. Solar Venero[2], M.M. Ricardi[3], M. Gómez-Lozano[4], L.J. Raiger Iustman[1 2], S. Molin[4], N.I. López[1].

[1]Dpto. QB, FCEyN, UBA, Bs As, Argentina. . [2]IQUIBICEN, CONICET, BsAs, Argentina . [3]IFIBYNE-CONICET FCEyN, UBA, BsAs, Argentina . [4]Novo Nordisk Foundation Center for Biosustainability, DTU, Denmark.

paulatrib@qb.fcen.uba.ar

Temperature is one of the most important factors for bacterial development. Cold environments are widely distributed on earth and microorganisms should develop different cellular adaptations to cope with the stress derived from low temperatures. *Pseudomonas extremaustralis* is an Antarctic bacterium able to grow under low temperatures. In this work, we analyzed the whole transcriptome of early exponential cultures of *P. extremaustralis* at 8°C by RNA deep-sequencing technology along with analysis of mutant strains of relevant genes. Transcriptomic data were analyzed by using Rockhopper software to determine differential expressed genes (P<0.05 and Q<0.05). Functional enrichment of differentially expressed genes was determined using Blast2GO software by assigning the GO category to all genome sequences and to the differentially expressed genes. We found that genes involved in primary metabolism, including TCA-related genes, as well as cytochromes, and amino acid metabolism coding genes, were repressed at this growth stage. Among up-regulated genes, those coding for transcriptional regulatory and signal transduction proteins were over-represented at cold conditions. These genes could result important for further growth and to reach the high biomass observed at 8ºC after 72h of culture. By deeply analyze mutant strains obtained from both a random library of mutants and site-directed deletion technique supported by genomic and transcriptomic analysis data; we were able to propose a novel role of a PQQ dependent pathway as essential for growth under low temperatures.

Código de Resumen: MM-002

Sección: Microbiología Molecular

Modalidad: Oral

IN VITRO AND *IN VIVO* CHAPERONE ACTIVITY OF THE PHASIN PhaP FROM *Azotobacter* sp. FA8

M.P. Mezzina[1], D.E. Wetzler[1], N. Dinjaski[2], A.M. Prieto[2], M.J. Pettinari[1].

[1]Instituto de Química Biológica de la Facultad de Cs. Exactas y Naturales, UBA (IQUIBICEN-CONICET). [2]Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CIB/CSIC).

marielamezzina@gmail.com

Phasins are a group of proteins associated to granules of polyhydroxyalkanoates (PHAs). Apart from their structural role as part of the PHA granule cover, different structural and regulatory functions have been found associated to many of them, and several biotechnological applications have been developed using phasin protein fusions. PhaP from *Azotobacter* sp. FA8 ($PhaP_{Az}$) is a representative of the prevailing type in the multifunctional phasin protein family. This protein has been cloned and expressed in *Escherichia coli*. Recombinant *E. coli* strains overexpressing $phaP_{Az}$ grow more, and accumulate more polymer, suggesting that $PhaP_{Az}$ exerts a growth promoting effect. Expression of $PhaP_{Az}$ was also observed to have an unexpected protective effect in *E. coli* strains that do not synthesize PHAs, under both normal and stress conditions, resulting in increased growth and higher resistance to both superoxide stress and heat shock. *In vitro* chaperone activity experiments were performed in order to shed light on the mechanisms by which $PhaP_{Az}$ exerts its protective effect. $PhaP_{Az}$ was shown to prevent *in vitro* thermal aggregation of the model protein citrate sinthase (CS) and to facilitate the refolding process of the enzyme after chemical denaturation. These experiments showed that $PhaP_{Az}$ presents *in vitro* chaperone activity. Fluorescence microscopy and electron transmission microscopy were used to study the role of $PhaP_{Az}$ in *in vivo* protein folding, protein aggregation and inclusion body dynamics. $PhaP_{Az}$ was shown to colocalize with inclusion bodies of PD, a protein that forms large inclusion bodies when overexpressed. A reduction in the number of inclusion bodies of PD was observed when the insoluble protein PD was coexpressed with $PhaP_{Az}$ or with the known chaperone GroELS. These results demonstrate that $PhaP_{Az}$ has chaperone like

functions both *in vitro* and *in vivo* in *E. coli* recombinants, and suggests that phasins could have a general protective role in natural PHA producers, that in these microorganisms is masked by the fitness enhancing properties of the polymer. These observations open the door for novel biotechnological applications of this protein, for example, in the production of recombinant proteins and other heterologous products in *E. coli*.

Código de Resumen: MM-003

Sección: Microbiología Molecular

Modalidad: Oral

A LONG-TERM EXPERIMENTAL EVOLUTION STUDY BY COMPARATIVE GENOMICS: MODULATION OF c-di-GMP HAS A KEY ROLE IN *Pseudomonas aeruginosa* ADAPTATION TO BIMODAL SWITCHING BETWEEN BIOFILM AND PLANKTONIC STATES

R.A. Tobares[1,2], A.M. Smania[1,2].

[1]CIQUIBIC-CONICET. [2]Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

atobares@fcq.unc.edu.ar

One of the most fascinating bacterial adaptive strategies during growth in fluctuating environments is phenotypic diversification into different niche specialists and subsequent selection of those adapted phenotypes by natural selection. We have been studying the opportunistic pathogen *Pseudomonas aeruginosa*, which is able to grow in communities known as biofilms. Biofilms can be considered as heterogeneous and stratified environments so that biofilm growth intrinsically implies phenotypic diversification. Small Colony Variants (SCV), one of the adapted morphotypes that arise from biofilm cultures, have been characterized as small size colonies formed by hyperadherent and highly biofilm producer cells with a diminished capability of movement. When SCV are grown in solid media, wild type-like (WT) morphotypes emerge from the colony edges, which show reversion of the SCV phenotypic traits. In this work, we analyzed the adaptability of *P. aeruginosa* using a combination of long-term experimental evolution assays and whole-genome sequencing. Three lines of *P. aeruginosa* UCBPP-PA14 were exposed to alternating and successive cycles of biofilm growth (conversion) and growth in solid media (reversion), and subjected to rounds of evolution in which clones with SCV or WT morphology were isolated from conversion or reversion rounds, respectively. Single SCV clones were propagated as founders in the next reversion round, as well as single WT clones were used to found the next round of conversion, leading to succeeding bottleneck events. Evolutionary histories were stopped when the frequency of emerged SCV or WT morphotypes in the respective rounds was less than 0.05%. Genomes from the ancestral and final clones obtained from each three lines were sequenced on an Illumina Hiseq1500 platform. Comparative genomic analysis demonstrated the acquisition of one non-synonymous mutation per round of evolution. Strinkingly, mutations in the three parallel evolving lines were found mostly in *wsp* and *yfiBNR* operon genes, codifying for chemosensory systems involved in regulating biofilm formation through modulation of c-di-GMP levels. We hypothesize that, in our experimental evolution assay, SCV conversion and reversion switching is based on mutations that produce stimulation or inhibition of diguanilate cyclase catalyzed c-di-GMP synthesis, respectively. Complementation experiments in the SCV clones obtained in the different evolving conversion rounds using the *P. aeruginosa* PA2133 phosphodiesterase produced a clear reversion of SCV traits, indicating the involvement of the underlying mutations in increasing c-di-GMP**.** So far, our results indicate that, in our experimental evolutionary model, adaptability of *P. aeruginosa* to bimodal switching between biofilm and planktonic states is based on compensatory spontaneous mutations in genes involved in the regulation of the intracellular levels of the second messenger c-di-GMP.

Código de Resumen: MM-004

Sección: Microbiología Molecular

Modalidad: Oral

X-ome-Q: A WEB-BASED SERVER FOR INTEGRATIVE OMICS ANALYSIS

G.F. Burguener[1], E.J. Sosa[1], L.A. Lucianna[1], L. Radusky[3], E. Lanzarotti[3,2], L. De Felipe[3,2], D.A. Fernández Do Porto[1], A.G. Turjanski[1,2], M. Martí[3,1].

[1]Plataforma Bioinformática Argentina, Instituto de Cálculo, FCEyN, UBA. [2]Departamento de Química Inorgánica, Analítica y Química Física / INQUIMAE - CONICET, FCEyN, UBA. [3]Departamento de Química Biológica, FCEyN, UBA.

ezequieljsosa@gmail.com

Modern online omics servers enable researchers to rapidly analyze data from different experimental sources. They perform tasks such as automatic assembly, annotation, visualization and molecular modeling of protein structures among others. However, each of these servers are focused on a particular type of analysis and cannot deal with different data sources. For a more comprehensive analysis of a given microorganism, users must manually collect and process results from different sources, and significant investment of time and resources are required. With this in mind, we developed a web based service to support microbial genome wide biological data analysis and processing. X-ome-Q is an easy-to-use web-based platform which allows genome wide based data consolidation from diverse sources at different processing stages including assembly, annotation, comparative genomics, metabolic pathway recognition, and modeling of proteins' structure. All this information can be easily navigated using keywords and different gene based annotations, including ontology and cog terms, protein family and metabolic pathways, etc. We hope X-ome-Q will thus enhance researchers capability of knowing genome to proteome characteristic of a given microorganism of interest, becoming a powerful tool to understand and probe its biology.

Código de Resumen: MM-005

Sección: Microbiología Molecular

Modalidad: Oral

TWO LuxR-TYPE TRANSCRIPTIONAL REGULATORS WITHIN A CYCLIC-LIPOPEPTIDE GENE CLUSTER ARE NOVEL TARGETS OF THE POST-TRANSCRIPTIONAL Gac/Rsm CASCADE IN *Pseudomonas protegens* CHA0

P. Sobrero[1], J. Frescura[1], M. Ongena[2], C. Valverde[1].

[1]*LBMIBS, DCyT, Universidad Nacional de Quilmes.* [2]*Université de Liege, Bélgica..*

valverdecl@hotmail.com

Pseudomonas protegens strain CHA0 regulates the expression of several traits involved in biocontrol of phytopathogenic fungi through a well characterized regulatory network that include an extracellular unknown quorum sensing-like signal, the GacS/GacA two component system, two RNA-binding proteins (RsmA and RsmE) and three small non-coding regulatory RNAs (RsmX, RsmYandRsmZ). The Gac/Rsm pathway orchestrates the induction of cyanide and antibiotics production, and of several extracellular hydrolytic enzymes. Based on structural and sequence requirements of the interacting elements of the cascade (proteins and RNAs) it is anticipated that expression of novel uncharacterized genes/operons may be subject to regulation by the Gac/Rsm system, and contribute to the ecological fitness and biocontrol activity of strain CHA0. In order to get a broader insight into the regulon of the Gac/Rsm network, we carried out an *in silico* prediction of novel Gac/Rsm targets by inspecting the genome sequence of strain CHA0 for the presence of RsmE-binding sites near or at their cognate ribosome binding site within the untranslated region (UTR) of all annotated genes; next, we filtered hits according to secondary structure folding, RNA-RsmE residue contacts, and conservation of the putative binding site among related pseudomonads. Among a set of 47 identified putative novel Gac/Rsm targets, we present here experimental evidences that the product of the gene annotations *PFLCHA0_c21910* (hereafter c21910) and *PFLCHA0_c21850* (hereafter c21850) are Gac/Rsm targets possibly involved in lipopeptide synthesis. Both annotations resemble LuxR-like transcriptional factor physically linked to operons responsible for cyclic lipopeptide (CLP) synthesis in different pseudomonads. In support of this, c21910 complemented CLP production in a *P. fluorescens* SBW25 mutant lacking the homolog transcriptional factor. Drop-collapse tests showed that the Gac/Rsm pathway regulates extracellular biosurfactant activity (CLP production) in CHA0. Moreover, the biosurfactant activity of wild type and Gac/Rsm mutants correlated with the CLPs profile of culture supernatants as determined by LC-MS analysis. Translational reporter fusions between c21910 or c21850 5'UTRs and a leaderless '*lacZ* mRNA were constructed and confirmed by sequencing. The expression pattern of these fusions along the growth curve of wild type CHA0 and their Gac/Rsm mutant derivatives showed that c21910 and c21850 expression was strongly repressed in both the *gacS* mutant and the *rsmX/X/Z* triple mutant in stationary phase, whereas it was activated in the *rsmA/E* mutant. Mutational analysis of the putative RsmA/E binding site suggests direct regulation of both genes by the translational repressor proteins. Our data indicate that CLP production in strain CHA0 is controlled by Gac/Rsm pathway and the mRNA product of the putative transcriptional regulator genes c21910 and c21850 are both direct and novel Gac/Rsm targets.

Código de Resumen: MM-006

Sección: Microbiología Molecular

Modalidad: Oral

THE MISMATCH REPAIR PROTEIN MutS CONTROLS Pol IV DEPENDENT-MUTAGENESIS INDUCED BY SUBINHIBITORY CONCENTRATIONS OF CIPROFLOXACIN

L. Margara[1], C. Argaraña[1], M. Monti[1].

[1] *CIQUIBIC-CONICET, Dpto. de Qca. Biol., FCQ-UNC, Córdoba. Argentina.*

lmargara@fcq.unc.edu.ar

The rapid emergence of *de novo* antibiotic resistance upon treatment of bacterial populations with subinhibitory antibiotic concentrations is a serious concern. It is, therefore, of considerable significance to understand the molecular mechanisms implicated in the emergence of resistance at low antibiotic concentrations. We previously found that subinhibitory concentrations of the quinolone antibiotic ciprofloxacin (Cip) increase the emergence of Cip resistant mutants of the pathogenic bacteria *Pseudomonas aeruginosa*. A DNA microarray assay revealed that *P. aeruginosa* genes encoding low fidelity DNA polymerases (Pol), including Pol IV, are up-regulated in response to Cip exposure. Another work demonstrated that subinhibitory concentrations of β-lactam antibiotics induce a Pol IV-dependent mutagenesis in *Escherichia coli*, *P. aeruginosa* and *Vibrio cholerea*. This mutagenesis also depends on the down-regulation of the mismatch repair (MMR) protein MutS by the small RNA SdsR in *E. coli*. We here investigated the contribution of both MutS and Pol IV factors in the mutagenesis induced by low Cip concentrations. In this sense, we have recently described a novel mechanism by which MutS controls the access to replication of Pol IV through its interaction with the processivity factor β clamp. Briefly, based on *in vitro* assays, we demonstrated that MutS inhibits Pol IV association to β clamp, which is absolutely required for the activity of this Pol. In addition, MutS limits the Pol IV-induced mutagenesis in cells growing under normal conditions. In this work, we first exposed a mutant *P. aeruginosa* strain harboring a chromosomal *mutS*$^{\beta}$ allele (which encodes a MutS mutant that does not interact with β clamp) and the parental strain (WT) to low Cip concentrations. Then, mutation rates of resistance to different antibiotics were determined using a fluctuation test. We observed that mutation rates increased in both strains suggesting that low Cip concentrations induce a global mutagenesis on the *P. aeruginosa* chromosome. However, this mutagenesis was higher in the *mutS*$^{\beta}$ strain compared to that observed in the WT strain. To study whether this is due to Pol IV activity, we constructed Pol IV-deficient strains by deleting *dinB*. Mutations rates were significantly decreased in the *mutS*$^{\beta}$ *dinB* strain relative to the *mutS*$^{\beta}$ strain indicating that Pol IV mutator activity is responsible of the increased mutagenesis observed in the *mutS*$^{\beta}$ background. Finally, and to determine if the MutS expression changed by the treatment with low Cip concentrations, protein levels were measured by Western blot assays. MutS expression levels were increased after Cip exposure compared to untreated cells. Accordingly, the small RNA SdsR sequence was not detected in the *P. aeruginosa* genome. Altogether, these findings suggest that Pol IV contribute to Cip induced mutagenesis in the *mutS*$^{\beta}$ strain but its activity is probably limited by MutS in parental strain.

Código de Resumen: MM-007

Sección: Microbiología Molecular

Modalidad: Poster

MUTATION OF THE RESPONSE REGULATOR *ragA* IN *Ensifer meliloti* NEGATIVELY AFFECTS SURVIVAL UNDER LOW PH

F.J. Albicoro[1], M.C. Martini [1], J. Nilsson [1], M.E. Salas [1], J.L. Lopez [1], M.J. Lozano[1], G. Torrez Tejerizo[1], A. Becker[2], A. Lagares[1], M.F. Del Papa[1].

[1] *Instituto de Biotecnología y Biología Molecular CONICET, La Plata, Argentina.* [2] *SYNMIKRO, LOEWE-Zentrum für Synthetische Mikrobiologie Vergleichende. Marburg, Alemania..*

albicoro@gmail.com

Ensifer meliloti, a root nodule bacterium, can establish symbiotic relationships with legumes resulting in atmospheric nitrogen being fixed to a form that can be utilized by the plant hosts. In its free-living state, this organism needs to overcome different unfavorable environmental conditions like soil acidity in order to survive, colonize the niche, reach the rhizophere and finally develop the nitrogen-fixing nodule in the legume roots. The so-called two-component systems (TCS) are part of the complex, diverse and broadly conserved strategy among bacteria for sensing and responding to external stimuli. TCS are typically comprised of a membrane-associated sensor kinase and a cytoplasmic response regulator. A better understanding of how this bacterium utilize TCS to sense acid stress condition and respond in consequence will help to elucidate the role of TCS in the rhizobial physiology. The aim of this work was to study the role of TCS involved in acid stress tolerance of *E. meliloti*. *A collection of E. meliloti* 2011 response regulator (RR) mutants were obtained either by a *Tn*5 transposon mutagenesis or by *single cross*-over method. Bacterial batch cultures, were performed in SG minimal medium under neutral (pH 7.0), sub-lethal (pH 5.6) or lethal acid conditions (pH 4.0). Differences were analyzed monitoring optic density at 600nm (OD_{600}) and colony-forming units (CFU) over time. In order to study the role of TCS in acid tolerance in *E. meliloti*, a collection of response regulators mutants was obtained. Growing rates were determined for each RR mutant as well as for the parental strain either in neutral (pH 7.0) or acid SG medium (pH 5.6). Measurement of OD_{600} over time allowed us to determine which mutants exhibited a differential growth under stress conditions. This analysis permitted us to select one mutant (Smc02366^{-}, putative response regulator *ragA*) that showed a reduced growth rate in the stressed condition that was confirmed monitoring CFU over time. We also found that this mutant has lower capacity to tolerate a sudden shift to lethal acid condition comparing with the parental strain. There is no precedence in the literature about *ragA* participation in acid tolerance. We found that *ragA* would be essential for enhanced cell viability under lethal acid conditions. Efforts are therefore currently underway to determine the role of the components of this TCS in symbiosis and to explore the participation and regulation in a diverse range of abiotic stresses

in *E. meliloti*.

Código de Resumen: MM-008

Sección: Microbiología Molecular

Modalidad: Poster

ADAPTATION OF *Staphylococcus aureus* TO THE HOST DURING CHRONIC INFECTION IS ASSOCIATED WITH DECREASED VIRULENCE

C.M. Suligoy[1], C.M. Dotto[1], A. Lombarte Serrat[1], A.N. Riviere[1], D.O. Sordelli[1], F.R. Buzzola[1].

[1] *Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM), UBA-CONICET.*

msuligoy8@gmail.com

Staphylococcus aureus is a highly prevalent opportunistic, multifactorial pathogen that can infect, replicate and persist in humans and domestic animals of economic importance. The *S. aureus* genome carries a vast array of genes coding for virulence and evasion factors. The capacity of *S. aureus* to cause a wide variety of diseases partially depends on its ability to express a number of these factors that, acting in concert, permit adaptation of the pathogen to distinct and changing environmental niches during infection. The evolution of *S. aureus* within affected tissue appears to play a key role in persistence of the microorganism and chronicity of the infection. Selection pressure exerted on *S. aureus* by host factors may determine the emergence of mutants better adapted to the evolving conditions at the infection site. There is undisputable evidence showing that certain diseases caused by *S. aureus*, such as osteomyelitis, start as acute and later develop into a chronic illness. This study identified changes that occur in *S. aureus* exposed to the host defense mechanisms during infection and evaluated whether these changes affect the virulence of the organism. To this purpose, we studied *S. aureus* isolates from single patient with chronic osteomyelitis and from the same infection site. In one case, strains SaT0 and SaT13 which were isolated 13-months apart, belonged in the same clonal complex group (CC1) and shared the same sequence type (ST188), PFGE type, *spa* type (t189) and Agr type (type I). Strain SaT0 expressed capsular polysaccharide type 8 (CP8) (at the beginning of the infectious process) whereas strain SaT13 did not (13 months later). Furthermore, the strain SaT0 expressed alpha and beta-haemolysins whereas strain SaT13 did not. It was demonstrated by real time PCR that these phenotypic differences were due to the lack of expression in strain SaT13 of the effector molecule of the global regulator *agr* (RNAIII). *S. aureus* strains were tested in a rat model of osteomyelitis and the bacterial load (CFU/tibia) and the morphometric osteomyelitic index (OI) were determined. The bacterial load and the OI of the *agr* deficient strain, which were unable to produce CP, were significantly lower than those of the parental wild-type. No significant differences were found in the bacterial load or the OI from rats challenged with the isogenic Reynolds strains (CP5, CP8 and NT non-typeable) indicating that lack of capsule expression alone was not the sole responsible for reduced virulence of the mutants. Small colony variants (SCVs) emerged in the tibias of rats experimentally infected with all *S. aureus* strains tested and the number of SCV CFUs increased with the length of the infection process. Whereas there is no doubt that lack of *agr*-dependent factors turns *S. aureus* less virulent, mutations that alter the *agr* functionality seem to permit a better adaptation of *S. aureus* to the host for persistence and infection chronicity.

Código de Resumen: MM-009

Sección: Microbiología Molecular

Modalidad: Poster

IN SILICO DETERMINATION OF POTENTIAL THERAPEUTIC TARGETS FOR LATENT PHASE *Mycobacterium tuberculosis*

L.A. Defelipe[1 2], D. Fernandez Do Porto[3], P.I. Pereira[5 4], M.F. Nicolás[4], L. Radusky[1 2], E. Sosa[3], A.G. Turjanski[1 3], M.A. Marti[1 3].

[1] *Departamento de Quimica Biológica, FCEN-UBA.* [2] *INQUIMAE-UBA/CONICET.* [3] *Plataforma Bioinformática Argentina, IC-FCEN-UBA.* [4] *Laboratório Nacional de Computação Científica, Petrópolis, Rio de Janeiro, Brazil.* [5] *Centro de Pesquisas Gonçalo Moniz, FIOCRUZ, Bahia, Brazil.*

ldefelipe@gmail.com

Tuberculosis (TB) continues to be the most frequent cause of illness and death from an infectious agent globally despite currently available drug treatments. Moreover, no new drugs have been introduced and the combination of the first-line drugs remains necessary for a long time which notably increases costs. Therefore, new drugs need to be developed, aiming to better treatment results, and to prevention of Multiple Drug Resistance (MDR) cases. Moreover, once inside the human host, *Mycobacterium tuberculosis* (Mtb), can remain alive for decades, hidden in the macrophagues facing nitrosative stress conditions. Reactive Nitrogen and Oxygen Species (RNOS) are known to present a concentration dependent mycobactericidal activity. Several new anti Mtb drugs are supposed to act by means of intracellular NO release, highlighting the relevance of RNOS for fighting the bacili and leading to the following idea: “if we know which enzymes are targeted by RNOS to suppress or

kill Mtb, we might be able to inhibit them with drugs impervious to TB nitrosative stress defenses." Therefore, with this in mind, we performed an Mtb whole proteome wide analysis of potential nitrosative stress sensitive and relevant drug targets. To achieve this aim we combined a nitrosative stress sensitivity predicting method, which is based on our knowledge of chemical reactivity of RNOS towards proteins, with an analysis of each protein expression profile in stress conditions, essentiality and druggability, in the context of Mtb metabolic network. Our results, which can be freely accessed at http://tuberq.proteinq.com.ar allowed us not only to identify several new potential Mtb drug targets, like I3PS or LipB expected to be critically sensitive under nitrosative stress conditions as those encountered *in-vivo*, but also to integrate and expand the knowledge for previous proposed targets. Moreover, the accessibility of the results and data surely represent an important tool for those researchers looking to new ways of killing Mtb.

Código de Resumen: MM-010

Sección: Microbiología Molecular

Modalidad: Poster

RECOMBINATION AMONG PLASMID-BORNE GENETIC PLATAFORMS CONTAINING *bla*$_{\text{OXA-58}}$ GENES IN THE PAN-DISSEMINATION OF CARBAPENEM RESISTANCE IN *Acinetobacter spp.*

M.M. Cameranesi[1], J. Morán-Barrio[1], A.S. Limansky [1], A.M. Viale[1].

[1] *IBR-CONICET - UNR, Microbiology, Rosario..*

marcelacameranesi78@hotmail.com

Over-production of carbapenem-hydrolyzing class D β-lactamases (CHDLs) is the most frequent cause of carbapenem resistance among multi-drug resistant (MDR) ACB complex members such as the *Acinetobacter baumannii, A. pittii* and *A. nosocomialis* which account for the majority of *Acinetobacter* infections. Short genomic sequences designated Re27 sequences are implicated in site specific recombination processes involved in the evolution of many plasmids. These sequences have been associated with particular replicase genes and could constitute favored insertion sites for structures carrying CHDL genes. We characterized here a non-conjugative plasmid (pAb242) from a carbapenem- and aminoglycoside-resistant MDR *A. baumannii* strain carrying a genetic platform containing *bla*$_{\text{OXA-58}}$ and*aphA6* genes, and searched for the presence of Re27-like sites in this structure. We also conducted a comparative analysis with similar platforms present in previously reported plasmids from other *Acinetobacter* spp. The pAb242 platform encompasses ~9 kbp and is bracketed by two Re27-like recombination sites, and is contiguous to a resolvase gene of the serine-recombinase family thus containing all necessary resources for intra-genomic mobilization. *bla*$_{\text{OXA-58}}$ is embedded in this platform into an imperfect composite Tn*3*-like transposon in which the upstream 5´-IS*Aba3* was targeted by an IS*Aba825* element. *araC*1 and *lysE* regulatory genes were found downstream of the second IS*Aba3* of the Tn*3* element, being *lysE* disrupted by Tn*aphA6*. MAUVE comparisons with other nine platforms from *Acinetobacter* plasmids showed in all cases a similar arrangement containing *bla*$_{\text{OXA-58}}$, *araC1* and *lysE* genes. On the contrary, distinctive features were found between similar platforms such as different ISs disrupting the 5´-IS*Aba3* element at different positions, and different insertions sites for Tn*aphA6* into *lysE*. Re27-like recombination sites were identified bordering the corresponding platforms in seven of the above arrangements. These Re27-like sequences allow the occurrence of multiple recombination processes, thus promoting different arrangements of *bla*$_{\text{OXA-58}}$ containing regions such as inversion mechanisms and plasmid concatenate formation. These platforms were identified in different context, *i.e.* 30-100 kbp plasmids, some of them containing more than one *rep* gene. We propose that Re27-mediated recombination processes of platforms containing resistance genes such as those described above account for the evolution of plasmids responsible of pan-dissemination of carbapenem resistance among the *Acinetobacter* population.

Código de Resumen: MM-011

Sección: Microbiología Molecular

Modalidad: Poster

CELLULOLYTIC ACTIVITIES OF A NOVEL *Cellulomonas flavigena* ISOLATE

F.E. Piccinni[1], Y. Murua[1], S. Ghio[1], P. Talia[1], M. Rivarola[1], E. Campos[1].

[1] *Instituto de Biotecnología, C.I.C.V.y A., Instituto Nacional de Tecnología Agropecuaria (INTA).*

fepiccinni@yahoo.com.ar

Lignocellulose degrading bacteria are important in many industries, such as feed and paper industries. They also show high potential as source of enzymatic extracts for scarification of cellulose in second generation bioethanol production. We have isolated *a Cellulomonas flavigena* strain, which we named B6, from forest soil, and characterized its cellulase and xylanase activities in cell-free culture supernatants. It was found that both cellulases and xylanases were mainly active in a range of pH close to neutral (5.5 to 8) and moderate temperature (between 40 and 60°C). Endoglucanase activity was maximal at pH 7 and

50°C, while xylanase activity was maximal at pH 6 and 60°C. The main reaction products were determined by HPLC, and consisted in xylobiose and xylose for xylanase activity and cellobiose for cellulase activity, indicating that the enzymatic extract has the capacity for full deconstruction of xylan but needs supplementation with beta-glucosidase activity for full deconstruction of cellulose. We have also obtained a draft version of the bacterial genome using the Illumina MiSeq sequencing platform. The data comprised 1.532.556 paired-end reads, with a length of 500 bp. It was assembled in 512 contigs and annotated by the IGS Annotation Pipeline (http://ae.igs.umaryland.edu). The genome has 74.7% of GC, in accordance with the reference strain of *Cellulomonas flavigena*. We have identified 16 new glycosyl hydrolases belonging to the CAZY GH families 6, 9, 48, 10, 11, 39 and 43, with a wide array of potential enzymatic activities, as well as 19 unclassified glycosyl hydrolases. Surprisingly, no beta-glucosidases were identified in the first annotation. A potential endoglucanase-xylanase belonging to GH6 family, had been previously identified in the supernatant as differentially expressed when the culture was grown on cellulose. The full coding sequence was identified in the genome, amplified and cloned for recombinant expression. These results indicate the high potential of this novel strain of *Cellulomonas flavigena* as a source of cellulolytic enzymes.

Código de Resumen: MM-012

Sección: Microbiología Molecular

Modalidad: Poster

EVOLUTION OF A NOVEL PLAMID-BORNE GENETIC PLATAFORM CARRYING *bla*$_{\text{NDM-1}}$ IN A CARBAPENEM-RESISTENCE *Acinetobacter bereziniae* CLINICAL STRAIN ISOLATED IN ROSARIO

M. Brovedan[1], P. Marchiaro[1 2], J. Morán-Barrio[1], L. Brambilla[1], G. Cera[2], M. Rinaudo[2], A.M. Viale[1], A. Limansky[1 2].

[1] *IBR-CONICET - UNR, Microbiology, Rosario..* [2] *Bacteriology, UNR, Rosario.*

brovedan@ibr-conicet.gov.ar

The emergence of Gram-negative clinical species harboring *bla*$_{\text{NDM-1}}$ gene encoding the metallo-β-lactamase NDM-1 has aroused public concern worldwide. Complete sequencing of plasmids carrying *bla*$_{\text{NDM-1}}$ provides important information of its genetic environment and a better understanding of its spread. We characterize here a novel plasmid, pNDM229, containing *bla*$_{\text{NDM-1}}$ isolated from a carbapenem resistant-*Acinetobacter bereziniae* clinical strain (HPC229) in Rosario, Argentina from an immunocompromised female inpatient. We also compare here the *bla*$_{\text{NDM-1}}$-containing genetic platform of pNDM229 with those present in other *Acinetobacter* plasmids. The whole genome sequencing of HPC229 disclosed the presence of *bla*$_{\text{NDM-1}}$ in a 44,560 bp plasmid containing 53 predicted ORFs, from which 31 encode proteins with BLAST scores compatible to sequences of attributed functions in databases. Phylogenetic analysis using representative N-terminal relaxase domains from the different MOB families indicated a close affiliation of the pNDM229 relaxase (TraA) to the MOBQ1 family, characteristic of broad host range plasmids. The immediate genetic environment of *bla*$_{\text{NDM-1}}$ in pNDM229 was similar to those reported previously for pNDM-BJ01-like plasmids. However, some differences were noted between all these plasmids including variations downstream of the Tn*125* element such as: i) a novel copy of IS*Aba14*-like in pNDM229, ii) an extra IS*Aba125* copy in *A. baumannii* pAbNDM, and iii) the absence of 3´ IS*Aba125* in pM131_NDM1 and its replacement by an IS*Aba11*. Other relevant differences between the above plasmids include deletions involving a number of genes composing the *bla*$_{\text{NDM-1}}$-containing platforms. Still, two regions are identical in all analyzed platforms which comprise from the IS*Aba14* element located upstream of the Tn*125* to *bla*$_{\text{NDM-1}}$, and the last 134-bp of its 3'end suggesting a common origin for these arrangements. Interestingly, the fact that the *bla*$_{\text{NDM-1}}$-containing Tn*125* in pNDM229 is bracketed by two IS*Aba14*-like suggests that this structure forms a novel composite transposon which could move itself as a whole. The presence of an IS*Aba14*-like copy in the HPC229 chromosome suggests that this novel arrangement could have been formed recently in this *A. bereziniae* strain. Finally, we propose that the mobilization of plasmids harboring *bla*$_{\text{NDM-1}}$ among different *Acinetobacter* hosts could account for the genetic variations (indels) observed in the above described *bla*$_{\text{NDM-1}}$-containing platforms.

Código de Resumen: MM-013

Sección: Microbiología Molecular

Modalidad: Poster

SCREENING OF AN AQUEOUS PLANT EXTRACT LIBRARY FOR ANTIBIOTIC ACTIVITIES AGAINST PhoP/PhoQ TWO-COMPONENT SYSTEM IN *Salmonella enterica*

M.G. Mediavilla[1], G. Viarengo[1], M.L. Travaini[2], G. Sosa[2], E. Ceccarelli[1], E. García Véscovi[1].

[1] *Instituto de Biología Molecular y Celular de Rosario (IBR) - CONICET/UNR.* [2] *INBIOAR SA.*

mediavilla@ibr-conicet.gov.ar

The *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) PhoP/PhoQ system is an orthodox two-component system that serves as a master regulator of this bacterium virulence. The regulon governed by this system is globally regulated by Mg^{2+} limitation and subsets of genes respond to different signals such as mild acidic pH, cationic antimicrobial peptides or unsaturated fatty acids. Taking into consideration the relevant role of PhoP/PhoQ system in the adaptation to environmental challenges during interaction of the bacterium with the host and that it can only be found in prokaryotes, low eukaryotes and plants, the system becomes an ideal target to search for new compounds in order to prevent/control *S.* Typhimurium virulence in mammalian hosts. Plant extracts are sources of compounds with exceptional chemical diversity that constantly gives rise to novel therapeutic agents. In this work, we carried out a screening of aqueous extracts of native plant species from Provincia de Chaco, Argentina, collected during a dry season to identify naturally occurring molecules that would modulate PhoP/PhoQ activity. To accomplish this task, we performed β-galactosidase activity assays in a 96-multiwell plate system to quantitatively asses the action of the extracts on the expression of PhoP-dependent reporters. We tested the activity of 6 different PhoP activated genes with transcriptional fusions to *lacZ* when bacteria were grown overnight in LB (activating condition), LB + $MgCl_2$ (repressing condition) and LB + plant extracts (test condition) either in 1 mL final volume in assay tubes or in 200 µL final volume in 96-multiwell plates. Of 38 initial lyophilized extracts we determined sub-inhibitory concentrations (in the 0,01 – 5 mg/mL range) and the regulatory effect on the different reporter strains. We selected 10 of these extracts to continue the characterization: 9 showed a repressing effect towards the reporter genes under study while only one (53H) unexpectedly showed repression of most reporters tested but activation of two of them (*virK::lacZ* and *pcgO::lacZ*). There is no previously reported relationship between these particular genes. We are currently pursuing the isolation and identification of the compounds responsible for the repressing/activating effects of the extracts, starting with a bioguided sub-fractionation approach. We are particularly focusing in extract 53H, to also determine whether there is a functional relationship between the activated genes. In sum, we successfully set up a protocol to test a large library of samples in a short time in order to establish a high throughput screening method to test and identify bioactive compounds with modulatory effect on the *Salmonella enterica* PhoP/PhoQ regulatory system.

Código de Resumen: MM-014

Sección: Microbiología Molecular

Modalidad: Poster

INTRACELLULAR PROTEOMICS ANALYSIS OF *Streptomyces* sp. MC1 DURING SULFATE AND Cr(VI) SIMULTANEOUS REMOVAL BY GEL-BASED AND GEL-FREE METHODS

J.O. Bonilla[1 3], E. Callegari[5], C. Estévez[2 4], M.J. Amoroso[2 4], L.B. Villegas[1 3].

[1] *INQUISAL-CONICET, San Luis.* [2] *PROIMI-CONICET, Tucumán.* [3] *Fac. Qca. Bioqca. y Fcia., UNSL.* [4] *Fac. Qca. Bioqca. y Fcia., UNT.* [5] *SD-BRIN Proteomics Facility. University of South Dakota, USA..*

jose.bonilla.mza@gmail.com

*Streptomyce*s sp. MC1 has shown capacity for Cr(VI) reduction. The presence of sulfate ion mitigated the inhibitory effect of Cr(VI) on bacterial growth. Moreover, sulfate and chromium removal by this bacterium simultaneously increased when both ions were in the medium. The present work focused on differential protein expression from *Streptomyces*sp. MC1 under Cr(VI) stress in the presence of sulfate, using gel-based and gel-free methods or “shotgun” analysis. The bacterium was grown in minimal medium containing glucose and 7.5 mM sulfate, with or without 20 ppm of Cr(VI) for 48h at 30°C and 180 rpm. Cells were chilled in liquid $N_2$and physically broken using mortar. The supernatants of cell lysates were used as protein samples. One aliquot of protein was concentrated by lyophilization for gel-free method and the other was concentrated by acetone precipitation for SDS-PAGE electrophoresis separation. Analysis of proteins samples mixtures were performed using 2 different approaches: 1) Identifying bands (SDS-PAGE) related to protein differential expression cut and gel digestion with trypsin, and 2) lyophilizing proteins dissolved in reaction buffer and trypsin digestion in solution. Tryptic peptides obtained were analyzed using 2D nano-Ultra Performance Liquid Chromatography, coupled to tandem mass spectrometry at SD Biomedical Research Infrastructure Network (SD BRIN) Proteomics Core Facility. Bioinformatics analysis for protein identification was performed by searching against Swiss Protein database with taxonomy filter for*Streptomyces coelicolor* A3, using Mascot server. ProteoIQ v2.8 program was used to organize the data after mass spectrometry analysis. The*S.coelicolor A3* (as model of actinomycetes)genome was the primary selection for orthologue database since the sequences from *Streptomyces*sp. MC1 are not yet available. From use of these methods concludes: they are complementary to each other improving the number of proteins identified, majority of the intracellular proteins have values of isoelectric points in the range of pH 4-7, and the presence of sulfate and Cr(VI) in the culture medium increases the expression of many proteins. These proteins are involved in energy production, biosynthesis of macromolecules, cell division, chaperones, transcription and regulation of supercoiling, and DNA replication and repair. In addition, many dehydrogenases involved in redox processes and possibly involved in the reduction of Cr(VI) were expressed. The response of Streptomyces *sp.* MC1 against stress caused by Cr(VI), was not specific, but

mechanisms of defense were activated based on the overexpression of proteins capable to cope the metal presence.This project demonstrated that combining gel electrophoresis with a gel-free approach improves the number of proteins identified, extends the available molecular weights and isoelectric points, and increases the dynamic range of the proteins, contributing to better quality and quantity of results

Código de Resumen: MM-015

Sección: Microbiología Molecular

Modalidad: Poster

NOVEL SEQUENCING OF ARGENTINEAN GENOMES OF *Leptospira*: SNPs ANALYSIS FOR EPIDEMIOLOGICAL PURPOSES

V. Varni[1], K. Caimi[1], P. Ruybal[2], A. Nagel[1], A. Amadio[3].

[1] *Instituto de Biotecnología, INTA.* [2] *Instituto de Microbiología y Parasitología Médica, Facultad de Medicina, UBA.* [3] *Estación Experimental Rafaela, INTA.*

varni.vanina@inta.gob.ar

Leptospirosis, caused by more than 250 different serotypes of the genus *Leptospira,* is one of the most common and widespread zoonotic disease worldwide. Infection is primarily spread through contact with water contaminated by urine of infected carrier animals. Leptospirosis is clearly an emerging and reemerging infectious disease.

In the last years the whole-genome sequencing has become a technology increasingly used for diagnosis and molecular epidemiology of various pathogens due to the fast data generation and the decrease of the costs involved. Our group has focused on the development of molecular markers based on the sequencing of gene regions (MLST). These studies led to the identification of the major genotypes circulating in isolates from Argentina, both in cattle and humans. However, these markers have a low resolution when investigating outbreaks with one predominant serogroup or genotype. The analysis of local genomic sequences can help in the development of genotyping methods that provide a more precise discrimination. The objective of this work was to generate the genomic sequence from a strain of serogroup Pomona, predominant in Argentina. From this starting point, we aim to perform comparisons with *Leptospira* genomes previously sequenced in order to identify SNPs that enable the discrimination, with the perspective of developing a high resolution typing tool.

Among the strains previously studied by MLST, we selected an Argentinean strain belonging to *Leptospira interrogans* serogroup Pomona for whole genome sequencing. It was performed using the Illumina "MySeq" technology. We also selected 8 *Leptospira* Pomona genomes from different countries, available in databases. The alignment of the 9 Pomona genomes to a reference genome (*Leptospira interrogans* Cophenageni,strain Fiocruz L1-130) allowed the identification of polymorphic positions. A phylogenetic analysis was performed using the SNPs obtained for each genome, in order to explore the differences among the strains.

A panel of 47,182 SNPs between the reference genome and the 9 Pomona were identified, where the 67 % were located in coding regions. The phylogenetic analyzes enabled the differentiation of strains that were previously indistinguishable by their genotype or serogroup. The clusters of isolates appeared to relate to their geographic origins.

The comparison of a local *Leptospira* genome with worldwide genomes of the same serogroup allowed the identification of SNPs. The analysis of those presents a promising perspective for local epidemiology of *Leptospira*, leading to the phylogenetic discrimination of isolates that could not be achieved by traditional molecular markers. The information thus generated, when validated in a larger set of strains, may lead to the development of a tool for high-resolution typing of this pathogen.

Código de Resumen: MM-016

Sección: Microbiología Molecular

Modalidad: Poster

CHARACTERIZATION OF AN INTEGRATIVE AND CONJUGATIVE ELEMENT IN A CLINICAL ISOLATE OF *Shewanella spp*.

G. Parmeciano Di Noto[1], D. Centrón[1], C. Quiroga[1].

[1] *Instituto de Investigaciones en Microbiología y Parasitología Médica- Facultad de Medicina-UBA.*

cc.quiroga@gmail.com

Shewanella is a gamma-proteobacteria composed by more than 50 species. It has the ability to reduce heavy metals and toxic compounds and is able to proliferate under aerobic and anaerobic conditions. *Shewanella* is an environmental bacterium, however it can cause clinical infections. Therefore it is considered an emerging opportunistic pathogen. Pathogenicity and

multirresistance in bacteria are inherently related with the acquisition of mobile genetic elements by lateral transfer. The aim of our study was to find and characterize mobile genetic elements encoded in the genome of clinical isolates of *Shewanella* spp.. We searched for mobile elements in the fully sequenced genome of a strain from our collection, *Shewanella* sp. Sh95, and found an integrative and conjugative element (ICE), named ICESh95. ICEs are mobile elements widely distributed among bacteria responsible for the lateral transfer of virulence determinants, antibiotic resistance genes and other bacterial traits. ICESh95 belongs to the SXT/R391 family of ICE, which was first described in *Vibrio cholerae.* To date, there is only one homolog of this element in a marine isolate of *Shewanella* spp*.* (ICESpuPO1). ICESh95 has all the necessary genes for its mobilization and transfer (*int*, *setD*, *setC* and *tra*) as well as the origin of transfer, *oriT*. Comparative sequence analysis between ICESh95 and ICESpuPO1 showed two different regions. While function of genes from the first variable region was unclear, the second region contained an SXT integron with an *intI9* integrase gene interrupted by an IS*4* insertion sequence. This integron had two gene cassettes, *qacH* and *drfA15*. The *dfrA15* gene cassette was found interrupted by a class C-attC group II intron highly identical to the C-attC intron of reference, *S.ma*.I2. Additionally, we tested for the presence of members of the SXT/R391 ICE family in 10 clinical isolates of *Shewanella* spp. by PCR using specific primers. As a result we observed that three of them also contained an SXT/R391 ICE. Insertion of SXT/R391 ICE always occurs at the *prfC* gene located in the chromosome. We analyzed the genetic surroundings of ICESh95 and we found that it is upstream of the *pabA* gene, instead of *prfC*, evidencing that this ICE invaded a novel site. Moreover, we conducted a conjugation assay that allowed us to prove that ICESh95 is capable of transferring into *Escherichia coli* HB101. ICE elements are multi-talented entities that can be involved in the dissemination of virulence and resistance genes. Taken together our results show the role of this element in the exchange of DNA between clinical and environmental niches thus participating in bacterial adaptation and evolution. ICESh95 not only acquired an SXT integron harboring antimicrobial resistance genes, it also adapted to invade a novel insertion site and proved its ability to use its own machinery for conjugation and transfer.

Código de Resumen: MM-017

Sección: Microbiología Molecular

Modalidad: Poster

FUNCTIONAL ROLES OF THE CATION DIFFUSION FACILITATOR FAMILY TRANSPORTERS IN VIRULENCE AND TRANSITION METAL HOMEOSTASIS IN *Pseudomonas aeruginosa*

A. Salusso[1], G. Elso-Berberián[1], D. Raimunda[1].

[1] *Instituto Mercedes y Martín Ferreyra (INIMEC)-CONICET-Universidad Nacional de Córdoba.*

draimunda@immf.uncor.edu

Pseudomonas aeruginosa, a gram-negative bacterium,is the prevalent pathogen in cystic fibrosis (CF) patients. Antibiotic resistance is often found as biofilm formation occurs. This process is likely driven by iron and zinc levels status in the bacteria which depends highly on transition metal (TM)-responsive transcription factors, TM-chaperones, and TM transporters. Transporters of the cation diffusion facilitator (CDF) family play their role exporting transition metals from the cytosol to the periplasmic space. *P. aeruginosa* presents an unusually high number of three CDF homologous genes, PA0397, PA1297 and PA3963. Protein sequence analyses predicted a broad specificity of transport for PA0397 and PA1297. Inhibition halos experiments showed that a ΔPA0397 strain was apparently less sensitive to Zn^{2+}. However, increased levels of the extracellular TM chelator pyoverdine were detected in this mutant in presence of Zn^{2+} and Ni^{2+} in an iron replete medium. In the non-pyoverdin producer double mutant Δ*pvdA*-ΔPA0397, Zn^{2+} and Ni^{2+} sensitivity was observed *vs*. the single mutant Δ*pvdA*. The strain ΔPA1297 was Co^{2+} sensitive and showed Co^{2+} accumulation when grown in presence of sub-lethal concentrations of the ion *vs*. WT. In a plant infection model ΔPA3963 and ΔPA0397 had a reduced fitness. These results highlight the role of *P. aeruginosa* CDFs transporters in TM homeostasis maintaining adequate intracellular Zn^{2+}, Co^{2+} and Ni^{2+} levels, and suggest their participation in infectious processes.

Código de Resumen: MM-018

Sección: Microbiología Molecular

Modalidad: Poster

EFFECT OF SALICYLIC ACID ON BIOFILM FORMATION BY *Staphylococcus aureus* STRAINS

C.M. Dotto[1], T. Grunert[2], N. Cattelan[3], A. Lombarte[1], C.M. Suligoy[1], O. Yantorno[3], D.O. Sordelli[1], F.R. Buzzola[1].

[1] *IMPaM-CONICET-UBA.* [2] *Institute of Functional Microbiology, Department of Pathobiology, University of Veterinary Medicine.* [3] *CINDEFI-CONICET-UNLP.*

cristianmdotto@hotmail.com

Staphylococcus aureus is the causative agent of a broad spectrum of infections due to its ability to attach to damaged epithelia and inanimate surfaces, and to adopt a biofilm lifestyle. Biofilm is involved in the development of device-related infections and chronic infections of diverse tissues. During the biofilm development, the polysaccharide intercellular adhesin (PIA) encoded by the *icaADBC* operon contributes to the bacterial agglomeration. The extracellular DNA (eDNA) from autolytic cells and several proteins are also involved in sticking the bacterial cells together. Unlike methicillin-resistant *S. aureus*, available evidence indicates that biofilm formation by methicillin-susceptible *S. aureus* isolates depends upon the PIA production. The environmental conditions and metabolic status of the bacteria affect the biofilm formation. Salicylic acid (SAL), the main aspirin metabolite produced *in vivo*, interferes with expression of several *S. aureus* virulence factors (such as capsular polysaccharide, Eap) and global regulators (such as *mgrA*, *saeRS*). The aim of the present study was to investigate the effect of SAL on biofilm formation by *S. aureus* and determine the nature of the biofilm's matrices after the treatment with SAL.Twenty-four hours biofilms formed by *S. aureus* in polystyrene microtiter plates, with or without SAL, were stained with crystal violet for spectrophotometric quantification. Detachment of preformed biofilms was determined after treating the biofilms with Dispersin B, proteinase K or DNAse I. Biofilms grown with or without SAL for 24 hours were processed and analysed by Fourier transform infrared spectroscopy (FTIR) in the spectral ranges of 1,200 to 800 cm^{-1} (polysaccharides region) and 1,800 to 1,200 cm^{-1} (amide and "mixed" region). Here, we demonstrated that SAL enhances biofilm production in all strains under study. The principal component analyses in the polysaccharide external and amide regions from the FTIR data determined that SAL caused polysaccharide and proteins perturbations in *S. aureus* biofilms. Dispersal of stablished biofilm by enzymatic treatments shown that the extracellular matrices of the SAL treated-biofilms are mainly of proteinaceous and polysaccharide nature. The contribution of the eDNA would be not relevant. Taken together the results suggest that SAL promotes the biofilm formation by *S. aureus* which would contribute to the persistence of infection.

Código de Resumen: MM-019

Sección: Microbiología Molecular

Modalidad: Poster

VARIATION IN STAPHYLOXANTHIN PRODUCTION AND BIOFILM FORMATION BY *Staphylococcus aureus* FROM CHRONIC INFECTIONS

A.N. Riviere[1], C.M. Dotto[1], M. Suligoy [1], A. Lombarte Serrat[1], D.O. Sordelli[1], M. Giacomodonato[1], F.R. Buzzola[1].

[1] *Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM), UBA-CONICET, CABA.*

cristianmdotto@hotmail.com

Staphylococcus aureus is one of the most important human pathogens both in the hospital and the community. This bacterium has the ability to cause chronic infections due to the formation of surface-associated aggregates or biofilms. The SigB alternative sigma factor is activated during biofilm formation and triggers the expression of genes associated with pigmentation and biofilm formation. The orange and yellow carotenoid pigments that produce *S. aureus* are the products of the staphyloxanthin (STX) biosynthetic pathway. The carotenoid pigment biosynthesis genes are organized in the operon *crtOPQMN*. STX is a virulence factor with antioxidant action and its production is positively regulated by the SigB factor. To further study the contribution of STX in the fitness of *S. aureus* in chronic infections, we compared the level of pigments produced with the biofilm biomass formed by *S. aureus* isolated from patients with osteomyelitis. We selected 18 *S. aureus* isolates from our laboratory collection of strains whose full genome was previously sequenced. The genotypic characterization (clonal complex, sequence typing, *agr* typing and *spa* typing) of each strain was performed by specific PCRs and sequence analysis. The pigment extracted was quantified by evaluation of the optical density (OD) at 450 nm and by correlation pigment production with the culture OD. Biofilms formed by *S. aureus* were measured by static microtiter plate assays. The distribution of the average OD values from the 18 isolates into quartiles permitted to classify the strains as high producers (HP: OD $\geq$ 0.62), producers (P: OD $\geq$ 0.4 and < 0.62), or non-producers (NP: OD < 0.4). The analysis of the levels of pigment extracted revealed that four strains were STX negative, other four isolates produced orange (A_{450}: 0.44 -1.03) pigment and the rest of the strains showed yellow (A_{450}: 0.28 - 0.43) pigment. The NP biofilm isolates expressed only yellow levels of STX. However, almost 30% of the P and HP biofilm strains were STX negative. A couple of genotypically identical (CC5, ST5, t002, *agr* type II) isolates that showed differences in pigment and biofilm production were selected for bioinformatical analysis. The SigB factor sequences were 100% identical among this pair of isolates whereas a single aminoacidic change at the 108 position of the *crtM* gene was observed in the STX negative producer strain under comparison. Taken together, these results indicate that STX production (orange pigment) correlates with increased biofilm formation in *S. aureus* isolates from patients with chronic osteomyelitis. However, a low percentage of the P and HP biofilm isolates were unable to produce orange or yellow pigments suggesting the presence of a genetic defect in the *crt* operon.

Código de Resumen: MM-020

Sección: Microbiología Molecular

Modalidad: Poster

THE ERROR-PRONE DNA POLYMERASES ImuB AND DnaE2 CONTRIBUTE TO STRESS-INDUCED MUTAGENESIS IN *Pseudomonas aeruginosa*

A.J. Moyano[1 2], A.M. Luján[1 2], S. Feliziani[1 2], A.M. Smania[1 2].

[1] *Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC) CONICET.* [2] *Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.*

amoyano@fcq.unc.edu.ar

DNA damage-induced mutagenesis is, in a major extent, an active process that requires specialized DNA polymerases able to perform translesion synthesis. The expression of these specialized polymerases is usually governed by the SOS system, a stress-inducible response that is activated when cells need to be rescued from severe DNA damage. These DNA polymerases, which belong to the Y family of DNA polymerases, diverge from the typical replicative DNA Pols I and III in their low fidelity and processivity, and the lack of 3'→5' proof-reading exonuclease activity. Thus, induction of the Y-family of DNA polymerases serves to increase the probability of survival under stressful conditions in exchange for an error-prone DNA synthesis, which bestowed them the name of "mutagenic polymerases".

Pseudomonas aeruginosa is an opportunistic pathogen and the main cause of morbidity and mortality in patients with cystic fibrosis (CF) due to chronic airways infections. It has been established that in order to persist, *P. aeruginosa* undergoes a mutation-based adaptive process. This process may therefore, be influenced by factors which are able to alter the mutation rate such as the activity of mutagenic polymerases. Inspection of *P. aeruginosa* genome reveals the presence of a *dinB*-enconded Pol IV and also of a three-gene cassette *imuA-imuB-dnaE2*. In this sense, it has been shown that Pol IV constitutes a SOS-dependent error-prone DNA polymerase of *P. aeruginosa*, being involved in the acquisition of prototypic CF adaptive phenotypes such as mucoidy and antibiotic resistance. However, little is known about other putative error-prone DNA polymerases such as *imuB* and *dnaE2*.

Here we have investigated the role of these two putative DNA polymerases in *P. aeruginosa* DNA-damage induced mutagenesis. The results obtained show that both genes contribute to UV-induced mutagenesis in this bacterium. DNA sequencing of the *rpoB* gene in rifampicin-resistant mutants suggests that ImuB and DnaE2 participate in the generation of A:T-T:A transversion mutations. Furthermore, UV treatment significantly increased the expression levels of *imuB* and *dnaE2* transcripts. Interestingly, expression of *imuB* and *dnaE2* was abolished in a *lexA*-deficient strain suggesting that both genes are controlled by the SOS regulon in *P. aeruginosa*. We subsequently analyzed the transcriptional organization of *imuB* and *dnaE2* and observed that these genes behaved as a single transcriptional unit, thereby constituting an operon.

On the whole, these results contribute to shed light on the wide repertory of adaptive responses of *P. aeruginosa* to thrive in hostile environments such as the airways of CF patients.

Código de Resumen: MM-021

Sección: Microbiología Molecular

Modalidad: Poster

GENOMIC ORGANIZATION OF *Bradyrhizobium* FLAGELLAR SYSTEMS

M.J. Althabegoiti[1], E.J. Mongiardini[1], J.I. Quelas[1], A.R. Lodeiro[1].

[1] *IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata-CONICET.*

mja@biol.unlp.edu.ar

Bacterial motility is an important trait for processes such as adherence to host cells, host cell invasion, protein secretion, and biofilm formation. *Bradyrhizobium diazoefficiens* and *B. japonicum* can live into plant nodules as a symbiont, or in a planktonic, free-living state in the soil, where they can swim and swarm self-propelled by their flagellar systems. *B. diazoefficiens* USDA 110 and *B. japonicum* USDA 6 possess two flagellar systems: a subpolar flagellum and some lateral flagella. This characteristic is shared with unrelated species such as *Vibrio parahaemoliticus*, *Aeromonas hydrophila*, *Chromobacterium violaceum*, *Rhodobacter shaeroides, Rhodopseudomonas palustris* and *Azospirillum brasilense* but not with other rhizobial species, which instead have a single peritrichous flagellar system similar to the lateral one of *Bradyrhizobium*. The presence of two flagellar systems in the same cell is intriguing, since each one consumes a large amount of cell energy and might interfere each other in motility and chemotaxis. Although in the above-mentioned species the lateral flagella were described as only required for motility

on surfaces, in *Bradyrhizobium* both flagellar systems are expressed in liquid medium. Therefore, elucidation of the roles of these two flagellar systems in *Bradyrhizobium* requires more investigation. A possible approach is a comparative study of the genomic organization of these systems. The most related species with this characteristic is *R. palustris*. In this work we compared the genome distribution and synteny of lateral and subpolar flagellar genes in *B. diazoefficiens* USDA 110, *B. japonicum* USDA 6 and *R. palustris* BisA53. Genes encoding lateral flagella are located in a single cluster in *Bradyrhizobium* and in *R. palustris*. The regions are syntenic and sequences are highly conserved: around 90 % of identity between *B. diazoefficiens* and *B. japonicum* and 75% identity between each *Bradyrhizobium* sp. and *R. palustris.* An inversion of this cluster was observed between *B. diazoefficiens* and *B. japonicum*. In the case of *Sinorhizobium meliloti* or *Rhizobium etli*, they also have a cluster enclosing all the genes for the synthesis of the flagellum. The genes that encode the subpolar flagellum of *B. diazoefficiens* are distributed among four regions in the genome. This pattern of dispersed genes is similar also in *B. japonicum* and *R. palustris* and they are more divergent than the lateral cluster. The above observations are in agreement with the proposal that the lateral flagellum was acquired by horizontal transfer. In this way we can think that the high conservation of the lateral cluster is due to a recent insertion in the genomes, while the subpolar (primary) system evolved together with the organism. These results will aid us to pursue the study on the roles of each flagellum in the life cycle of *Bradyrhizobium* sp.

Código de Resumen: MM-022

Sección: Microbiología Molecular

Modalidad: Poster

GENOMIC ANALYSIS OF BUTANOL PRODUCTION IN A NOVEL THERMOPHILIC BUTANOL PRODUCER

R. Díaz Peña [1], B.S. Méndez[1], M.J. Pettinari[1].

[1] *Dpto de Química Biologica. FCEyN- Universidad de Buenos Aires.* [2] *IQUIBICEN- CONICET.*

rociodiazpena@gmail.com

Over the past decades, development of biofuels has raised a great interest as an alternative to fossil fuels which generate large amounts CO_2, emissions and toxic byproducts (causative agents of greenhouse effect), and are non-renewable resources. In this context, butanol has attracted special attention due to its advantages over ethanol. Not only it is capable of producing more energy if properly harnessed but is also less corrosive and water soluble, so it can be used in blending with gasoline or diesel. Moreover its use does not require modifications to existing engines, it has a lower vapor pressure and is less hygroscopic than ethanol, a characteristic that enables its transportation without altering the product. The microbial production of butanol by bacteria of the genus *Clostridium* has been studied in detail. Such is the case of *C. acetobutylicum*, which has been used in industrial processes for the synthesis of acetone and butanol from different substrates. In our laboratory we have isolated and characterized a new species, originally named *C.thermopapyrolyticum*, an anaerobic thermophilic bacterium that is capable of producing butanol once it has reached stationary state. To complete the genetic characterization of this microorganisms its genome has been sequenced. Genome to genome comparisons with previously sequenced bacteria revealed that it is most probably a member of the genus *Thermoanaerobacterium*, and not *Clostridium*. In view of this, we denominated it as *Thermoanaeobacterium* sp. GCU5. To identify genes encoding the key enzymes in the metabolic pathway of n-butanol production, we performed a genomic analysis using RAST annotation Server and Blast algorithm. Eight genes involved in this pathway were identified: *thl*, *hbd*, *crt*, *bcd*, *etf*A, *etf*B, *adh*, *bdh*. It was observed that the genes *thl*, *hbd*, *crt*, *bcd*, *etf*A, *etf*B are organized in the operon *bcs*, whereas in *C. acetobutylicum* the gene *thl* is not part of this operon. Furthermore, we observed that *Thermoanaerobacterium* sp. GCU5 presents genes coding for enzymes involved in the metabolism of xylan (hemicellulose main component), cellobiose and sucrose, all components present on sugar cane agroindustrial residue. Knowing this bacterium has all genes responsible for butanol production as well as those involved in xylan, we decided to evaluate its ability to grow on xylose and arabinose (monosaccharide present in xylan), sucrose and cellobiose. We demonstrate that *Thermoanaerobacterium* sp. GCU5 is able to grow on arabinose, xylose, sucrose and cellobiose as well as galactose. Ongoing studies are focused on analyzing butanol production from these substrates.

Código de Resumen: MM-023

Sección: Microbiología Molecular

Modalidad: Poster

INVESTIGATING THE ROLE OF N-ACETYLGLUCOSAMINE ON THE S-LAYER PROTEINS OF *Lysinibacillus sphaericus*

J. Tarsitano[1], C. Sánchez Rivas[1], S. Ruzal[1], M. Palomino[1], J. Fina Martin[1], P. Waehner[1], M. Prado Acosta[1], L. Malone[1].

[1] *Departamento de Química Biológica, FCEN-UBA, IQUIBICEN - CONICET.*

juliantarsitano@gmail.com

Lysinibacillus sphaericus is a spore forming Gram positive bacteria whose vegetative cell surface is covered with S-layer proteins. This species is able to produce during the sporulation process, protein crystals associated to the spore wall with enthomopathogenic activity against mosquito larvae. *L. sphaericus* is unable to use sugars as carbon source other than *N*-acetylglucosamine (GlcNAc) for which a phosphoenolpyruvate (PEP) sugar phosphotransferase system (PTS) is present. We have shown that S-layer proteins remain associated to the spores contributing not only to their ability of retaining metals (Allievi et al, 2011) but also acting as synergic agents in larvicidal activity against *Aedes aegypti* larvae, a known carrier of viruses causing Dengue and Chikungunya fevers, both diseases representing a global threat (Allievi et al, 2014). We have observed variations in the molecular masses of S-layer proteins produced in different growth stages in related strains. The role of glycosylation of *Bacillus* proteins in several strains' pathogenicity has been documented. Therefore, we have decided to study post translational modification on S-layer proteins on different strains and specifically the role of GlcNAc, as glycosil group donor of those modifications. The different strains used for these studies are ASB13052 and its isogenic mutant, bearing a deletion in the GlcNAc transport system *(Δpts)*. The presence of S-layer proteins and a variation on the protein pattern depending on the growth phase of the bacteria and the strain used was verified by western blot. In addition it was also observed that the mutant had an altered sporulation process and therefore altered spore quality (heat resistance) as well as S-layer proteins of diverse molecular masses. Ongoing studies are focused on demonstrating whether these two characteristics are related to the different forms of S-layer observed and the role of N-acetylglucosamine on the larvicidal activity.

Código de Resumen: MM-024

Sección: Microbiología Molecular

Modalidad: Poster

ANALYSIS OF ADAPTABILITY TO BIMODAL SWITCHING BETWEEN BIOFILM AND PLANKTONIC STATES IN MUTATOR STRAINS OF *Pseudomonas aeruginosa*

C.A. Colque[1 2], R.A. Tobares[1 2], A.M. Smania[1 2].

[1] *CIQUIBIC-CONICET.* [2] *Departamento de Química Biológica, Facultad de Ciencias Químicas, UNC.*

asmania@fcq.unc.edu.ar

Pseudomonas aeruginosa is an opportunistic pathogen, which cause chronic biofilm infections of humans with underlying predispositions. During biofilm growth, *P. aeruginosa* rapidly diversifies into different niche specialists by a process known as adaptive radiation. Adaptive radiation can be easily visualized by the emergence of variants with distinct colony morphologies. Importantly, we have previously shown that high mutation rates in *P. aeruginosa* MRS deficient strains (*mutS*-) is related to an increase in phenotypic diversification, particularly during the biofilm mode of growth. Small Colony Variant (SCV) is one of these biofilm adapted phenotypes, which are characterized by a diminished capability of movement, and a hyperadherent and autoaggregative behavior. Together, these phenotypes result into high biofilm producers and very small size colonies relative to the wild-type (WT) phenotype. When SCV is grown on solid media, the WT phenotype emerges from the edges of the colony indicating a bimodal switching between two phenotypic phases. To evaluate the adaptability of *P. aeruginosa*, an evolutionary assay was performed by exposing the bacteria to alternating and successive rounds of SCV conversion in biofilms and WT reversion in solid media. Previous results in WT strains showed that SCV conversion and reversion involved the acquisition of mutations, mainly in *wsp* and *yfiBNR* operons, that were accumulated one per round as compensatory mutations. These genes have been recently associated to the modulation of the levels of the second messenger c-di-GMP and biofilm formation. If adaptability to bimodal switching between SCV conversion in biofilms and reversion on solid media is based on the acquisition of mutations, how is this process affected by an increase in mutation rate? In this work we analyzed the genetic basis of SCV conversion and reversion in *mutS*- strains. First results indicate that bimodal switching between this two phases in *mutS*- strains was able to go on for over 16 rounds, while WT strains showed much less capacity. Then, whole genome sequencing of ancestral and final clones was carried out. Our results showed that *mutS*- strains have acquired a significantly higher number of mutations per round (≈19/round) over mutations seen in WT strains (1/round). The analysis of mutational spectra in *mutS*- strains was strongly skewed towards the typical spectrum of MRS deficient strains, dominated by transitions and small indels. Most mutations affected genes involved in motility and attachment (*pelB*, *wsp* system, *flgL*, among others). However, in the first three cycles of SCV conversion/reversion, *wsp* and *yfiBNR* genes alternatively accumulated 1 mutation per round. Then, the modulation of c-di-GMP levels may play a central role in the adaptation of *P. aeruginosa mutS*- strains, while the rest of observed mutations could have a fine tuning or neutral role in the adaptive process as a consequence of a higher mutation rate.

Código de Resumen: MM-025

Sección: Microbiología Molecular

Modalidad: Poster

MODIFICATION OF THE CELL-WALL POLYMERS OF *Lactobacillus casei* GROWN IN HIGH SALT CONDITION

L.M. Malone[1], M. Allievi[1], J. Fina Martin[1], E. Dieterle[1], M. Piuri[1], S.M. Ruzal[1].

[1] *Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales-UBA, IQUIBICEN-CONICET.*

malonelucia@gmail.com

The bacterial cell envelopes have an important role in the maintenance of the shape, integrity and survival of the species as well as communicating with the environment and bacteriophage interaction. In *Lactobacillus* species we have found that growth in high salt condition results in profound changes to deal with osmotic stress adaptation. We have characterized a TCA soluble fraction obtained from cell walls (CW) as the responsible of the phage interaction when assayed for its adsorption inhibition. Polymers of non-proteinaceous composition are found in this purified fraction of the cell envelope visualized by PAGE and TLC. Biochemical analysis showed that differences are obtained when growth in 0.7M NaCl. Putative components of the fraction are wall teichoic acids (WTA) and rhamnose containing glycans (RWPS). Those differences could be attributed to two possible reasons: loosely bind polymers due to modified envelope structure or a differential level of expression of the biosynthesis pathways. To determine which factor is responsible for the lower level of polymers recovery we verified gene expression by qRT-PCR of probable functions encoded in the genome of *L. casei* BL23. The coding functions related to the synthesis of RWPs wall and neutral sugars are *isgC*, *epsC, wze, cps1A-J, rmlA-D2.* We have analyzed growth supernatants to address increased release of wall polymers in high salt. Therefore we concluded that a mix of genotypic and phenotypic effects is the responsible of the differences observed. These findings open new insight in the developing strategies to avoid infections with phages during dairy fermentation processes by pre-growing starters in high salt, which will be further evaluated.

Código de Resumen: MM-026

Sección: Microbiología Molecular

Modalidad: Poster

PROTECTIVE EFFECT OF PHASINS PhaP FROM *Azotobacter* sp.FA-8 AND PhbP FROM *Pseudomonas extremaustralis* IN RECOMBINANT *Escherichia coli*

D.S. Alvarez[1], M.P. Mezzina[1], M.J. Pettinari[1].

[1] *Departamento de Química Biológica, Facultad de Cs Exactas y Naturales, Universidad de Buenos Aires.*

alvarezdanielasoledad@gmail.com

Phasins are proteins associated to intracellular polyhydroxyalkanoate (PHA) granules, a biodegradable polymer accumulated by many bacteria under unfavorable conditions as a carbon and energy reserve. Phasins not only play an important structural role in the polymer accumulation and the number and size of the granules, but have also been shown to have regulatory functions. It has been reported that PHA accumulation in recombinant *E.coli* strains causes stress in cells, evidenced in the increased expression of stress-related proteins such as chaperones IbpA and DnaK. This suggests that PHA production induces the heat shock response by titrating available cytoplasmic chaperones onto the surface of the PHA granules. The presence of $PhaP_{Az}$ in these PHA-accumulating strains exhibited a decrease in the heat shock response and, consequently, the expression of these chaperones. Furthermore, $PhaP_{Az}$ expression also has a protective effect in non-PHA-producing *E. coli* strains, evidenced in the increased growth and higher resistance to heat shock and superoxide stress. The effect described for this phasin is not only passive but involves an active role in promoting protein folding, and preventing unfolding due to its chaperone-like function. The aim of this work is to analyze if the protective effect observed for $PhaP_{Az}$ is a common feature among phasins. To help elucidate this, we studied the effect of the expression of phasin PhbP from *Pseudomonas extremoaustralis* ($PhbP_{Pe}$) in recombinant *E. coli*. An *in silico* comparative analysis of $PhaP_{Az}$ and $PhbP_{Pe}$ revealed that both phasins have similar secondary structures, consisting of α-helices and random coil regions. *phbP* was amplified by PCR from *P. extremoaustralis* genomic DNA and cloned in a plasmid that allowed overexpression in *E. coli*. The protective effect of this phasin was studied by analyzing the level of expression of the small heat shock protein IbpA, which is a stress indicator. For this purpose, strain ADA100, which carries a fusion of *lacZ* to the *ibpA* promoter in the chromosome, was transformed with this plasmid in order to analyze *ibpA* expression levels by the β-galactosidase activity assay, in normal and heat stress conditions. In addition, resistance to heat shock was also studied by determining cell viability after incubation at 52ºC, by the colony count method. As previously observed for $PhaP_{Az}$, $PhbP_{Pe}$ was also shown to protect *E. coli* from heat shock. If the effects described for $PhaP_{Az}$ are a common feature among phasins, these proteins could play an important role in protein homeostasis both in natural and recombinant PHA producers, complementing the resources provided from polymer degradation with specific chaperone activities that could protect the cells from different types of stress.

Código de Resumen: MM-027

Sección: Microbiología Molecular

Modalidad: Poster

LIGHT MODULATES GENE EXPRESSION OF COMPONENTS OF THE EFFLUX PUMP AdeIJK IN *Acinetobacter baumannii*

G. Müller[1], A. Golic[1], M.S. Ramírez[2], M.A. Mussi[1].

[1]Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI- CONICET). Rosario, Argentina.. [2]Center for Applied Biotechnology Studies, Dpt. of Biological Science, California State University F.

mussi@cefobi-conicet.gov.ar

We have recently showed that light modulates *Acinetobacter baumannii* susceptibility to antibiotics clinically relevant such as minocycline (MIN) and tigecycline (TIG), which represent important weapons against multidrug resistant (MDR) bacteria. In fact, the application of light resulted in variation in MIC values to TIG from 2 (in the dark) to 128 μg/ml (under blue light) in the case of some strains such as ATCC 19606. In a similar way, the XDR clinical strain A42 shows fluctuation in MIC values to MIN from <0.125 in the dark to 16 μg/ml under illuminated conditions. These impressive differences point out the profound influence light can exert on antibiotic resistance, unnoticed until now. Beyond this, the response to light might enhance bacterial persistence until more favorable conditions or additional resistance determinants are accumulated. The magnitude of the phenotype depends on the culture media, being maximal in Luria Bertani (LB) Difco, reduced in the presence of iron and null in Müeller Hinton (MH) media. Also, the phenomena was found to be temperature dependent, as it occurs at 24 ºC but reduced at 37 ºC. The generation of $^1O_2$ results in the same phenotype as the application of blue light, i.e. reduced susceptibility to MIN and TIG, providing strong evidence that light might exert its effects through $^1O_2$ production. This is not surprising since the production of $^1O_2$ by light in the presence of photosensitizers has been extensively described. In addition, we showed that light, as well as $^1O_2$, induce the expression of genes previously related to resistance to TIG, such as those coding for the efflux pump AdeABC. The objective of this work is to study the effect of light in the expression of genes coding for the efflux pump AdeIJK, which was previously shown to act synergistically with AdeABC in strain BM4454. For this purpose, we performed qRT-PCR on the same cDNA samples as those used above, generated from 19606 or A42 cells, but using specific primers for *adeI*, *J* and *K* transcripts, and normalized the levels to those of *recA*. Our results show that in cells grown in LB plates incubated overnight at 24ºC, as well as in cells grown to exponential phase in liquid LB media, specific gene components of the AdeIJK efflux system are induced under blue light respect to dark conditions. Most interestingly, in liquid LB cultures supplemented with sub-MIC concentrations of TIG and grown to exponential phase, the induction by light was much more increased. As control, no differences in the levels of *AdeIJK* transcripts were detected between light and dark conditions in MH, a condition in which no differences in resistance to MIN or TIG were observed between light and dark. The overall results show that *adeIJK* genes are modulated by light, reinforce the notion of a correlation between reduction in susceptibility to TIG and induction of efflux pump resistance genes by light, and suggests that the efflux pump AdeIJK might act synergistically to AdeABC.

Código de Resumen: MM-028

Sección: Microbiología Molecular

Modalidad: Poster

FIRST STUDY OF *fbsA* GENE CARRIED BY *Streptococcus agalactiae* CLINICAL STRAINS ISOLATED IN MISIONES

M. Novosak[1 2], F. Bobadilla[1 2], M. Laczeski[1 2], M. Vergara [1].

[1]Universidad Nacional de Misiones - Facultad de Ciencias Exactas, Químicas y Naturales. [2]Instituto de Biotecnología Misiones - InBioMis.

marinanovosak2008@gmail.com

Streptococcus agalactiae, Group B streptococcus (GBS), is part of the human intestinal and genital flora. In pregnant women between 35-37 weeks of gestation detection of GBS is relevant for the probable vertical transmission to the newborn. GBS causes the most severe infection that can affect the human beings in their first days of life, causing septicemia, pneumonia and meningitis.The severity of neonatal disease is largely determined by components of the capsule, which allow the differentiation of ten GBS serotypes (Ia, Ib, II-IX), and surface proteins which mediate virulence.

Recently, genes directly involved in the development of severe invasive disease have been described, including the *fbsA* gene that encodes a protein that protects the bacteria from the opsonization and promotes adherence to epithelial and brain endothelium cells, key events in the pathogenesis of GBS. It is necessary to identify the genes necessary for the establishment and maintenance of infection to understand the mechanism by which this pathogen causes disease.

The objective of this study was to detect the presence of virulence gene *fbsA* in isolated strains from pregnant women with 35-37 weeks of gestation.

A total of 200 GBS stains, that were recovered from storage at -80 ° C in 20% skim milk on agar plates supplemented with 5% sheep blood and tested by biochemical test and serological group identification (Phadebact Streptococcus Test, Swedeen), have been studied to confirm their purity. The gene *fbsA* was investigated by PCR conventional technique. Automated sequencing of PCR products was performed by using the Automatic Sequencing Service of Macrogen Korea.

The *fbsA* gene was detected in 100% of the studied strains. A fragment of 156 bp was obtained by PCR using 5'TGTAGCTAATGGACCGATGTT3' and 5'TTTTCATTGCGTCTCAAACC3' primers and sequenced and analyzed biocomputationally by comparing with the NCBI database using BLASTn. The analysis was based on the similarity with the sequences aligned in the BLAST search. The search results showed a 97% identity with the *fbsA* gene (GenBank: CP006910.1).

This study confirms the presence of *fbsA* gene in GBS strains, which encodes a protein that acts like an adhesin and link the passage of the microorganism to meninges. The study of genes thatencode surface proteins that act as virulence factors is necessary to contribute to the epidemiological knowledge of the disease and determine GBS antigenic epitopes that can be used in the strategy of building a regional vaccine.

Código de Resumen: MM-029

Sección: Microbiología Molecular

Modalidad: Poster

IDENTIFICATION OF MOBILE GENETIC ELEMENTS ENCODED IN CLINICAL ISOLATES OF UROPATHOGENIC *Escherichia coli*

A. Rivolta [1], M. Irusta[1], G. Parmeciano Di Noto[1], D. Archuby[2], L. Derdoy[2], C. Quiroga[1].

[1] *Instituto de Investigaciones en Microbiología y Parasitología Médica-Facultad de Medicina-UBA.* [2] *Sección Microbiologia-Laboratorio Central-Htal Dr J.M.Ramos Mejía..*

cc.quiroga@gmail.com

Escherichia coli is a versatile bacterium that thrives in a wide variety of niches. This microorganism is commensal of the gastrointestinal tract of humans; however some clones can acquire virulence and antimicrobial determinants by way of incorporation of mobile elements into their genome. Uropathogenic *E. coli* (UPEC) is a pathogenic variant that infects and colonizes the urinary tract. In recent years, it has been observed an increment on the resistance and virulence of UPEC strains as a result of the acquisition of broad-host range (BHR) plasmids and pathogenicity islands. The aim of this work was to evaluate the presence of key mobile elements responsible for the dissemination of antimicrobial resistance and virulence factors in the genome of UPEC isolated from a public hospital from Buenos Aires city. We used twenty-one (n=21) *E. coli* isolates from urine samples with different antimicrobial susceptibilities; eleven of which were resistant to third and fourth generation of cephalosporins. Detection of BHR plasmids from incompatibility groups IncW, IncP and IncN was done by standard PCR using specific primers. Fifteen out of 21 isolates harbored BHR plasmids, in which the IncP group was the most prevalent element (10/15). Only two isolates harbored two plasmids, both from IncW and IncP groups. Overall, no correlation was observed for the presence of these BHR plasmids and the antimicrobial susceptibility. We also searched for the presence of the pathogenicity island PAI-1, one of the most disseminated PAI in UPEC strains, as well as for class 1 and 2 integrons. We observed that most UPEC isolates from our study code for this PAI (14 out of 21), confirming that this element is also widely spread in our population. In addition, we noticed a co-ocurrence of PAI-1 and the BHR plasmids tested here (7/21). On the other hand, only seven isolates were positive for *intI1* whereas no *intI2* was detected. Six out of 7 *intI1* positive strains were consistent with resistance to third and fourth generation of cephalosporins, which suggests that this platform may contain genes coding for their respective beta-lactamases. Last, only 3 strains positive for *intI1* harbored a BHR plasmid, which indicates that the remaining integrons may be located in plasmids from other incompatibility groups or in genomic islands. Our work evidences that the UPEC isolates used in this study acquired preferably plasmids from the IncP incompatibility group and the element PAI-1. We also observed the co-occurrence of mobile elements that could be beneficial for the survival of these pathogens. The presence of BHR plasmids in UPEC isolates reflects the adaptation and evolution of this microorganism that may result in a steady and troublesome increment of multirresistance in the community.

Código de Resumen: MM-030

Sección: Microbiología Molecular

Modalidad: Poster

QUORUM SENSING INTERCELLULAR COMUNICATION SYSTEM PARTICIPATES IN *Yersinia enterocolitica* BIOFILM FORMATION

C. Lucero Estrada[1 2], N. Di Marco[2], J. Weirich[3], E. Bohn[3], A. Zorreguieta[4 5], I. Autenrieth[3].

[1] Instituto Multidisciplinario de Investigaciones Biológicas. CONICET, San Luis. [2] Área de Microbiologia. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. [3] Instituto de Medicina, Microbiología e Higiene, Tubinga, Alemania.. [4] Instituto de Investigaciones Bioquímicas de Buenos Aires. CONICET, CABA. [5] Laboratorio de Microbiologia Molecular y Celular, Fundacion Instituto Leloir, CABA.

nataliadimarc@gmail.com

Yersinia enterocolitica (*Ye*) causes acute gastroenteritis which may result in severe post-infectious complications as reactive arthritis. It is able to form biofilms onto biotic and abiotic surfaces but till the moment it is not clear the function of biofilm formation in its pathogenesis. Every species of the genera *Yersinia* is able to produce and release *N*-acyl-homoserin lactones (AHLs), diffusible molecules that are part of a complex intercellular system known as *Quorum Sensing* (QS). In *Ye* the YenI/R system, which presents significant analogy to the LuxI/R family, participates in synthesis and recognition of AHLs. The aim of this work was to obtain mutant strains with *yen*I/R deletion in order to know if QS participate in *Ye* biofilm formation. The reference strain *Y. enterocolitica* WA 1B/O:8 (bio/serotype), was used to obtain mutant strains. The Gibson assembling reaction was used to obtain a suicide plasmid that was cloned into *Escherichia coli* pir^+ by electroporation. After obtaining multiples copies of the plasmid, the *E. coli* b2168Dnic35 strain was transformed. The latter strain mated with *Ye* and then suicide plasmid was integrated into *Yersinia* chromosome via homologous recombination creating partial diploids (merodiploid) with tetracycline resistance. The suicide plasmid also contains the counter selection marker sacB. The SacB enzyme metabolites yield the cytotoxic product sucrose. *Ye* merodiploids were cultured on sucrose plates therefore clones that lost the suicide plasmid were selected in this medium. The looping out of the suicide plasmid can either restore the original wild type allele or yield the desired mutant allele. The result of the looping out reaction was tested by colony PCR and also, mutants were validated by sequencing the mutated genes and analyzing the mRNA expression. The crystal violet technique in a 96-well polystyrene plate (PE) was used to observe the biofilm formed after 24 h of incubation at 25°C without shaking in trypticase soy broth added with 0.25% glucose (TSBG). Laser Scanning Confocal Microscopy (LSCM) was used to observe biofilms fixed on glass and stained with DAPI. With the Gibson cloning technique it was possible to obtain the *yen*I/R mutants without antibiotic resistance. The biofilm observed with mutant strains was significantly thinner than with wild type (WT) strain; the mean values were *Ye*Δ*yen*I 0.54±0.09, *Ye* Δ*yen*R 0.64±0.08 and*Ye* WT1.70±0.23 ($p<0,001$). Although, all strains were able to firmly attach to the glass chambered slide, establishing bacterial aggregates or microcolonies, the biofilm formed by WT strain was stronger than the observed with mutant strains. A functional QS system is necessary during biofilm formation in *Ye* 1B/O:8 strains under these culture conditions. More work is needed to know the importance of QS and biofilm formation during *Y. enterocolitica* transmission and infection.

Código de Resumen: MM-031

Sección: Microbiología Molecular

Modalidad: Poster

EFFECTS OF DIFFERENT FACTORS ON BIOFILM FORMATION IN *Streptococcus uberis* STRAINS

M.V. Moliva[1], M.C. Lasagno[1], E.B. Reinoso[1].

[1] Universidad Nacional de Río Cuarto.

mmoliva@exa.unrc.edu.ar

Streptococcus uberis is one of the main infectious agents responsible for causing mastitis. Previous studies carried out in our laboratory showed that *S. uberis* strains isolated from milk are able to produce biofilm. It is speculate that one of the reasons behind the prevalence of *S. uberis* infections is its ability to form biofilm. This study investigated the influence of different factors as: lactose (5%), skim milk (0.5%), alpha-casein (3mg/ml), bovine serum albumin (5 mg/ml) and proteases inhibitors as cysteine protease inhibitor E-64 (1 mM) and metalloprotease inhibitor 1-10 phenantroline (100 M). In addition the stability of *S. uberis* biofilm against protease treatment with proteinase K was tested. Thirty two *S. uberis* strains previously identified and characterized for biofilm formation were used. Our study indicated that the substrates skim milk, alpha casein and bovine serum albumin appear to have a positive effect on its ability to form biofilms. These results suggest that the milk protein composition of the host might affect the growth mode of *S. uberis* and the development of infection, promoting adherence and internalization. On the other way, lactose had no significant effect on the biofilm formation under these conditions. However the effects of the different factors seem to vary between the strains. The treatment of biofilms with proteinase K resulted in complete detachment of biofilms, suggesting that extracellular proteins play a crucial role in formation of biofilm. E-64 and 1-10 showed to increase biofilm production in the presence of alpha casein. Our results provide a valuable insight into molecular specificities of this pathogen. Future work will concentrate in associating *S. uberis* ability to form biofilms *in vivo* and intramammary infection. This will allow for development of strategies to better manage and prevent mastitis caused by this pathogen.

Código de Resumen: MM-032

Sección: Microbiología Molecular

Modalidad: Poster

ROLE OF THE GlnK PROTEIN (PII PROTEIN) IN THE NITROGEN STRESS RESPONSE CIRCUIT IN *Bradyrhizobium diazoefficiens* USDA 110

F. Lamelza[1], V.A. Hegel[1], M.F. López[1], S.L. López García[1].

[1] *Instituto de Biotecnología y Biología Molecular (IBBM) - CONICET- Facultad de Cs. Exactas - UNLP.*

flopy_lamelza@hotmail.com

Symbiosis between rhizobia and legume plants is a model of nutritional complementation. Plants reduce atmospheric CO_2 and provide carbon compounds to the bacteria inside the nodule while rhizobia use these compounds and fix atmospheric N_2 to ammonia, a nitrogen (N) source that plants can metabolize. *Bradyrhizobium diazoefficiens* USDA 110, our bacteria of study, is an alphaproteobacterium that is able to exist as a free-living organism growing at the expense of soil nutrient, or in symbiotic association with soybean plants. This symbiotic interaction requires limited amounts of soil N sources to take place. However, agricultural soils are N fluctuating environments and free-living bacteria have developed specific mechanism to acquire and metabolize N in an efficient way in order to survive. The Nitrogen Stress Response (NSR) circuit is an example of these mechanisms and it has been studied in rhizobia such us *Ensifer meliloti and other bacteria like Escherichia coli and Rhodopseudomonas palustris*. Nevertheless, we don´t know anything about this response in *B. diazoefficiens* USDA 110. In most microorganisms, the core elements of the NSR regulation cascade include a bifunctional uridylyltransferase/uridylyl-cleavage enzyme GlnD (*glnD*) and two PII proteins: GlnB (*glnB*) and GlnK (*glnK*). The uridylylation state of these PII proteins is regulated by the α-ketoglutarate/glutamine intracellular ratio. In nitrogen-starved cells, when the concentration of α-ketoglutarate is high, GlnD uridylylates PII proteins which, in turn, activate the bacterial NSR, leading to more efficient ammonia assimilation by increasing glutamine synthetase (GS) activity. Using bioinformatics tools we found homologous genes in *B. diazoefficiens* USDA 110 that codify two forms of PII proteins: one copy of *glnB* and two copies of *glnK* (*glnK1* and *glnK2)*. Generally, rhizobia have only one copy of *glnK*, so we proposed to find out whether both copies are functional as well as to understand the role of these PII proteins in the complex nitrogen metabolism. To achieve our objective, null mutants of *glnK1* (ΔglnK1 strain) and *glnK2 (*ΔglnK2 strain) were generated. Although in other rhizobia the deletion of *glnK* don´t produce striking phenotypes, it seems to be different in *B.diazoefficiens*. The results obtained when GS activity was assayed led us to think that both copies of GlnK are functional in our bacteria. Moreover, preliminary growth studies of ΔglnK1 strain showed a significant difference when a nitrate source was added to the culture after ammonium starvation. The ΔglnK1 strain was not able to grow as well as the wild type strain with this N source and the final OD500nm was lower. This result suggests that GlnK proteins could be involved, not only in ammonium assimilation but also in nitrate metabolism or uptake; so it is necessary to continue studying the performance of both mutant strains in media with nitrate as N source.

Código de Resumen: MM-033

Sección: Microbiología Molecular

Modalidad: Poster

EFFECT OF PHENOLIC COMPOUNDS ON *Staphylococcus aureus* BIOFILM DEVELOPMENT

A. Lombarte Serrat[1], C. Dotto[1], M. Hrast[3], M. Sova[3], S. Gobec[3], M. Suligoy[1], D. Sordelli[1], L. Saso[2], M. Giacomodonato[1], F. Buzzola[1].

[1] *IMPaM, UBA-CONICET, Buenos Aires.* [2] *Department of Physiology and Pharmacology Vittorio Erspamer, Sapienza University of Rome, Italy.* [3] *Faculty of Pharmacy, University of Ljubljana, Slovenia..*

andrea_pn-rj@hotmail.com

Staphylococcus aureus causes a wide range infectious diseases in humans and animals. Biofilms have been linked to bovine mastitis since *S. aureus* biofilm-producer strains showed an increased ability to attach to mammary mucosal surfaces and cause persistent infections compared with non-biofilm forming strains. *S. aureus* biofilm formation partially depends upon the production of polysaccharide intercellular adhesin (PIA), coded by the *ica* operon. Antibiotic therapy is becoming increasingly ineffective in the treatment of biofilm-associated infections. Therefore, the search for new chemical compounds with anti-biofilm properties becomes necessary. The aim of this study was to investigate the inhibitory effect of four phenolic compounds (named F1-F4 for simplicity) prior-to and post- biofilm formation by *S. aureus*. To avoid the identification of strain-specific hits, the study was performed on five *S. aureus* isolates from milk of bovines with mastitis and two laboratory reference (Newman and SA113) *S. aureus* strains. The isolates were selected for their ability to produce large amounts of biofilm by either *ica*-dependent or -independent mechanisms. Bacteria were treated with compounds before biofilm formation takes place (prior-to-exposure) and 24 h after biofilms were formed (post-exposure). The biofilm biomass was stained with crystal violet for spectrophotometric

quantification. The initial screening in the Newman strain allowed to classify the phenolic compounds as inactive (F1), moderately active (F2, F4) or highly active (F3). Only *N*-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-hydroxybenzamide (F3) presented a moderate but significant dose-dependent effect on the inhibition of biofilm formed by the strains under study. The different phenolic compounds showed no bacteriostatic or bactericidal activity. The prior-to-exposure evaluation revealed that F2 induced 89%, 86% and 43% biofilm inhibition in AR99, RF122 and SA113 *S. aureus* strains, respectively. F3 was able to inhibit by 77% to 23% the biofilm formation in all strains under scrutiny except AR77. F4 decreased biofilm production by 23% only in the Newman strain. No compound affected the AR77 biofilm formation. Results obtained by post-exposure of phenolic compounds indicated that F3 showed an anti-biofilm effect in most strains analyzed except V329 and RF122. Data obtained from F1 experiments did not show any significant effect on biofilm inhibition or inactivation in any of the strains under study confirming its inactivity. It is likely that in the presence of F3, certain bacterial cells are able to attach and form biofilms, but their maturation process is significantly hampered. This study highlights the potential of F3 as a successful agent that can act prior-to and post- biofilm development.

Código de Resumen: MM-034

Sección: Microbiología Molecular

Modalidad: Poster

CHARACTERIZATION OF NtrY, A SENSOR PROTEIN OF A TWO COMPONENT SYSTEM IN *Bradyrhizobium diazoefficien*s USDA 110

V.A. Hegel[1], M.F. López[1], F. Lamelza[1], S.L. López García[1].

[1] *Instituto de Biotecnología y Biología Molecular (IBBM), La Plata -CONICET- Fac. de Cs. Exactas, UNLP.*

valeriahegel@gmail.com

Rhizobia are soil bacteria able to establish a nitrogen-fixing symbiosis within the root nodule cells of legume host plants.The transition from the free-living to the symbiotic state is accompanied by drastic changes in bacterial metabolism, eventually leading to the formation of bacteroids specialized for nitrogen (N) fixation. Our research focuses on the symbiotic relation between soybean legume and *Bradyrhizobium diazoefficiens* USDA 110. We have already advanced in the study of nutritional effects of N in *B. diazoefficiens* cultures in free-living bacteria as well as in different stages of the symbiotic interaction. Moreover, we have demonstrated that N-limited cultures stimulate early events of symbiosis and N_2 fixation. The major aim of our work is the comprehension of the role of the regulators involved in N metabolism. One of these regulators is NtrBC, a two-component system (TCS) that senses the intracellular variations of C/N ratio and controls the expression of some genes implicated in ammonium assimilation. When the C/N ratio is high (under nitrogen starvation) NtrB (sensor protein) phosphorylates NtrC (response regulator) that in turn, activates transcription of genes required for nitrogen uptake and metabolism. *B. diazoefficiens*, as other alphaproteobacteria, has a second two-component system named NtrY-NtrX located immediately downstream of the *nifR3-ntrB-ntrC* operon and whose function remains unknown. NtrY exhibits a high degree of homology with sensor histidine kinase proteins while NtrX with response regulator proteins. This system has been reported in *Azorhizobium caulinodans, Azospirillum brasilense, Rhodobacter capsulatusand Brucella* spp. to be involved in nitrogen metabolism, symbiotic nodulation and low-oxygen response. Taking into account that *nifR3-ntrB-ntrC* is located next to *ntrY-ntrX,* we studied if these genes belong to the same operon analysing by RT-PCR the intergenic region. These results demonstrated that *ntrY-ntrX* are located in a different operon than *ntrB-ntrC*. In order to study the role of NtrY/X in N metabolism, we generated *ntrY* null mutant strain (named LP4489) in *B. diazoefficiens* USDA 110. This mutant was generated by crossover-PCR so as to avoid polar effect in *ntrX*. We compared the growth of this mutant with the wild type strain in Evans media with high concentration of N (20 mM of NH_4Cl) and in Evans media with low concentration of N source (0.1 mM of NH_4Cl). In both cases we found that there was no significant difference in the OD 500nm neither in the colony forming unit per milliliter (cfu/ml). However, it is necessary to analyze the growth of this mutant in other N sources. Finally, swimming assays were performed in AG media with 0.3% w/v of agar. Unexpectedly, LP4489 strain was less motile than the parent USDA 110. These results could suggest that NtrY has an effect on bacteria motility. Indeed, more studies are required to investigate the role of this sensor protein in *B. diazoefficiens*.

Código de Resumen: MM-035

Sección: Microbiología Molecular

Modalidad: Poster

BIOGENESIS OF OXA-58 FROM *Acinetobacter baumannii* CLINICAL ISOLATE IN GRAM-NEGATIVE BACTERIA

C. Fabbri[1], M.M. Cameranesi[1], A.S. Limansky[1], A.M. Viale[1], J. Morán-Barrio[1].

[1] *IBR-CONICET U.N.R. Microbiology, Rosario..*

fabbri.carolina@gmail.com

Acinetobacter baumannii is an important Gram-negative opportunistic pathogen responsible for a variety of nosocomial infections. It can rapidly evolve multi-drug resistance (MDR) when confronted with antibiotic therapy, and the emerging resistance to carbapenems among nosocomial strains represents a major concern worldwide. One of the mechanisms proposed to play a significant role in carbapenem resistance in *A. baumannii* is the overexpression of carbapenem hydrolyzing class D, OXA-type, β-lactamases (CHDL). Many studies were aimed to uncover the genetic location and context of the bla_{OXA} genes, and to kinetically characterize the encoded enzyme. However, less attention has been given to OXA lactamase biogenesis, a process that includes translocation of the newly synthesized precursors from the cytoplasm across the inner membrane, and folding in the actual subcellular location. Most β-lactamases belonging to classes A, B and C are periplasmic soluble enzymes in the periplasm of Gram-negative bacteria, but still no information exists about CHDL. We analyze here OXA-58 biogenesis regarding to the level of protein production and carbapenem resistance conferred, using *A. baumanni* ATCC 17978 as well as *E. coli* K12 as model hosts of Gram-negative bacteria. Plasmid pWH1266-bla_{OXA-58} was used for OXA-58 production in *A. baumannii*, and pBAD-derived plasmids were constructed for *E. coli*. We also developed different protocols and optimized procedures for proper subcellular localization analysis. Periplasmic contents were obtained by osmotic shock procedures. Membrane fractions were obtained by Sarcosynate treatment of sonic extracts and ultracentrifugation, thus allowing partition of inner and outer membranes. SDS-PAGE analysis indicated that OXA-58 was associated to the inner membrane fraction in *A. baumannii* when produced in low quantities, and also in the periplasmic space as a soluble protein when over-produced. In these cases the levels of OXA-58 showed correlation with the MIC values obtained towards IPM. In *E. coli* however, though different conditions inducing bla_{OXA-58} expression were used, no carbapenem resistance was observed when the complete bla_{OXA-58} gene was expressed. Substituting the original signal sequence with that of PelB resulted in OXA-58 production as judged by SDS-PAGE analysis and *E. coli* ampicillin resistance. Altogether, these results indicate the association of OXA-58 to the *A. baumannii* inner-cell membrane probably as a mechanism of enzyme stabilization.

Código de Resumen: MM-036

Sección: Microbiología Molecular

Modalidad: Poster

PLASMIDS FROM ENVIRONMENTAL STRAINS ISOLATED FROM HIGH ALTITUDE ANDEAN LAKES

D. Kurth[1], O.F. Ordoñez[1], M.E. Farías[1].

[1] *Planta Piloto de Procesos Industriales y Microbiológicos (PROIMI-CONICET), 4000, Tucuman.*

dgkurt@gmail.com

High-altitude Andean Lakes (HAAL) are pristine environments with extreme conditions such as high UV radiation, high heavy metal content (mainly arsenic), high salinity, and oligotrophy. Survival in these conditions depends on resistance mechanisms that might be encoded either in genomes or mobile elements. Several linear plasmids have previously been found in actinomycete strains, and a number of genes have been proposed to function as resistance mechanisms. In this work, we searched for plasmids in other HAAL strains. We analysed isolates identified as *Exiguobacterium,Acinetobacter* or *Pseudomonas*, as they were among the most abundant within the HAAL collection. Using standard plasmid extraction protocols for Gram(+) and Gram(-) bacteria, small plasmids were isolated from two highly UV-B resistant strains, namely *Acinetobacter* sp. Ver3 and *Exiguobacterium* sp. S17. For the *Exiguobacterium* plasmid, a partial sequence was obtained from the draft genome, and completed and confirmed using PCR. For the *Acinetobacter* plasmids, restriction fragments were cloned and sequenced, and comparison with the draft genome allowed identifying a full 10kb plasmid and at least one more megaplasmid. Using a different methodology, namely PFGE, it could be further assessed that the *Exiguobacterium* strain has in fact two more megaplasmids which were not purified with the standard extraction protocol. The sequence of the isolated megaplasmids was also determined from restriction analysis, sequencing and comparison to the draft genome. Some of the genes might be important for survival in the environmental extreme conditions. We present here the sequences obtained and aim to characterize the role of these mobile genetic elements, both in survival to extreme conditions and in putative transfer of this information to the HAAL microbial community. Characterization of these genetic elements could also help to develop tools for the poorly studied *Exiguobacterium* genus.

Código de Resumen: MM-037

Sección: Microbiología Molecular

Modalidad: Poster

PHYLOGENETIC CHARACTERIZATION OF A *Pseudomonas syringae* STRAIN, ISOLATED FROM LOCAL OAT AND CAPABLE OF PRODUCING A VARIETY OF VIRULENCE FACTORS

E.D. Primo[1], C. Pereyra Duarte[1], A.T. Lisa[1].

[1] *Depto Biol Molecular, UNRC. Río Cuarto, Cba-Argentina..*

emiprimo@gmail.com

P. syringae is widely spread in nature and infects a large number of agriculturally important plant species. The ability of the organism to deliver virulence factors across the plant cell wall is a key to its pathogenicity. It causes a variety of symptoms in leaves, stems, and fruit. In our laboratory, a strain of *P. syringae* was isolated from the spots of leaves of diseased oat plants from a field in southern Córdoba. It was identified by metabolic profiling and named as *P. syringae* S5. Some virulence factors as biofilm, motility, homoserine lactones and protease production, etc., were also studied. The strain S5 exhibited phytotoxic activity measured by the chlorosis induced on leaves, different than oats, such as soybeans and peanuts. In this work, to better characterize the isolated S5 strain, we performed phylogenetic analysis of partial nucleotide sequences of the 16S rRNA gene. It showed that the S5 strain clustered together with different pathovars of *P. syringae*, and had a greater phylogenetic relationship with the pv. *atropurpurea.* The nucleotide sequences were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank) under accession number KJ830937. Subsequently, BOXPCR fingerprints were generated for S5, reference strains (*P. syringae* pv. *tomato* DC3000 and pv. *syringae* 61) and for other strains of the same geographical region (*P. syringae* pv. *glycinea* EM1, pv. *glycinea* A5, *P. syringae* VT2, Q, C13 and LS3). The dendrogram generated from BOX fingerprints clustered S5 strain with *P. syringae* pv. *syringae* 61 and with both of the *P. syringae* pv. *glycinea* strains (EM1 and A5) isolated from plants of our area. *P. syringae* produces several types of virulence factors but the role of its toxins is particularly significant during symptom development. Then, the current study was also focused on the toxins production by S5 strain. Besides the tabtoxin production, we conducted a bioassay for detection of syringomycin, which is through growth inhibition of *Geotrichum candidum*. A zone of inhibition around the bacterial growth (or supernatant) was detected, and it was clear evidence of syringomycin production by S5. To check the presence of *syrA* and *syrD* (the genes involved in the synthesis of syringomycin) PCR assays were designed with specific oligonucleotides, but the genes could not be detected; so we assume that the inhibition of the fungus was caused by another amphipathic compound similar to syringomycin. It was also detected a fungal growth inhibition when PAO1 *algR* (overproducer of surfactant compounds) or a commercial surfactant (Silwet L77) were used in bioassays. From the data shown here, we conclude that the S5 strain is phylogenetically related with *P. syringae* pv. *atropurpurea* and, by Box-fingerprints clustered with *P. syringae* pv. *syringae* 61 and with other strains isolated from our area. Besides, S5 strain produces tabtoxin and a surfactant compound different to the syringomycin, which could be the key to the pathogenicity of the strain.

Código de Resumen: MM-038

Sección: Microbiología Molecular

Modalidad: Poster

Salmonella-SPECIFIC TRANSCRIPTIONAL REGULATORS INVOLVED IN BIOFILM FORMATION

N.R. Figueroa[1], M.V. Humbert[1], S.K. Checa[1], F.C. Soncini[1].

[1] *Instituto de Biología Molecular y Celular de Rosario, CONICET-UNR.*

figueroa@ibr-conicet.gov.ar

Salmonellosis is among the most common foodborne diseases, with millions of human cases occurring worldwide every year. One of the key aspects of *Salmonella*'s life cycle that contributes to its high prevalence is its ability to form biofilms. This multicellular behavior allows the pathogen to survive hostile environmental conditions, and confers resistance to both host defenses and antimicrobial agents. Such resilience against extreme challenges is provided by means of a self-produced extracellular matrix which also contributes to attachment of sessile bacteria to each other and to both biotic and abiotic surfaces. Cellulose and curli fimbriae are major constituents of the *Salmonella* extracellular matrix. Expression of their biosynthetic genes is controlled at the transcriptional level by the master regulator CsgD. This transcriptional activator is in turn finely tuned by several transcription factors that integrate different environmental cues. We identified new *Salmonella*-specific transcription factors that participate in the control of biofilm-formation of this enteric bacterium. Deletion, as well as overexpression of the genes coding for these factors in different genetic backgrounds markedly affected motility, resistance to antibiotics and modified the development of characteristic biofilm morphotypes of this species. We showed that these factors affect production of cellulose and expression of *csgD* through different mechanisms. These results indicate that these *Salmonella*-specific transcription factors trigger biofilm formation, and hence control the switch between planktonic and sessile lifestyles.

Código de Resumen: MM-039

Sección: Microbiología Molecular

Modalidad: Poster

A METABOLOMIC APPROACH TO CHARACTERIZE ACID-ADAPTED BATCH CULTURES OF *Sinorhizobium meliloti*

W.O. Draghi[1], M.F. Del Papa[1], M.C. Martini[1], A. Barsch[2], A. Pühler[2], K. Niehaus[2], A. Lagares[1].

[1] *IBBM - Instituto de Biotecnología y Biología Molecular, UNLP-CONICET, La Plata, Argentina..* [2] *CeBiTec. Center for Biotechnology. University of Bielefeld. Germany.*

martini.mcarla@gmail.com

Sinorhizobium meliloti is a soil bacterium with the ability to establish a nitrogen-fixing symbiosis with different species of *Medicago, Melilotus and Trigonella*. In natural soil environment both partners need to surpass diverse biotic and abiotic stresses to achieve a successful symbiosis. In particular, the *Medicago-S. meliloti / medicae* symbiosis has been largely recognized as highly sensitive to (even moderate) acid stress. Thus, to cope with extracellular acidity *S. meliloti* is able to induce an adaptive acid tolerance response (ATR+), which not only promotes better survival rates but also improves symbiotic competitiveness. To characterize the biochemistry of such adapted (ATR+) rhizobia, a metabolomic approach was used. Results showed changes in several compounds of the central C-metabolism (mainly those involved in the pentose phosphate pathway) with an decrease in the intracellular NAD(P)H/NAD(P)+ ratio. The lower availability of reducing power is in agreement with a decreased concentration of polyhydroxybutirate. Results of the observed metabolic remodelling suggest that the balance of reducing power could be a relevant factor for maintaining the cellular homeostasis under acid stress. Further analysis will be necessary to progress in understanding how the metabolic changes here reported mediate the higher acid tolerance of the rhizobia and their more competitive phenotype on the host roots nodulation under acidic conditions.

Código de Resumen: MM-040

Sección: Microbiología Molecular

Modalidad: Poster

AMMONIUM EXCRETION IN *glnA* CONDITIONALLY-LETHAL MUTANT STRAINS OF *Azotobacter vinelandii*

R. Ambrosio[1 2], J.C. Ortiz Marquez[1 2], L. Curatti[1 2].

[1] *Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET).* [2] *Fundación para Investigaciones Biológicas Aplicadas, Mar del Plata, Argentina. .*

lcuratti@fiba.org.ar

Current demand of food and bioenergy pushes agriculture to relay on extensive use of fertilizers to improve crop productivity. However, extensive use of these agrochemicals is harmful to the environment and represents a significant share of agricultural costs. The aim of our work is to advance in the development of N-biofertilizing bacteria by metabolic engineering of *Azotobacter vinelandii*. Thus, we have isolated strains impaired in the switch-off of N2-fixation genes as a response to an increment in the intracellular levels of ammonium, and strains with a point mutation in the *gln*A-encoded glutamine synthetase (GS) that are deficient in ammonium assimilation. Although both kinds of strains release ammonium into the medium, diazotrophic growth was compromised up to a level that could prevent excretion of higher levels of ammonium. Nevertheless these strains performed well as N-biofertilizers when inoculated into algae cultures. In this work, we further explored the effect of fine-tuning GS activity on ammonium excretion. As a first approach we challenged *A. vinelandii* cells with different concentrations of the GS- inhibitor MSX and observed how changes in the level of GS activity resulted in differences in the maximum amplitude of ammonium excretion and in the time course of ammonium release. Next, a tunable genetic control of GS activity, mediated by IPTG transcriptional induction, was stably introduced into the *A. vinelandii* chromosome. The system comprises *Escherichia coli lacI*q (*lac* repressor), *lac*O (*lac* operator), the *trc*-promoter and a selectable marker. When using *gln*A as a target gene, as expected, we obtained conditionally lethal strains, since *A. vinelandii* only assimilates ammonium into amino acids by this enzyme. Cells cultivated overnight in the presence of ammonium and 25 µM IPTG and then for 6 h in the absence of IPTG (for depletion of GS) were transferred to fresh medium lacking ammonium and containing different concentrations of the inductor. In the absence of IPTG, mutant cells produce very high levels of ammonium up to 4 mM, lower concentrations at 0,1 and 10 µM, respectively, but did not release ammonium at higher concentration of IPTG. Conversely, bacterial growth was critically dependent on increased concentrations of IPTG. These findings suggest that fine-tuning GS activity might result in a proper balance between growth and ammonium release that could be further exploited to improve N-biofertilizers.

Código de Resumen: MM-041

Sección: Microbiología Molecular

Modalidad: Poster

THE *Sinorhizobium meliloti TRANS*-ENCODED SMALL RNA Sm8 (MmgR) MODULATES CARBON STORAGE UNDER NUTRIENT IMBALANCE

A. Lagares (h)[1], A. Becker[2], C. Valverde[1].

[1] *LBMIBS, DCyT, Universidad Nacional de Quilmes.* [2] *SynMikro, Universidad de Marburg, Alemania.*

valverdecl@hotmail.com

Bacterial gene expression is known to be regulated at various levels. Among them, riboregulation stands for post-transcriptional tuning of expression that is orchestrated by RNA molecules. The nitrogen-fixing legume symbiont *Sinorhizobium meliloti* expresses ca. 450 small *trans*-acting non-coding RNAs with potential regulatory functions. sRNA-mediated processes may play a distinctive role in the fine-tuning of regulation of gene expression in *S. meliloti*, both in free-living conditions and during the symbiotic association with the plant host *Medicago sativa*. The *S. meliloti* small non-coding RNA Sm8 has been of our particular interest because it has a remote evolutionary origin and shows a high conservation among the alpha-proteobacteria.Sm8 is a *trans*-encoded sRNA of 77 nucleotides in length that does not codify for a protein. Sm8 is kept at basal expression levels during a nutrient-balanced saprophytic growth and begins to accumulate at the stationary phase of growth after the onset of carbon-independent nutrient limitation. An *S. meliloti* Δ*sm8* mutant accumulates higher cellular biomass when growth is arrested as a consequence of imbalanced nutrient availability in the presence of an excess of a carbon source. The difference in biomass production observed between wild type and Δ*sm8* is due to a higher accumulation of the storage compound polyhydroxybutyrate (PHB) in the absence of Sm8. In this sense, Δ*sm8* is not able to limit the extent of stored PHB at increasing amounts of available C source when growth is arrested, as normally does the wild type. As expected, Sm8 expression from a plasmid gene copy in the mutant genomic background restored the wild type behavior. As further supporting evidence, ultrastructural analysis of N-starving wild type and Δ*sm8* stationary bacteria revealed differences in the amount and size of PHB storage granules between both strains.Transcriptomic and proteomic exploration of the Sm8 regulon revealed that Sm8 regulates -direct or indirectly- several genes known to be involved in PHB metabolism. Based on this evidence, we renamed the Sm8 transcript as MmgR, i.e., a regulatory RNA whose mutation results in a strain that makes more PHB granules. Further experiments should be conducted to explore the symbiotic relevance of MmgR as a regulator of C reserves accumulation.

Código de Resumen: MM-042

Sección: Microbiología Molecular

Modalidad: Poster

FLIL EFFECTS ON SWIMMING AND SWARMING BEHAVIOR OF *Bradyrhizobium diazoefficiens*

F. Mengucci[1], J. Carriño[1], C. Dardis[1], A. Lodeiro[1], E.J. Mongiardini[1], J.I. Quelas[1].

[1] *Instituto de Biotecnologia y Biologia Molecular (IBBM), CCT-La Plata CONICET, UNLP.*

florenciamengucci@gmail.com

Bradyrhizobium diazoefficiens is a motile soil α-proteobacterium that can live in free-living state or in N_2-fixing symbiosis with soybean. Thanks to this ability, *B. diazoefficiens* is used as commercial inoculants for soybean crops. Previous works in our lab showed that motility and distribution of these rhizobia in soil are determining factors of their symbiotic efficiency. There are diverse modes of bacterial motility among which swimming of individual cells in liquid media, and swarming of multicellular layers on solid or semi-solid surfaces. Both are propelled by flagella. *B. diazoefficiens* has two different flagellar systems: a subpolar system that is constitutively expressed and a lateral system that is inducible. Each system is composed of different sets of proteins, and both contribute to swimming and swarming. Lateral flagella induction is affected by the type of carbon source, viscosity, pH, or growth in liquid or semi-solid agar. FliL is a flagellar protein that is important for bacterial motility, whose role remains unclear despite it has been studied in several bacterial species. It seems to be associated to the flagellar basal body and would contribute to the stability of the flagellar structure when flagellar load increases. *B. diazoefficiens* USDA 110 have two putative fliL, each one related to the subpolar or lateral flagellar gene clusters (fliL1 and fliL2, respectively). According to TMHMM 2.0 server, both FliL have a single trans-membrane domain in the N-terminal position, but it seems that FliL2 is periplasmic, whereas all FliL1 appear to reside in the cytoplasm. In order to study their roles, we obtained unmarked in-frame deletional mutants for each gene in the *B. diazoefficiens* type strain USDA 110. These two mutants (named LP5826 and LP6868 for fliL1 and fliL2 deletions) were evaluated for swimming in liquid, semi-solid and viscous media. Also, LP6868 was analyzed for swarming. In semi-solid medium, only the diameter of LP6868 swimming halo was smaller than the wild-type, even when the subpolar and lateral filaments were expressed normally, as judged by SDS-PAGE of flagellins and electron microscopy of whole cells. This difference in motility was steeper in viscous medium (5% PVP). These results suggest that in this *fliL2* deletion mutant the lateral flagella were stopped and/or had less torque than the wild-type flagella. In contrast, LP6868 can swarm a little

more than the wild-type, and the edge of swarming colony presented elongated cells. Similar results were obtained in a previous work in *Proteus mirabilis* fliL mutants. In summary, our results showed that FliL2, but not FliL1, have effects on *B. diazoefficiens* swimming and swarming behavior. The role of FliL1 in subpolar flagellum remains unclear.

Código de Resumen: MM-043

Sección: Microbiología Molecular

Modalidad: Poster

REGULATION OF THE SUBPOLAR FLAGELLUM SYNTHESIS IN *Bradyrhizobium diazoefficiens*

C. Dardis[1], F. Mengucci[1], M.J. Althabegoiti[1], G. Parisi[2], A. Lodeiro[1], J.I. Quelas[1], E.J. Mongiardini[1].

[1] *Instituto de Biotecnología y Biología Molecular (IBBM). CCT-La Plata CONICET, UNLP..* [2] *Unidad de Bioinformática Estructural, Centro de Estudios e Investigaciones, UNQ.*

carolinadardis@hotmail.com

Bradyrhizobium diazoefficiens is an α-proteobacterium with high agronomic importance due to its ability to fix atmospheric nitrogen in symbiosis with soybean. Our previous studies showed that a hyper-motile strain of *B. diazoefficiens* is able to compete for nodulation and produce higher soybean grain yields than the wild type in certain conditions. This observation led us to study different aspects of bacterial motility, including the regulation of the synthesis of its dual flagellar systems. The flagellar synthesis is an energy-expensive process, which occurs in several steps that are tightly controlled by master regulators. These proteins trigger an ordered transcription cascade which means that the expression of one gene at a given level requires the transcription of another gene at a higher level. Applying bioinformatic tools, we identified putative regulators of *B. diazoefficiens* USDA 110 involved in the synthesis of the subpolar flagellum using *Caulobacter crescentus* as a model. We found the following candidates: *ctrA* (master regulator), *flbD* (class II regulator), *fliX* (trans-activation factor of *flbD*), *flbT* (class III regulator) and *flaF* (trans-activation factor of *flbT*). To confirm the role of these genes, mutations by insertion or deletions of each one were raised. However, initial attempts to obtain *ctrA* mutants were unsuccessful probably because the absence of CtrA causes deleterious phenotype. In this work, we constructed and partially characterized *flbD* and *fliX* mutants in *B. diazoefficiens* USDA 110. Both mutants were obtained by double-crossing over. For the *flbD*, we used the counter selection method in order to avoid polar effects, while the *fliX* mutant was obtained by insertion of an antibiotic cassette inside the *ORF*. All these mutants were checked by PCR and sequencing. When we began the mutant characterization, we noticed that none of these were able to produce extracellular subpolar flagellins, although the lateral flagellins were present in both of them. In addition, we observed that swimming in soft-agar plates was reduced in each mutant compared to the wild-type. These new findings suggest that both genes are involved in the regulation of the subpolar flagellum synthesis in *B. diazoefficiens* independently of the regulation of lateral flagella. In this sense, the model of regulation seems to be similar to *C. crescentus* but not to *Salmonella*, as was thought on the first studies. From now on, we will continue working to determine which genes are under the control of these two proteins in order to construct a hierarchy model for the regulation of the synthesis of this flagellum.

Código de Resumen: MM-044

Sección: Microbiología Molecular

Modalidad: Poster

INTERACTION OF CELL DIVISION PROTEINS WITH PBP2b AND EFFECTS IN MORPHOGENESIS OF *Streptococcus pneumoniae*

N. Yandar[1], A.G. Albarracín Orio[1], G.E. Piñas[1], N. Reinoso[1], C. Falcon[1], P.R. Cortes[1], J. Echenique[1].

[1] *CIBICI (CONICET) - Facultad de Ciencias Quimicas, Universidad Nacional de Córdoba.*

nubia.yandar@gmail.com

The penicillin-binding proteins (PBPs) are enzymes involved in cell wall synthesis and cell division in *Streptococcus pneumoniae*. We have previously described that Cp1015 strain containing *pbp2b* mutations (from clinical strains that confer β-lactam resistance) showed a pneumococcal subpopulation with bacillary shape, atypical septum formation and positioning, frequent asymmetrical divisions, and a PBP2b and FtsZ delocalization. These results suggested that PBP2b is involved in essential processes related to cell shape determination and cell division. By two-hybrid assays, we revealed that PBP2b interacted with proteins such as EzrA, MreC, RodA and FtsA, which were involved in cell division in *S.pneumoniae*. We constructed the *mreC*, *mreD*, *rodA*, *ftsA* and *ezrA* mutants by insertion-duplication mutagenesis. This mutants displayed small colonies, fitness, morphological and cell-wall biosynthesis alterations by vancomycin-fluorescein staining. In this study we obtained double mutants by sequential transformation of this proteins with PBP2b and $PBP2b^{28}$-GFP. They revealed elongated phenotype, a variety of morphology defects: “twisted-towel” shape, bind together chains, terminal thickens and aggregates of PBP2b and $PBP2b^{28}$-GFP. These results suggested that ErzA, MreC, MreD, RodA and FtsA could contribute, together with PBP2b, to determine the pneumococcal cell shape of *S. pneumoniae.*

Código de Resumen: MM-045

Sección: Microbiología Molecular

Modalidad: Poster

ORIGIN, ADAPTATION AND BIOGEOGRAPHY OF THE *intI1* GENE

V.E. Álvarez[1], L. Chamosa[1], M. Nardelli[2], P. Power[3], M.P. Quiroga [1], D. Centrón[1].

[1] *IMPAM (UBA/CONICET).* [2] *Universidad de Luján.* [3] *Universidad de Buenos Aires.*

valvarez@gmail.com

Class 1 integrons are the most successful mechanism associated to Lateral Antimicrobial Resistance Genetic Transfer among Gram-Negative clinical isolates. Recent studies showed they can be found in different habitats; however, a detailed study has not been conducted yet. The aim of this work was to identify the origin and the variability of circulating alleles of *intI1* type gene from class 1 integrons. *IntI1* gene sequences from GenBank running a BLASTn query using AF313471 as reference (April 2015) were analyzed for its origin according to the degree of human impact, the continent where they had been firstly isolated, the presence of Tn*402* platform and the type of site *attI1*associated. AWK and Bioperl scripts, ClustalW algorithm in MEGA v6.0, phylogenetic trees using the Neighbor Joining and Maximum Likelihood algorithms and Jalview v2.8.2 were used in order to study circulating alleles. Seventy one alleles of *intI1* belonging to the environment (n = 36), clinic (n = 29) and 6 shared by both habitats were detected. Within the latter category, 4 alleles had a frequency higher than 20% and they were spread over the 4 continents evaluated. Furthermore, 81.7% were unique alleles. Only 1.72% of the *intI1* genes (11/640) were inserted into the complete Tn*402* transposon. We detected several variants of the *attI1* site linked to *intI1*. Although we identified 116 *attI1* variants, one prevalent was detected in 83.1% of the *intI1* alleles, while the second in frequency was detected in 9.86% of them. Mutations over the entire *intI1* gene were identified, some with a low rate of preservation of the physical and chemical properties of amino acids. Moreover, other mutations which led to the generation of pseudogenes were seen. RHRY conserved tetrad characteristic of tyrosine integrase recombinases was analyzed. This tetrad is specifically related to the orientation of DNA in the proper conformation needed for the site-specific recombination. We confirmed that even at very low frequencies, the residue H was the only mutated in the tetrad, replaced in one allele by an amino acid that has the same physicochemical properties. These studies evidenced an active flow of *intI1* alleles, both within the clinic and the environment. Although a small number of alleles flowed in both habitats, they corresponded to the 90.93% of the strains, suggesting the generalist intrinsic nature of this gene, which is functional in different niches. The hypothesis of this two-way flow was also supported by the identification of unique clinical *intI1* alleles which have emerged recently, whose class 1 integron 3' ends are different from the known 3'-CS. These results suggest a scenario in which the first *intI1* alleles that started to circulate among clinical strains, have disseminated all around the world, with sporadic and continue captures of novel alleles from the open environment.

Código de Resumen: MM-046

Sección: Microbiología Molecular

Modalidad: Poster

VARIABILITY AND EVOLUTION OF THE *aadA1* GENE CASSETTE

A.V. Galán[1], M.P. Quiroga[1], D. Centrón[1].

[1] *Universidad de Buenos Aires.*

angiegalan@gmail.com

Gene cassettes (GC) are mobile elements associated to horizontal gene transfer inserted in integrons. The integron/cassettes system allows bacteria to capture and express GC which are promoterless open reading frames (ORF) with a recombination site called *attC*. *AadA* is one of the most widespread resistance GC in the clinic. The aim of this study was to analyze the variability and evolution of both full GC and its individual components, ORF and *attC*. A search was conducted on INTEGRALL database for different alleles of the *aadA GC.* Full GC sequences and their individual components were studied through ClustalW and phylogenetic trees with MEGA 6 program. We made a further study in GenBank database with *aadA1 attC* GC reference sequence to identify associated GC, and the GC position in different Integrons. We found 37 alleles of *aadA* from Integrall database. Through the alignment of their *attCs* sites we found 18 different sites. As expected, the alignment occurred mainly by the nucleotide bases located at the beginning and the end of the sequence of different *attCs* sites, specifically those designated 1L and 1R, according to consensus sequence (RYYYAAC; GTTRRY R: Purine Y: Pirimidine), which are important for recombination. These sequences are not highly modified despite the high diversity of alleles found in this analysis. In the *aadA9* allele, a tandem duplication of 1L was observed. Regarding to 2L and 2R regions (GTTCRARY; RYTYAAC), which are associated to the formation of an extra-helical base, 30 alleles have the consensus sequences, 25 remained with 100% of identity with the *aadA1 attC* site. The *attC* sites in 3 alleles modified their consensus sequences. However, we found modifications of *attC* sites in 2 alleles, leading to the loss of the extra-helical base in 2L. The maximum likelihood algorithm tree

based on the *attC* sites evidenced that the predominant *att*C site was associated to 12 *aadA* alleles. This prevalent *attC* site is included in one cluster together with other 15 *attCs* sites, while the two remaining *attCs* sites corresponding to *aadA1c* and *aadA1bq* alleles were found further away from the rest. Trees of full cassettes and ORFs were identical and only have differences with the *attC* sites tree. The location of 200 *aadA1* GC was analyzed; we found they were inserted into class 1 or 2 integrons. In class 1 integrons *aadA1* was mainly located in the second position within the variable region of the integron and downstream of *dfrA1* GC, but also frequently as the only cassette in the variable region. In class 2 integrons *aadA1* was found mostly in third place, also associated to *dfrA1* GCand less frequently downstream of *sat2 or catB8* GCs. The *aadA1* GC is highly widespread in class 1 and 2 Integrons. Exchangeable ORF associated to one *attC* site and viceversa was a common finding for the *aadA* GC.

Código de Resumen: MM-047

Sección: Microbiología Molecular

Modalidad: Poster

DISSEMINATION OF PLASMIDS RELATED TO COLEII-TYPE IN CLINICAL AND ENVIRONMENTAL STRAINS

L.S. Chamosa[1], V.E. Alvarez[1], F. Falcone-Diaz [1], M. Almuzara[1], M.P. Quiroga[1], D. Centrón[1].

[1] *Laboratorio de Investigaciones en Mecanismos de Resistencia a Antibióticos, IMPaM, UBA-CONICET.*

luchamosa@hotmail.com

pDCPR1 and pDL209 plasmids belong to a new incompatibility group, ColE2-*like*, which has the particularity of having an hybrid structure composed by genes with homology to the replicase (*repA*) of *Pseudomonas savastanoi*, and to partition genes (*parA* and *parB*) from *Xanthomonas albilineans.* Both species are pathogens of some commercially important plants. In this work, we investigated the distribution of this new incompatibility group in clinical and environmental strains from Argentina in order to determine the presumable direction of the gene flow. PCRs were performed using specific primers for *repA*, *parA* and *parB* in DNA extractions with DNA Puriprep-GP Kit (Inbio Highway) of 88 strains isolated between 2004-2014 belonging to genera *Pseudomonas*, *Serratia*, *Escherichia*, *Citrobacter, Enterobacter, Proteus, Salmonella, Klebsiella* and *Yersinia* from hospitals (n=76) and environment (n=12). In addition, *repA* and *parA* trees were constructed using Neighbour Joining of all accession numbers with up to 60% identity at the protein level (n= 65 for *repA*, n= 34 for *parA*). Fifteen *repA* positive amplifications were obtained. These corresponded to the genus *Pseudomonas* including *P. putida, P. fluorescens* and *P. mendocina*, as well as *P. aeruginosa,* and *Serratia marcescens* (n = 1). The 26,67% of *repA* positive strains also possessed *parA*, although 3,41% of the total isolates had only *parA* or *parB* gene. We identified a higher frequency of genes associated with this incompatibility group (*repA, parA* and *parB*) in "interface" species than in the other strains. Regarding the *repA* gene, we observed adaptations to different niches either plants, clinical and environmental niches, including increased host range plasmids. At the same time, we noted a differential spread of plasmids between the two hemispheres, with two trends: dissemination in *P. syringae* in the northern hemisphere, and horizontal genetic transfers in the southern hemisphere. With respect to *parA*, we observed two clusters disseminating both in several ecological niches and species. Our results showed at an epidemiological level the hybrid feature of ColE2-*like* replicons. These results indicated a wide distribution of components of this replicon particularly in *Pseudomonas* spp. strains, which, although isolated in the clinic, they are usually found in the open environment such as *P. putida, P. fluorescens* and *P. mendocina*. The fact that some of these genes are mostly found in environmental strains would demonstrate that clinical settings select those mobile elements from different habitats which confer adaptive advantages for successful dispersion of pathogenic strains. This view supports the presence of a bidirectional flow between environment and clinic, to which a transitional step or "interface" at a species level is added, exemplified by environmental strains isolated in the clinic, that could act as reservoirs and vectors.

Código de Resumen: MM-048

Sección: Microbiología Molecular

Modalidad: Poster

PHOTODYNAMIC ACTION OF 1-METHYL ETHER RUBIADIN, A NATURAL ANTHRAQUINONE, OVER *Candida tropicalis* BIOFILMS

J. Marioni[1], J.L. Cabrera[1], S.C. Núñez Montoya[1], M.G. Paraje[1].

[1] *IMBIV-CONICET. Fac. Cs. Exactas, Físicas. y Naturales, Universidad Nacional de Córdoba.* [2] *IMBIV-CONICET. Dpto. Farmacia, Fac. Cs. Qcas, Universidad Nacional de Córdoba.*

paraje@efn.uncor.edu

Rubadin 1-methyl ether (RME) is a anthraquinone (AQ) isolated from the bioactive extracts of a phototoxic shrub, *Heterophyllaea pustulata* Hook f. (Rubiaceae), which grows in northwestern of Argentina. We have established that this AQ along with others present in the bioactive extracts of this plant, exhibit photosensitizing properties. In this work, we assessed the *in vitro* antifungal activity of RME over *Candida tropicalis* biofilms, under darkness and irradiation in order to establish whether this effect could be photoestimulated; we also quantified the production of reactive oxygen species (ROS) and reactive nitrogen intermediate (RNI) in the two evaluated experimental conditions. RME, purified from benzenic extract, was identified by their RMN data. Biofilm quantification of *C. tropicalis* NCPF 3111 and *C.tropicalis* clinical was performed by the O'Toole & Kolter method. RME was tested at three concentrations in triplicate, under darkness and irradiation. The supernatant was used to measure $O_2^{\bullet-}$ production by the Nitro-Blue Tetrazolium (NBT) reaction, and RNI for nitric oxide (NO) generation by Griess reagent. The total system antioxidant capability was determined by FRAP assay and the activation of SOD by NBT assay. Amphotericin B (AMP B) was used as antifungic control. The biofilms formation of both strains was reduced by RME action only under irradiation. Thus, RME achieved a percentage reduction (%R) of approximately 81.8 ± 2.6 % on the clinical strain biofilm at the three tested concentrations, whereas on the biofilm of the reference strain only generated a 47 ± 10 %R at a single concentration (15.6 µg/mL). In addition, RME was more active than AMP B (61.2 ± 4.8 % at CIM). We noted that biofilms reduction is correlated with a large increase in the production of $O_2^{\bullet-}$ and NO. Moreover, we observed an inactive SOD and an increase in total antioxidant system. In conclusion, we demonstrated that the *in vitro* antifungal activity of RME against biofilms of *C. tropicalis* is due to the photodynamic mechanism Type I, and the activation of the total antioxidant system not allowed the complete biofilm eradication. Therefore, it would be promising evaluate the effect of consecutive periods of irradiation to eliminate the biofilms.

Código de Resumen: MM-049

Sección: Microbiología Molecular

Modalidad: Poster

COMPARISON OF ANTIFUNGAL ACTIVITY OF NATURAL PRENYLATED FLAVANONES AGAINST *Candida albicans* BIOFILMS

M. Peralta[1], M.A. da Silva[2], M.G. Ortega[1], J.L. Cabrera[1], M.G. Paraje [2].

[1]IMBIV- CONICET. Dpto. Farmacia, Fac. Cs. Qcas., Universidad Nacional de Córdoba . [2]IMBIV--CONICET. Cát Microbiología, Fac.Cs. Exactas, Físicas y Naturales, Univ. Nacional de Córdoba .

paraje@efn.uncor.edu

The formation of biofilms is an important virulence factor that allows *C. albicans* to cause many types of infection and is responsible for most cases of candidiasis at both mucosal and systemic sites. It has been reported that *Candida* biofilms are 30-2000 times more resistant to several antifungal agentscompared to their planktonic counterparts. The continuing emergence of infections with antifungal resistant *Candida* strains requires a constant search for new antifungal drugs. The present study compared the antifungal effect of a new prenylated flavanones of *Dalea boliviana* Britton (2S)-5,7,2-trihydroxy-5-(1,1-dimethylallyl)-8-prenylflavanone (1) and (2S)-5,7,2-trihydroxy-8,3-diprenylflavanone (2) and a natural prenylflavonoid *D. elegans* (2,4-dihydroxy-5-(1,1-dimethylallyl)-8-prenylpinocembrin-8PP), on *Candida albicans* biofilms, and compared this with an azole antifungal (fluconazole). The fluconazole sensitive (SCa) and azole-resistant (RCa) *C. albicans* strains were used, with biofilm formation being studied using crystal violet (CV) and confocal scanning laser microscopy (CSLM). The minimal inhibitory concentration for sessile cells (SMIC) was defined as the concentration of antifungal that caused a 50% (SMIC 50) and 80% (SMIC 80) reduction of treated biofilms. Biofilms were grown on disks and examined by CSLM using Calcofluor-White, a UV-excitable dye that binds chitin and beta-glucan, which has long been used to highlight fungal cell walls. Our results show that 8PP has similar pronounced antibiofilm effects against sensible and resistant *C. albicans* strains. While the flavonoid (8PP) concentration is higher than the antifungal of a comparative reference (fluconazole), the biofilm formation was strongly inhibited (>85%) by 8PP at 100 µM. It was observed that the cellular viability of biofilms decreased with increased concentration of the compounds assayed, with the results showing a correlation between the CV assay and CFU/ml. The hazy biofilm appearance was due to diffuse staining of the extracellular material with Calcofluor-White, and implies that this material was composed of mainly cell-wall-like polysaccharides. In the antifungal-treated C. albicans biofilm, the majority of C. albicans cells were present as blastospores (yeast forms) attached to the surface of the disk, which appeared as a haze-like film covering the fungal microcolonies. Biofilms treated with 8PP showed a significantly reduced thickness, with cells being fewer and of less density compared to those of the untreated control ($p < 0.001$). Similar images were obtained with fluconazole. Our data suggest that 8PP may be useful for the treatment of biofilm-related *Candida* infections, 8PP may also have a therapeutic potential in *C. albicans* infections. Further studies are still necessary in order to increase the understanding of the mechanisms of antibiofilm activity of this compound.

Código de Resumen: MM-050

Sección: Microbiología Molecular

Modalidad: Poster

INFLUENCE OF EXOPOLYSACCHARIDES IN BIOFILM FORMATION IN *Mesorhizobium loti*

A. Supanitsky[1], D.M. Russo[2 3], A. Zorreguieta[2 3], V. Lepek[4].

[1] *Instituto Nacional de Tecnología Industrial, INTI.* [2] *IIBBA-CONICET.* [3] *Fundación Instituto Leloir.*
[4] *Instituto de Investigaciones Biotecnológicas, INTECH, Universidad Nacional de General San Martín.*

drusso@leloir.org.ar

Biofilm lifestyle plays an important role in survival of bacteria under different environmental stresses. The interaction between symbiotic microorganisms and the environment, in particular with the host plant, depend on superficial and extracellular molecules. *Mesorhizobium loti* produces several exopolysaccharides that participate in the symbiotic process with the host plant. In previous works, our results suggested that EPS production is crucial for biofilm formation in *M. loti Ayac* 1 BII. Analysis of the *M. loti* MAFF303099 sequenced strain revealed the presence of a cluster of *exo* genes involved in EPS biosynthesis, polymerization, transport, and post-production processing, and a distant locus putatively related to EPS biosynthesis. By directed mutagenesis, we generated two EPS mutants of *M. loti* MAFF303099, interrupting the *mll5252* exoY and *mlr6756* genes. *mll5252* gene encodes a protein homologue to the galactosyltransferase ExoY, which is involved in exopolysaccharide I (EPS I) production in *S. meliloti.* The *mlr6756* gene encodes a putative exopolysaccharide biosynthetic protein. Biofilm formation was assayed in polystyrene multiwell plates using crystal violet staining. EPS levels were determined by Anthrone reaction, and cellulose production was evaluated by Congo red staining. Biofilm formation was reduced in *mll5252* mutant in comparison with WT whereas the *mlr6756* mutant showed no significant differences compared with the WT. *mll5252* mutant formed non-mucoid colonies that were strongly stained with Congo red in comparison with the WT strain, while *mlr6756* formed mucoid colonies that overproduced EPS. Finally, nodulation experiments were carried out on *Lotus tenuis* plants inoculated with WT strain or mutants. *mll5252* mutant strain showed a significant reduction in the total number of nodules in comparison with the WT. Plants inoculated with *mlr6756* mutant developed an increased number of nodules that seemed to develop earlier than the WT. Our results suggest that in *M. loti* MAFF303099, the production of EPS(s) is required for biofilm development and nodulation.

Código de Resumen: MM-051

Sección: Microbiología Molecular

Modalidad: Poster

INFLUENCE OF DNA HETEROLOGIES ON THE STRAND EXCHANGE REACTION CATALYZED BY RecA OF *Pseudomonas aeruginosa*

V. Borgogno[1], M. Monti[1], C. Argaraña[1].

[1] *CIQUIBIC-CONICET, Dpto Qca Biológica, Fac de Cs Químicas, UNC.*

vborgogno@fcq.unc.edu.ar

Homologous recombination (HR) plays key roles in the generation of genetic diversity by reassembling DNA sequences from total or partially homologous DNA molecules. It allows rapid acquisition of novel functions, driving adaptation and promoting speciation. However, creation of new sequence combinations by HR has to be balanced to give the highest chance of functionality maintenance of coded proteins. In bacteria, the initial stages of recombination are catalyzed by RecA, which promotes the alignment of two DNA sequences and initiates strand exchange between them. An important property of RecA is its tolerance to a limited extent of heterology which determines the efficiency of strand exchange between divergent DNA sequences and the fidelity of HR. Besides RecA, the ability to tolerate heterologies during recombination is held in check by the Mismatch Repair System (MRS). Thus, when MRS is either downregulated or inactivated the heterologies discrimination by RecA may be decisive to maintain the integrity of the genome and ultimately to avoid mutations. In the present work, we study the influence of heterologies on HR catalyzed by RecA of *Pseudomonas aeruginosa*. By a systematic *in vitro* study of oligonucleotides strand exchange using fluorescence resonance energy transfer (FRET) techniques, we examined the efficiency of RecA-catalyzed strand exchange in presence of 12 different types of single mismatches in three positions (close to the 5', middle or 3' end) of the incoming strand, as well as the presence of small loops. Here we show that most mismatches close to the 5' end of the incoming strand had strong inhibitory effect and this was variable depending on the type of mismatch. On the other hand, most mismatches located in the middle or 3' end of the incoming strand did not show an inhibitory effect and the strand exchange efficiency was similar among different types of mismatches. Furthermore, a direct correlation between strength of inhibition and the reversibility of strand exchange reaction was observed. Therefore, RecA recognizes different types of mismatches depending on its position suggesting that differences in the intrinsic properties of DNA owing to mismatch type, neighboring nucleotide sequences and the position of the mismatch may play a role during the strand exchange. In addition, we found that the presence of small loops (+/- 1 or 2 nucleotides) diminished RecA-mediated strand exchange reaction. These results may suggest that RecA maintains a guarding role against frameshift mutations, such as it was previously described for

the MRS proteins. In conclusion, RecA can effectively compare DNA segments with different types of heterologies in its search for homology.

Código de Resumen: MM-052

Sección: Microbiología Molecular

Modalidad: Poster

Burkholderia cepacia EPIDEMIC STRAIN MARKER (BCESM) DETECTED IN *Burkholderia contaminans* ISOLATES RECOVERED FROM CYSTIC FIBROSIS PATIENTS COULD BE ASSOCIATED WITH TRANSMISSIBILITY AND PERSISTENCE

M. Leguizamón[1], C. Prieto[1], P. Martina[1], F. Vignolles[1], M. Bettiol[2], P. Montanaro[3], M.L. Cazzola[4], S.S. Pérez[4], O. Yantorno[1], A. Bosch[1].

[1]CINDEFI CONICET-CCT La Plata, Facultad de Cs. Exactas, UNLP, La Plata. [2]Hospital Sor Maria Ludovica, La Plata, Sala de Microbiología. [3]Hospital Santísima Trinidad de Córdoba, Servicio de Bacteriología. [4]Hospital HIGA R. Rossi, Sala Bacteriología.

marianaaleguizamon@gmail.com

Burkholderia cepacia complex (Bcc) species have emerged as highly problematic human pathogens causing severe respiratory infections in cystic fibrosis (CF) patients. Among the 20 Bcc closely related species currently described, *B. multivorans, B. cenocepacia, and B. cepacia* have been reported to be the most frequently recovered species worldwide. Nevertheless, in a local epidemiological study we found a remarkable high representation of *B. contaminans* arising to almost 60% in CF patients. Although for some patients only a transient respiratory tract infection may occur, the acquisition of *B. contaminans* most typically results in a chronic infection with acute exacerbations and a gradual decline in lung function. The BCESM genomic region, part of a genomic island BcenGI11, was encountered in all the *B. cenocepacia* strains belonging to the ET12 lineage which have caused major outbreaks in CF patients in Canada, the UK and other European countries (3). Besides, in *B. cenocepacia* and other species of the complex the BCESM positive strains have generally been associated with high transmissibility, virulence and mortality. Additionally, it was recently reported for the *B cenocepacia* J2315 (belonging to the highly transmissible ET12 lineage) that this genomic marker contains an operon associated with quorum sensing (QS) signals expression, and is involved in persistence, biofilm formation and virulence. The objective of this work was to analyze the prevalence of *B. contaminans* clinical isolates harboring the BCESM genomic region and investigate its possible association with transmissibility and/or persistence. A total of 107 *B. contaminans* isolates recovered from sputum samples of cystic fibrosis patients attended in Argentina were analyzed. BCESM was detected by PCR using *B. cenocepacia* specific primers. For the BCESM genomic region sequencing analysis specific primers were designed. QS signals were identified by means of AHLs biosensors. Our results showed a high prevalence of clinical isolates harboring the BCESM in both, isolates belonging to first infections (63%) and isolates recovered from CF patients with chronic lung infection (62 %). We found that in the population here analyzed the presence of the BCESM genomic region was positively correlated to the expression of QS signals. These results together with the BCESM sequence analysis showed that although *B. contaminans* local clinical isolates seem to shear some of the characteristics of *B. cenocepacia* strains belonging to the ET12 lineage, more studies are needed to understand its high transmission and persistence in CF local patients.

Código de Resumen: MM-053

Sección: Microbiología Molecular

Modalidad: Poster

ATOMIC FORCE SPECTROSCOPY OF SINGLE ADHESIN ON LIVING *Bordetella pertussis´*s SURFACE

L. Arnal[1], N. Cattelan[1], M.I. Villalba[1], F. Castez[2], G. Longo[3], S. Kassas[3], M.E. Vela [2], O.M. Yantorno[1].

[1]CINDEFI-CONICET-UNLP, 50 No 227, La Plata, 1900,Argentina. [2]INIFTA-CONICET-UNLP.Suc. 4 CC 16, La Plata, Argentina. [3]Institut de Physique des Systèmes Biologiques, Lausanne, Switzerland.

yantorno@quimica.unlp.edu.ar

Pertussis is a human´s respiratory tract disease caused mainly by *Bordetella pertussis*. Even after the introduction of vaccination, 60 years ago, pertussis remains a serious public health problem. Recent reports indicate that Bp might grow as biofilm attached to the upper respiratory tract as a mean to persist in the host and becoming an important risk of spread of these bacteria. Filamentous haemaglutinin (FHA) is the mayor adhesin of Bp and is involved in different steps of biofilm formation. Here, using Atomic Force Spectroscopy, we studied single interactions between purified FHA and a specific antibody attached

to Si_3Ni_4 cantilevers and detected single FHA molecules in the surface of fixed and living Bp Tohama I reference strain cells. Bp Tohama I (Pasteur Institute, France) and BpGR4 (BpFHA-, Bp Tohama derivative mutant lacking expression of FHA) strains were used through this study. Cultures were done in Erlenmeyer flasks in Stainer Sholter liquid medium, at 37ºC and under agitation (160 rpm). After 24 h of growth cells were harvested at 8000g for 5 min and incubated onto polyethyleneimine (PEI) coated glass slides (0,1% PEI in distillated water, overnight, 4ºC) for 1 h. For purified FHA, samples were prepared on muscovite mica (SPI v1 grade), by incubating 1% APTES solution for 1 min, dried with N_2 current and incubating 30 µl of a 20nM solution of FHA in PBS for 15 min at room temperature and then washed with Milli-Q water three times and dried with N_2. Samples were mounted on a NanoWizardII AFM´s liquid cell (JPK, Germany). Si_3Ni_4 cantilevers were modified with specific antibodies against FHA by linking them with glutaraldehyde 0.5%. Data were analyzed using OpenFovea software and JPK data processing software. A Force vs. log of loading rate curve was built and could be very well fitted with a linear function showing typical dependence of specific interactions with loading rate. Single cell spectroscopy was made at a scan rate of 500nm/s. At a first step we used fixed Bp Tohama I cells and several individual cells were mapped. Elasticity and force interaction maps were built from the recorded fv images, showing specific interactions on bacterial surface for wild type cells but not for FHA. We also tested the specificity of the interactions by making the force spectroscopy assay after incubating the cells with free antibody solution blocking the interaction´s sites. Results showed that proteins seem to be assembled into nanodomains or clusters of adhesins which also share location with the most rigid domains of the cell. The distribution of FHA in nanodomains could mean that the bacterium clusters FHA in certain regions of the membrane reaching biggest and strongest domains of interactions which could be advantageous for cell-substrate and cell- cell interactions during biofilm development.

Código de Resumen: MM-054

Sección: Microbiología Molecular

Modalidad: Poster

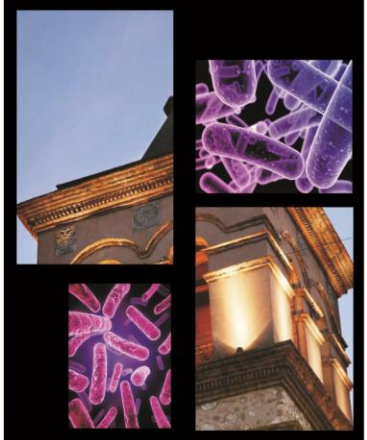
STRUCTURAL CHARACTERIZATION OF PILIN PROTEINS FROM *Xanthomonas citri* subsp. *citri*

S. Petrocelli[1], M.N. Cabrini[2], A.C. Casabuono[2], A.S. Couto[2], L.M. Moreira[3], E.G. Orellano[1 4].

[1] FBIOyF-UNR. [2] CIHIDECAR-Dpto. Qca. Orgánica, FCEN-UBA.. [3] DECBI y NUPEB-Universidade Federal de Ouro Preto, Brasil.. [4] IBR-CONICET.

petrocelli@ibr-conicet.gov.ar

The type IV pili (Tfp) are among the most widely distributed and best studied adhesins in pathogenic bacteria. Tfp are essential for eukaryotic cell adhesion, natural transformation, biofilm and twitching motility. Moreover, in plant pathogenic bacteria, the Tfp could be involved in host colonization and in the pathogenesis process. *Xanthomonas citri* subsp. *citri* (Xac) is the phytopathogen responsible for citrus canker. Three structural subunit-related genes of Tfp, *fimA*, *fimA1* and *pilA* were identified in Xac genome. In a previous work we have constructed a *pilA* mutant strain from Xac (*Xac*Δ*pilA*) and we have characterized the physiology of wild type and mutant bacteria. We also studied the role of the Tfp during the bacterial interaction with the host plant. The objective of this work was to characterize pilin subunits of the Tfp from Xac. *In silico* analysis showed that *pilA* and *fimA/A1* are placed on different locus and that PilA and FimA/A1 proteins present conserved structural elements and similar spatial distribution. A structural study of the purified Tfp fractions from Xac wild-type and *Xac*Δ*pilA* showed that pilins are glycosylated in both strains andthat FimA and FimA1 are structural components of Tfp. Finally, functional interaction networks between Tfp- and pathogenesis-related genes from Xac revealed interaction with genes related to Diffusible Signal Factor, xanthan production, type-two secretion system and pilus biogenesis. Our results demonstrate that the minor *pilA* gene is involved in the function of Tfp and that the pili is a glycosylated heteropolymer composed of FimA and FimA1 proteins.



EDUCACIÓN EN MICROBIOLOGÍA

Código de Resumen: EM-001

Sección: Educación en microbiología

Modalidad: Oral

LIBReciencia: AN IBR OUTREACH PROJECT AND MICROBIOLOGY AS A TOOL FOR TEACHING SCIENCE

E. García Véscovi[1 2].

[1] *Instituto de Biología Molecular y Celular de Rosario- IBR-CONICET.* [2] *Facultad de Ciencias Bioquímicas y Farmacéuticas - UNR.*

garciavescovi@ibr-conicet.gov.ar

LIBReciencia es un proyecto de vinculación surgido en el IBR en el que participan voluntariamente un equipo de trabajo conformado por estudiantes doctorales, post-doctorales e investigadores del Instituto*. En una primera etapa, LIBReciencia se ha enfocado en la generación de una serie de propuestas destinadas a que profesores y alumnos de la Escuela Secundaria cuenten con herramientas para experimentar de forma directa el proceso según el cual se elabora el saber científico, y puedan acercarse a la manera en que los científicos desarrollan su actividad habitual. Con esta finalidad, se diseñaron dispositivos didácticos compuestos por tareas experimentales asequibles, y material orientativo escrito y audiovisual. A lo largo de 2014, estos desarrollos fueron transferidos mediante el dictado de Talleres teórico-prácticos a los que asistieron numerosos docentes de escuelas de nivel secundario de la ciudad de Rosario (Santa Fe, Argentina). A posteriori, se efectuaron tareas de soporte para la implementación de las tareas en el Aula. En el transcurso de los Talleres se intercambiaron detalles de implementación práctica, se brindaron pautas orientativas para el trabajo, se discutieron potenciales problemas, alternativas y sugerencias que retroalimentaron la propuesta de LIBReciencia. De los diseños didácticos elaborados, tres actividades utilizan a la Microbiología como recurso didáctico debido a su factibilidad de implementación y su cercanía con fenómenos cotidianos: “Bacterias, están ahí?”, “Bichos en la cocina” y “Al pan-pan…y a las levaduras….?”. En esta presentación se discutirán características, modalidades de ejecución, la continuidad en la tarea y la demanda actual en el ámbito educativo de estas formas de vinculación de la actividad científica con la sociedad.

Código de Resumen: EM-002

Sección: Educación en microbiología

Modalidad: Poster

TEACHING MICROBIOLOGY IN TECHNICAL CARRERS IN CHEMISTRY FOR A PROPER PROFESSIONAL PERFORMANCE IN THE FUTURE

D.L. Vullo[1].

[1] *Universidad Nacional de General Sarmiento, Los Polvorines, Buenos Aires.*

dvullo@ungs.edu.ar

La Tecnicatura Superior en Química (TSQ) es una carrera de pregrado implementada en la UNGS desde el año 2012. Se basa en la formación de personal técnico universitario para cubrir el área de vacancia sobre todo en empresas pertenecientes a parques industriales de la zona de influencia de la universidad. El plan de estudios está programado para 3 años de cursada en donde se centraliza el aprendizaje de habilidades en el laboratorio en las diferentes disciplinas, sustentado por conceptos teóricos dentro de la modalidad de dictado teorico-práctica. Como el área docente es única, todas las materias se desarrollaron con sus respectivos programas analíticos encadenados de manera que se complementan todos los contenidos, haciendo una mirada integradora de los diferentes aspectos de la Química, entre ellos la Química Biológica y la Microbiología. En el caso particular de la Microbiología, ésta es fundamental en la formación de un técnico a la hora de poder ejercer su profesión en sectores de control de calidad o desarrollo de productos. No se dicta en un único curso sino que los diferentes temas se tratan en distintos cursos acorde a la temática abordada y el grado de dificultad que posean. Por ejemplo: Química de la Vida cubre temas de Microbiología General y experiencias de laboratorio de ese nivel básico; Química Ambiental incluye aspectos de Ecología Microbiana y Microbiología Ambiental; Química Analítica II trata algunas técnicas de diagnóstico microbiológico y Laboratorio III - que es una materia netamente experimental y una de las últimas de la carrera- contiene el desarrollo de análisis microbiológicos que se utilizan normalmente en la industria. Lo importante de esta estructura de enseñanza es no compartimentalizar el conocimiento que se transmite, sino tener una integración de todos los aspectos que se deben tener en cuenta en el trabajo profesional, reflejando situaciones reales a las cuales se podrán enfrentar los futuros técnicos.

Código de Resumen: EM-003

Sección: Educación en microbiología

Modalidad: Poster

ENSEÑANDO MICROBIOLOGÍA EN EL NIVEL MEDIO

E.B. Reinoso[1], M.V. Moliva[1], M.E. Gualtieri[2], E.E. Gualtieri[3].

[1] Universidad Nacional de Río Cuarto. [2] Instituto Técnico Carnerillo. [3] Instituto San Francisco de Asís.

mmoliva@exa.unrc.edu.ar

La enseñanza de la microbiología se ve enfrentada en ocasiones a las dificultades para explicar fenómenos biológicos en organismos que son invisibles a los ojos de los estudiantes. A esto se suman los conceptos necesarios para explicar las distintas temáticas, de difícil comprensión por parte de los alumnos. Las estrategias para la enseñanza de la microbiología constituyen una propuesta didáctica de conceptos que se requieren para la comprensión de cada uno de los contenidos. Mediante un proyecto de Divulgación Científico – Tecnológica CONICET 2012 los científicos fuimos a la escuela para mostrar el mundo microbiano a través de distintas actividades dirigidas a alumnos de cuarto curso de educación media. Durante la primera clase se propuso leer y comprender textos relacionados a la microbiología, reconociendo el papel de los microorganismos en la vida cotidiana. Posteriormente, en la segunda clase, se realizó un trabajo práctico sobre la importancia del lavado de manos para contribuir en los hábitos de higiene y prevención de enfermedades. Los alumnos deslizaron las yemas de sus dedos en placas con medios de cultivo, previo y posterior al lavado de manos. Luego de la incubación de las placas a temperatura ambiente (25°C) durante 2-3 días (tercera clase) los alumnos pudieron observar la presencia de microbiota viable obtenida de sus propias manos, mediante la visualización macroscópica de colonias de bacterias como así también de hongos. Para identificar las diferentes morfologías microbianas al microscopio, se realizó una tinción de Gram, y se explicaron las características de las especies bacterianas y fúngicas más sobresalientes. Al finalizar la actividad se hizo hincapié en preguntas de discusión de los resultados, destacando que un simple lavado de manos con agua y jabón reduce el número de microorganismos presentes, como así también su repercusión en la salud y la sanidad pública. La discusión más relevante con los alumnos fue la comprobación de que nuestro propio cuerpo es un verdadero cultivo de vida microbiana, compuesto por microorganismos que nos ayudan y otros que pueden resultarnos perjudiciales. El tiempo estimado para la realización de la actividad es de 3 clases de 60 min cada una. Al final de las actividades los alumnos realizaron afiches con los resultados obtenidos, los cuales fueron expuestos en la escuela para conocimiento de otros alumnos. Con el desarrollo de este tipo de actividades se espera motivar a los alumnos en el estudio de los microorganismos de manera agradable, promover la ciencia en las escuelas de nivel medio de manera de despertar vocaciones científicas y contribuir de algún modo a la "alfabetización científica". Finalmente, un mayor conocimiento del mundo microbiológico contribuye indirectamente a la modificación de hábitos en materia de higiene y salud.

Código de Resumen: EM-004

Sección: Educación en microbiología

Modalidad: Poster

FACULTAD DE CIENCIAS AGRARIAS DE LA UNIVERSIDAD NACIONAL DE MAR DEL PLATA: RETHINKING TEACHING STRATEGIES

M.A. Pereyra[1].

[1] Facultad de Ciencias Agrarias. Universidad Nacional de Mar del Plata .

pereyra.alejandra@gmail.com

En los distintos talleres que se han organizado para los docentes en el ámbito de la Facultad de Ciencias Agrarias (FCA) UNMdP, hemos consensuado que los estudiantes aprenden cada año con mayor dificultad. Fueron muchas las causas que se plantearon, la mayoría de ellas centradas en los estudiantes: falta de formación, desinterés, problemas económicos, exceso de tecnología, falta de madurez. Ante esta situación, el interrogante es, qué herramientas utilizaremos los docentes para revertirla. Queda claro que el estudiante actual es diferente, y que el docente debe repensar las estrategias de enseñanza para mejorar la comunicación. Debe tender hacia una actitud proactiva, estar dispuesto a acompañar en el aprendizaje implementando buenos diseños pedagógicos y herramientas actualizadas para su desarrollo, y sugerir caminos utilizando las nuevas tecnologías de comunicación. Pero este cambio de mentalidad debe involucrar a toda la comunidad universitaria, teniendo en cuenta que el aumento en el interés y la motivación de los estudiantes redundarán en un mayor ingreso a las carreras y en su retención a lo largo de las mismas. Desde CONEAU se envían directivas para aumentar las horas-práctica en los planes de estudio. Se entiende como horas-práctica aquéllas en las que el estudiante se enfrenta a situaciones similares a la de su futura práctica profesional para resolver problemáticas que involucren los conocimientos adquiridos en el nivel de estudios alcanzado. En este marco, la práctica profesional final es excluyente y obligatoria. Para los niveles básicos, los laboratorios de la FCA pueden ser utilizados para simular situaciones prácticas apropiadas. También se cuenta con laboratorios de investigación de alta calidad, en los que los estudiantes con inclinación a la investigación, pueden realizar una tesis de graduación. Pero el mayor

inconveniente encontrado es la concreción y mantenimiento del contacto con las empresas o productores del medio, en cuyas instalaciones se realizarán las prácticas finales de las carreras. Se deben repensar las estrategias para estas prácticas, proveyendo a los estudiantes de un instructivo claro con las tareas concretas a realizar (preproyecto de trabajo), con el plazo a cumplir, y con las características del informe final a redactar. Las Empresas o productores también deben conocer la finalidad del trabajo del estudiante, para evitar falsas expectativas de todos los participantes. Afianzar el rol del docente como guía para la formación de profesionales capacitados para actuar en el medio es una preocupación actual en la FCA, y se están repensando las estrategias para cubrir las falencias detectadas.

INDICE DE AUTORES

A

B

Carranza, C.S.	BB-010, BB-002, BB-008, BB-009
Carrau, A.	IN-009, IN-008
Carriño, J.	MM-042
Carrizo, G.P.	BB-015
Carrizo, S.L.	BF-012
Casabuono, A.C.	MM-054
Casalongué, C.A.	IN-015
Casco, D.S.	BD-007
Castellano, P.	BF-003
Castellanos de Figueroa, L.	BB-015, BB-019
Castez, F.	MM-053
Castro, M.F.	BB-018
Catalano, P.	BB-024
Cattelan, N.	MM-053, MM-018
Cazzola, M.L.	MM-052
Ceccarelli, E.	MM-013
Centrón, D.	MM-045, MM-046, MS-014, MM-047, MM-016
Cera, G.	MM-012
Cerezo, J.M.	BF-038
Cervantes, L.	MS-010
Chalón, M.	BF-034
Chamosa, L.	MM-045, MM-047
Checa, S.K.	MM-038
Chiotta, M.L.	MS-018, MS-012, MS-011
Chulze, S.	MS-011, MS-018
Clementi, R.	FM-020
Collavino, M.	MS-009
Colque, C.A.	MM-024
Combina, M.	BF-018
Contreras, M.	BD-009, BD-001
Coronel, C.D.	BF-028
Corso, A.	BD-008
Cortes, P.R.	MM-044
Costa, C.	BB-024
Couto, A.S.	MM-054
Crespo Andrada, K.	FM-019
Curatti, L.	BF-028, MM-040, IN-011
Curia, V.	BF-030

D

Manzoni, C.	BF-009
Marchiaro, P.	MM-012
Margara, L.	MM-006
María, P.J.	BF-013
Marioni, J.	MM-048
Marti, M.A.	MM-009
Martin, M.P.	BB-007
Martina, P.	MM-052
Martina, P.F.	BD-007, FM-013
Martínez Beguerí, R.	BB-013, BF-018
Martínez, F.L.	MS-008
Martínez, M.A.	BF-025, BB-018, BF-027, BF-029
Martini, M.C.	MM-007, MS-014, MM-039
Martos, G.I.	BF-014
Mateyca, C.	FM-006
Maturano, Y.	BF-018
Mediavilla, M.G.	MM-013
Medici, E.	BF-009
Medina, E.	MS-016, MS-017
Medina, R.	FM-002, BB-001
Melchiorre, M.	IN-016
Melian, C.	BF-003
Méndez, B.S.	MM-022
Mendieta, J.R.	IN-015
Mengucci, F.	MM-043, MM-042
Mentel, M.	BF-008
Merletti, G.	BF-034
Merlo, C.	MS-007
Mestre, M.	BF-018
Mezzina, M.P.	MM-002, MM-026
Minahk, C.	BF-034
Minnaard, J.	IN-010
Mirettia, M.	FM-020
Miyazaki, S.S.	MS-020
Mlewski, C.	BD-006
Molin, S.	MM-001
Moliva, M.V.	EM-003, MM-031
Monetta, P.	MS-017

O

Ocampo, A.	BB-007
Olsina, R.	BB-018
Ongena, M.	MM-005
Ordóñez, M.V.	FM-021
Ordoñez, O.F.	MM-036, BD-002
Ordoñez, O.R.	BB-016
Orellano, E.G.	IN-007, IN-013, IN-008, IN-009, IN-002, MM-054
Orihuel, A.	FM-004, FM-007
Ortega, M.G.	FM-017, MM-049
Ortiz Marquez, J.C.	MM-040
Ottado, J.	IN-014

P

Pacciaroni, A.	BF-004
Páez, P.L.	FM-016, FM-017, FM-019, BF-031
Paggi, R.	BF-032
Palazzini, J.M.	MS-011
Palomino, M.M.	BF-002, MM-023
Paños, N.H.	BF-007
Paraje, M:G:	FM-019, FM-016, MM-048, MM-49
Parisi, G.	MM-043
Parmeciano Di Noto, G.	MM-029, MM-016
Paroldi, H.	MS-017, MS-016
Pascual, L.	BB-005, BB-004, BB-012, BB-020, BB-006
Pastor, A.	BF-004, MS-004
Pedrozo, L.P.	BB-019
Pegels, E.	BD-007
Pellegrino, M.S.	BB-014
Pera, L.M.	BF-008, BF-022, BF-040
Peralta, M.	MM-049
Pereira, P.I.	MM-009
Pereyra Duarte, C.	MM-037
Pereyra, M.A.	EM-004
Perez Chaia, A.	FM-015, FM-014
Perez, M.	BB-023
Perez, P.F.	IN-010
Pérez, S.S.	MM-052

Saguir, F.M.	BF-035, BF-039, FM-012
Sajur, S.A.	BF-039
Salas, M.E.	MS-013, MM-007
Salazar, M.J.	BB-026
Salerno, G.L.	BD-004, BF-028
Salloum, M.S.	MS-007
Salto, I.P.	MS-010
Salusso, A.	MM-017
Salvador, R.S.	BF-017, BF-019
Salvatierra, H.N.	BF-040
Sánchez Rivas, C.	MM-023
Sanchez Rizza, L.	BF-028
Sánchez, L.A.	BF-020, BF-021
Sanz Smachetti, M.E.	BF-028
Sarti, G.C.	MS-020
Saso, L.	MM-033
Savoy de Giori, G.	BF-005, BF-006
Scott, C.Y.	FM-009
Senese, A.	BF-007
Sgro, G.	IN-014
Smania, A.M.	BB-003, MM-003, MM-020, MM-024
Sobrero, P.	MM-005
Sola, C.	BD-008
Solar Venero, E.C.	MM-001
Solchaga, J.I.	FM-021
Soncini, F.C.	MM-038
Sordelli, D.O.	MM-008, MM-018, MM-019, MM-033
Soria, M.	BD-002, BD-005
Sosa Marmol, S.M.	FM-012
Sosa, E.J.	MM-004, MM-009
Sosa, G.	MM-013
Sosa, V.	BF-004
Sova, M.	MM-033
Strasser de Saad, A.M	FM-011
Suligoy, M.	MM-008, MM-018, MM-019, MM-033
Supanitsky, A.	MM-050

T

Talia, P.T.	BF-019, BF-017, MM-011
Tano, J.	IN-009
Taranto, M.P.	BF-036, BF-037
Tarsitano, J.	BF-002, MM-023
Tempesti, T.	FM-020
Terada, C.	MS-006
Tobares, R.A.	MM-003, BB-003, MM-024
Toldrá, F.	BF-003
Tondo, M.L.	IN-002, IN-013
Tonelli, M.L.	IN-006
Tonón, C.V.	IN-015
Toro, M.E.	BB-013, BB-015, BB-019, MS-016, MS-017, BF-018
Torres Soporsky, M.A.	BF-035, BF-039
Torres Tejerizo, G.A.	MS-010, MM-007
Travaini, M.L.	MM-013
Tribelli, P.M.	MM-001
Turjanski, A.G.	MM-009, MM-004

V

Valdez, E.	BD-007
Valetti, L.	MS-019
Vallejo, C.V.	FM-011
Valverde, C.	MM-041, MM-005, BB-021
Varela, P.	MS-004, BF-004
Varni, V.	MM-015
Vázquez, C.	MS-007
Vázquez, A.	BF-010, BF-009
Vazquez, F.	BB-013, BB-015, MS-016, BB-019, MS-017, BF-018
Vázquez, N.	FM-001
Vega Avila, A.	MS-016, MS-017
Vela, M.E.	MM-053
Vélez, P.S.	BD-003, BB-017
Ventosa, A.	BD-009
Venturini, M.	BB-023
Vergara, M.	MM-028
Viale, A.M.	MM-035, MM-012, MM-010
Viarengo, G.	MM-013