Diseño de Tapa: Adela Luján y Andrea Smania

SAMIGE

Sociedad Argentina de Microbiología General

VI Congreso Argentino de Microbiología General

21 al 23 de Octubre del 2009
Villa Carlos Paz, Córdoba, Argentina

Comisión Organizadora Local

Teresita Lisa, Andrea Smania, Carlos Argaraña, Adriana Fabra

Colaboradores Locales

Paola Beassoni, Marina Forrellad, Sofía Feliziani, Agustina Llanos, Adela Luján, Natalia Morero, Mariela Monti, Alejandro Moyano, Diego Sánchez

Comisión Asesora SAMIGE 2009

Marcela Ferrero, Nancy López, Beatriz Méndez, Daniela Russo , Claudia Studdert, Claudio Valverde, Diana Vullo, Ángeles Zorreguieta

Comisión SAMIGE

Mario Aguilar, Héctor Álvarez, Néstor Cortez, Graciela De Antoni, Marcela Ferrero, Augusto García,Antonio Lagares, Diego de Mendoza, Nancy López, Beatriz Méndez, Graciela Salerno, Graciela Savoy, Liliana Semorile, Faustino Siñeriz, Claudio Valverde, Adrián Vojnov, Diana Vullo, Osvaldo Yantorno, Ángeles Zorreguieta

Comité de Honor

Marcelo Dankert, Gabriela Favelukes, Horacio Pontis

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

Las siguientes Instituciones han financiado y auspiciado la organización de SAMIGE 2009:

Agencia Nacional de Promoción Científica y Tecnológica

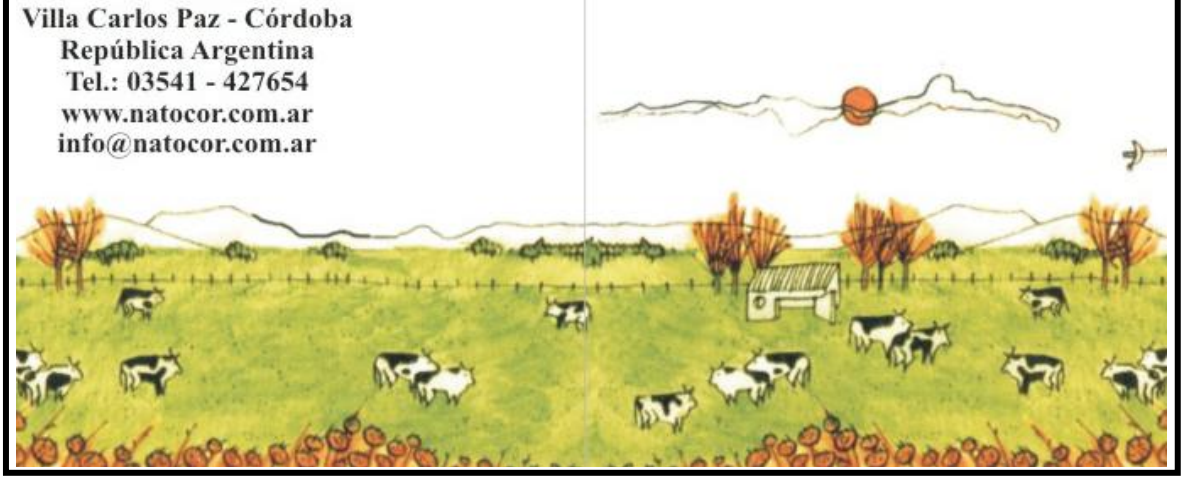
Consejo Nacional de Investigaciones Científicas y Técnicas

Ministerio de Ciencia y Tecnología de la Provincia de Córdoba

Facultad de Ciencias Exactas, Fisicoquímicas y Naturales de la Universidad Nacional de Río Cuarto

Facultad de Ciencias Químicas de la Universidad Nacional de Córdoba

Las siguientes Empresas han auspiciado la organización de VI SAMIGE 2009:



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SAMIGE 2009. CONGRESS OVERVIEW

II Argentine Workshop of *Pseudomonas* and *Burkholderia*	**VI Annual Meeting of SAMIGE**	
Wednesday, October 21st	Thursday, October 22nd	Friday, October 23rd
8:00-9:00 Registration	9:00-11:00 **Oral Communications**	9:00-11:00 **Oral Communications**
9:00-11:00 **Symposium I**	11:00-11:30 Coffee break	11:00-11:30 Coffee break
11:00-11:30 Coffee break	11:30-12:30 **Plenary Lecture** Dr Eleonora García-Véscovi (Argentina)	11:30-12:30 **Plenary Lecture** Dr Mario Aguilar (Argentina)
11:30-12:30 **Plenary Lecture** Dr. Søren Molin (Denmark)		
13:00-15:00 **Lunch and Posters**	13:00-14:30 Lunch	13:00-14:30 Lunch
15:30-17:30 **Symposium II**	15:00-17:00 **Oral Communications**	15:00-17:00 **Oral Communications**
VI Annual Meeting of SAMIGE		
17:30-19:00 Registration 19:15 **Opening Ceremony** Dr Ángeles Zorreguieta	17:30-19:30 **Posters and Coffee** BD (P01-P06) BB (P01-P13) PM (P01-P07) MM (P01-P13) EM (P01-P10) IN (P01-P06) BF (P01-P11)	17:30-19:30 **Posters and Coffee** BD (P07-P12) BB (P14-P26) PM (P08-P14) MM (P14-P26) EM (P11-P19) IN (P07-P12) BF (P12-P23)
19:30-20:30 **Opening Lecture** Dr Elizaveta Bonch-Osmolovskaya (Russia)	19:30-20:30 **Plenary Lecture** Dr Enrique Morett (México)	19:30-20:30 **Closing Lecture** Dr Søren Molin (Denmark)
	20:30 SAMIGE Assembly	20:30 **Closing Ceremony**
	22:00 Cocktail	

PROGRAM

WEDNESDAY, October 21st 2009

17:30 – 19:00 **Registration**

19:15 **OPENING CEREMONY**

Dr Ángeles Zorreguieta
Fundación Instituto Leloir, Buenos Aires, Argentina
"Homage to Dr Rodolfo Ugalde"

19:30 – 20:30 **OPENING LECTURE**

Dr Elizaveta Bonch-Osmolovskaya
Laboratory of Hyperthermophilic Microbial Communities. Russian Academy of Science, Russia
"Diversity of thermophilic prokaryotes, from genomes to new thermostable enzymes"

Chairperson: *Dr. Faustino Siñeriz, PROIMI-CONICET, Tucumán*

THURSDAY, October 22nd 2009

9:00-11:00 **ORAL COMMUNICATIONS**

Chairpersons: *Dra. Marcela Ferrero, PROIMI-CONICET, Tucumán – Dr. Osvaldo M. Yantorno, CINDEFI-CONICET. Facultad de Ciencias Exactas. UNLP, La Plata..*

-Environmental Microbiology-

9:00-9:15, MA-01

"Metagenomic analysis of nitrogen fixing bacterial populations from soils with different agricultural management from the Humid Pampa region"

Verónica M. Bergottini[1], Mónica Collavino[1], Daniel H. Grasso[2], O. Mario Aguilar[1]
[1]Instituto de Biología Molecular y Biotecnología, UNLP, CCT-La Plata CONICET. [2] Inta Castelar.

9:15-9:30, MA-02

"Assessment of microbial community function and structure in soil microcosms exposed to glyphosate"

María C. Zabaloy[1,2] , Jay L. Garland[3], Marisa A. Gómez[1,2]
[1] Departamento de Agronomía, Universidad Nacional del Sur [2] CERZOS-CONICET [3] Dynamac Corp., Kennedy Space Center, Nasa

-Bioremediation and Biocontrol-

9:30-9:45, BB-01

"Dynamics of the production of cyclic lipopeptides by *Bacillus spp.* and its foliar application on soybean"

Florencia Alvarez[1], Pablo Rodríguez[2,3] ,Ubaldo Estanga[2], Lucrecia Couretot[4], Jorge Cozzi[2], Gladys Mori[1], Edgardo Jofré[1]
[1]Dpto. de Ciencias Naturales, Universidad Nacional de Río Cuarto. [2]Dpto. de Desarrollo, NITRAP SRL. [3]Cátedra de Terapéutica Vegetal, Dpto. de Tecnología, Universidad Nacional de Luján. [4]Estación Experimental Agropecuaria, INTA Pergamino.

9:45-10:00, BB-02

"Isolation, selection and characterization of bacterial strains for high organic matter content wastewater treatment"

Florencia A. Ficarra[1,2] , Martín Espariz[1,2] , Sebastián H. Lagorio[2], Christian Magni[1,2]

[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET) [2]Facultad de Cs. Bioquímicas y Farmacéuticas de Rosario (UNR)

10:00-10:15, BB-03

"Selection of epiphytic yeasts for the biocontrol of *Penicillium expansum* and *Botrytis cinerea* in postharvest pears in Patagonia"

Cecilia M. Lutz[1], Christian A. Lopes[1], Cristina Sosa[2], M P. Sangorrín[1]

[1]Laboratorio de Microbiología y Biotecnología.IDEPA. CONICET-U.N.Comahue [2]Laboratorio de Fitopatología. IDEPA. CONICET-U.N.Comahue

10:15-10:30, BB-04

"PAH-degrading microorganisms capable to withstand the environmental stress conditions of Patagonia Central"

Laura Madueño[1], Héctor M. Alvarez[2], Irma S. Morelli[1]

[1]CINDEFI (UNLP, CCT-La Plata, CONICET), La Plata, Argentina. [2]CRIDECIT (UNPSJB) and CONICET, Comodoro Rivadavia, Chubut, Argentina

10:30-10:45, BB-05

"Antagonism of *Clostridium difficile* by bifidobacterium bifidum cidca 5310: *in vivo* and *in vitro* studies"

Fernando M. Trejo[1], Pablo F. Pérez[1,2] , Graciela L. De Antoni[1,3]

[1] CIDCA-UNLP-CONICET CCT La Plata, [2] Cátedra de Microbiología, FCE UNLP [3] Comisión de Investigaciones Científicas (CIC-PBA).

10:45-11:00, BB-06

"Analysis of the inhibitory capacity of *Lactobacillus plantarum* on biofilm and quorum sensing signals of *Pseudomonas aeruginosa*"

Alberto N. Ramos[1,2] , Diego Noseda[2], Alejandra Bosch[2], Osvaldo M. Yantorno[2], Juan C. Valdez[1]

[1] Cat. de Inmunología, Inst. de Microbiol, Fac. de Bioq., Qca., Fcia. y Biotec. Univ. Nac. de Tucumán [2] CINDEFI. Conicet. Facultad de Ciencias Exactas. Universidad Nacional de La Plata

11:00 – 11:30 **Coffee break**

11:30 – 12:30 **PLENARY LECTURE**

Dra. Eleonora García Véscovi

IBR-CONICET, Universidad Nacional de Rosario, Argentina

"Regulatory mechanisms and pathogenesis in Serratia marcescens"

Chairperson: *Dr. Claudio Valverde, Departamento de Ciencia y Tecnología, UNQ, Buenos Aires*

13:00 – 15:00 **Lunch**

15:00-17:00 **ORAL COMMUNICATIONS**

Chairpersons: *Dra. Daniela Russo, Fundación Instituto Leloir, Buenos Aires – Dra. Diana Vullo, Instituto de Ciencias Universidad Nacional de General Sarmiento, Buenos Aires.*

-Biodiversity-

15:00-15:15, BD-01

"Bacterial Community Structure in Coastal Marine Sediments, Assessed by Pyrosequencing of 16S rRNA genes"

Magalí S. Marcos[1], Mariana Lozada[1], Mónica N. Gil[1], Walter D. Di Marzio[2], Hebe M. Dionisi[1]
[1]Centro Nacional Patagónico (CENPAT-CONICET), Puerto Madryn, Chubut [2]Universidad Nacional de Luján, Luján, Buenos Aires

-Physiology and metabolism of microorganisms-

15:15-15:30, FM-01

"Phosphatidylcholine synthase is involved in the synthesis of phosphatidylcholine in *Pseudomonas putida* A ATCC 12633 grown with tetradecyltrimethylammonium and Al3+"

Paola S. Boeris[1,2] , Andrés S. Liffourrena[1], Mario A. Salvano[1], Isabel M. López-Lara[2], Gloria I. Lucchesi[1]
[1]Dpto. Biología Molecular. FCEFQyN. UNRC. Río Cuarto. Córdoba [2]Centro de Ciencias Genómicas, UNAM, Mexico

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15:30-15:45, FM-02

"Isolation of a photosynthetic bacterium from extreme environments in high-altitude Andean wetlands"

Cecilia B. Di Capua[1,2] , María E. Farias[3], Néstor R. Cortez[1,2]
[1] Instituto de Biología Molecular y Celular de Rosario (IBR - CONICET) [2] Fac. de Cs. Bioq. y Farm. - Universidad Nacional de Rosario (UNR) [3] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI- CONICET)

15:45-16:00, FM-03

"Characterization of sucrose metabolism proteins and their encoding genes in a bloom-forming cyanobacteria"

Maria A. Kolman[1], Laura E. Giarrocco[1], Graciela L. Salerno[1]
[1]CEBB-MdP, CIB, FIBA. Vieytes 3103, CC 1348, 7600 Mar del Plata, Argentina.

16:00-16:15, FM-04

"Role of alkaline/neutral invertases in *Nostoc* sp. PCC 7120"

Carolina N. Nishi[1], Laura E. Giarrocco[1], Walter A. Vargas[2], Graciela L. Salerno[1]
[1]Centro de Estudios de Biodiversidad y Biotecnología de Mar del Plata (CEBB-MdP), CIB, FIBA, MdP [2]The J. Craig Venter Institute (JCVI), Rockville, U.S.A

16:15-16:30, FM-05

"β-carotene is involved in NO production in *Methylobacterium extorquens* AM1"

Martín E. Tagliotti[1], Celeste Molina Favero[1], Cecilia M. Creus[1], Lorenzo Lamattina[2]
[1]Unidad Integrada Balcarce FCA, UNMdP - EEA INTA [2]IIB, FCEyN, UNMdP

-Molecular Microbiology-

16:30-16:45, MM-02

"Characterization of the PhoP/PhoQ system in *S. marcescens* and its role in pathogenesis"

Julieta Barchiesi[1,2] , Maria E. Castelli[1,2] , Eleonora García Véscovi[1,2]
[1]Instituto de Biología Molecular y Celular de Rosario. CONICET. [2]Facultad de Ciencias Bioquimicas y Farmaceuticas .UNR.

17:30-19:30 **POSTERS AND COFFEE**

19:30-20:30 **PLENARY LECTURE**

Dr Enrique Morett
Instituto de Biotecnología de la Universidad Nacional Autónoma de México, México
"Next generation sequencing methodologies: applications in comparative genomics and transcriptomics"

Chairperson: *Dra.Angela T. Lisa, Dpto. Biología Molecular, FCEFQyN, UNRC, Río Cuarto.*

20:30 **SAMIGE ASSEMBLY**

22:00 **Cocktail**

FRIDAY, October 23rd 2009

9:00- 11:00 **ORAL COMMUNICATIONS**

Chairpersons: *Dr. Nancy López, Dpto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, UB, Buenos Aires – Dr. José L. Echenique, CIBICI-CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas,UNC, Córdoba.*

- Molecular Microbiology-

9:00-9:15, MM-03
"The regulation of plcH gene of *Pseudomonas aeruginosa* depends on environmental condition"
Marina A. Forrellad[1,2] , Maria J. Massimelli[1], Fernando Govantes-Romero[2], Angela T. Lisa[1]
[1]Dpto. Biologia Molecular, FCEFQyN, UNRC. [2]CABD, Universidad Pablo Olavide, Sevilla, España.

9:15-9:30, MM-04
"The ability to form biofilms is not always correlated with the production of quorum-sensing signals in clinical isolates of *Acinetobacter baumannii*"
Laura E. Friedman[1], Catriel E. Gatto[1], Carlos Vay[2], Mirta A. Franco[1]
[1] Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, UBA [2] Laboratorio de Bacteriología, Dpto de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, UBA

9:30-9:45, MM-05
"Rapid identification and differentiation of *Burkholderia cepacia* Complex isolates by PCR-RFLP patterns of *gyrB* and *recA* genes"
Pablo Martina[1], Gonzalo Sequeira[1], Alejandra Bosch[1], Marisa Bettiol[2], Carlos Vay[3], Laura Galanternik[3], Claudia Hernández[3], José Degrossi[4], Patricia Montanaro[5], Osvaldo Yantorno[1]
[1]Cindefi-Conicet, Facultad De Ciencias Exactas, Unlp, 50 E/115 Y 116, La Plata 1900, Argentina. [2]Hospital De Niños, La Plata. [3]Hospital De Clinicas, Hospital Gutierrez, Hospital Garraham. Bs.As. [4]Facultad De Farmacia Y Bioquimica, Uba. [5] Hospital Santisima Trinidad, Cba.

9:45-10:00, MM-06
"Simple sequence repeats and mucoid conversion: biased *mucA* mutagenesis in Mismatch Repair-deficient *Pseudomonas aeruginosa*"
Alejandro J. Moyano[1,2] , Andrea M. Smania[1,2]
[1]Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), CONICET. [2] Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

10:00-10:15, MM-07

"Novel insights about class 2 integrons from experimental and genomic epidemiology"

María Soledad Ramírez[1], *Silvia Piñeiro*[2], *Argentinian Integron Group*[1], *Daniela Centrón*[1]

[1]*Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, UBA, Argentina.* [2]*School Of Medicine, University Of Maryland Allied Health Building, Baltimore, USA.*

10:15-10:30, MM-08

"Insight into the secretion and maturation pathways of the subtilisin-like extracellular protease from the haloalkaliphilic archaeon *Natrialba magadii*"

Diego M. Ruiz[1], *María I. Giménez*[1], *Rosana E. De Castro*[1]

[1]*Instituto de Investigaciones Biológicas, CONICET-UNMDP*

10:30-10:45, MM-09

"St5-iva genotype: as a major cause of invasive infections by ca-mrsa at a pediatric hospital in Córdoba, Argentina"

Claudia Sola[1], *Analía Garnero*[2], *Catalina Culasso*[3], *Lydia Carvajal*[3], *Patricia Montanaro*[3], *Ana L. Egea*[1], *Eduardo Glatstein*[2], *José L. Bocco*[1]

[1]*Departamento de Bioq.Clínica, Fac. Cs. Químicas, UNC-CIBICI-CONICET* [2] *Servicio de Infectología Hospital de Niños de La Santísima Trinidad* [3] *Servicio de Microbiología, Hospital de Niños De La Santísima Trinidad*

10:45-11:00**,** MM-10

Identification of a hutc-mediated regulatory network: the histidine utilization repressor recognizes more than one promoter

Gastón M. Arocena[1], *Rodrigo Sieira*[1]

[1]*Instituto de Investigaciones Biotecnológicas - UNSAM*

11:00-11:30 **Coffee break**

11:30-12:30 **PLENARY LECTURE**

Dr. O. Mario Aguilar

Instituto de Bioquímica y Biología Molecular de la Universidad Nacional de La Plata, Argentina

"*Phaseolus vulgaris* nodulation affinity by different *Rhizobium etli* strains"

Chairperson: *Dra. Adriana Fabra, Departamento de Ciencias Naturales, FCEFQYN, UNRC, Río Cuarto*

13:00 – 15:00 **Lunch**

15:00-17:00 **ORAL COMMUNICATIONS**

Chairpersons: *Paola Beassoni, Dpto. Biología Molecular, FCEFQyN, UNRC, Río Cuarto - Claudia Studdert, IIB-FCEyN-CONICET/UNMDP, Mar del Plata.*

-Prokaryote-Eukaryote Interactions-

15:00 – 15:15, IN-01

"Rhizobial Nod factors are required for nodule primordia development in the crack entry infection process in peanut"

Fernando Ibañez[1], Herminda Reinoso[1], Adriana Fabra[1]
[1] Departamento de Ciencias Naturales, FCEFQYN, Universidad Nacional de Río Cuarto

15:15-15:30, IN-02

"Adhesion factors and signaling pathways involved in the interaction between *Bacillus cereus* and cultured human enterocytes"

Jessica Minnaard[1,2] , Ivanna S. Rolny[1,2] , Pablo F. Pérez[1,2]
[1]CIDCA (CONICET. La Plata) [2]Cátedra de Microbiología. Facultad de Ciencias Exactas, UNLP.

15:30 – 15:45, IN-03

"Role of exopolysaccharide synthesis and biofilm formation during the establishment of the *Sinorhizobium meliloti*-alfalfa symbiosis"

Luciana V. Rinaudi[1], Fernando Sorroche[1], Ángeles Zorreguieta[2], Walter Giordano[1]
[1]Dpto. Biología Molecular, Universidad Nacional de Río Cuarto. Río Cuarto, Córdoba. [2]Fundación Instituto Leloir, CONICET, FCEYN, Universidad de Buenos Aires. Buenos Aires.

15:45 – 16:00, IN-04

"Circadian variation in hydrogen cyanide-mediated paralysis of *Caenorhabditis elegans* by *Pseudomonas fluorescens* strain CHA0"

Andrés Romanowski[1], María Laura Migliori[1], Claudio Valverde[2], Diego Golombek[1]
[1] Laboratorio de Cronobiología, DCYT, UNQ [2] Programa Interacciones Biológicas, DCYT, UNQ

16:00 – 16:15, IN-05

"Role of members of the autotransporter family in adhesion and invasion of *Brucella suis* to host cells"

Veronica Ruiz[1], Diana M. Posadas[1], Fernando A. Martin[1], Angeles Zorreguieta[1]
[1] Fundación Instituto Leloir, IIBBA CONICETt, FCEN UBA

-Biotechnology and Fermentations-

16:15 – 16:30, BF-01

"A rapid-bod biosensor based in lyophilized *Klebsiella pneumoniae*. effects of oxygen, ferricyanide and microbial concentration"

María C. Bonetto[1,2] , Natalia J. Sacco[1,2] , Eduardo Cortón[1,2]
[1] QB, FCEYN, UBA. [2] CONICET

16:30 – 16:45, BF-02

"Mineral phosphate solubilization by *G. diazotrophicus"*

Juan M. Crespo[1], Verónica Guidi[1], Maria L. Molinari[1], Jose L. Boiardi[1], María F. Luna[1,2]
[1] CINDEFI (UNLP; CCT-La Plata, CONICET), Facultad de Ciencias Exactas [2] CIC-PBA

16:45- 17:00, BF-O3

Genetic engineering of nitrogen fixation towards optimization of synthetic microbial communities for next generation biofuels

Juan C. Ortiz Marquez[1,2] , Leonardo Curatti[1,2]
[1] CEBB-MDP-CONICET [2] CIB-FIBA

17:30-19:30 **POSTERS AND COFFEE**

19:30-20:30 **CLOSING LECTURE**

Dr. Prof. Søren Molin
Department of Microbiology Systems, Technical University of Denmark, Denmark.
"Bacterial Biofilms - after a decade of intense global research"

Chairperson: *Dr. Andrea M. Smania, CIQUIBIC-CONICET, Departamento de Quimica Biológica, Facultad de Ciencias Químicas, UNC, Córdoba*

Comunicaciones Orales

Oral Communications

BD-O1

BACTERIAL COMMUNITY STRUCTURE IN COASTAL MARINE SEDIMENTS, ASSESSED BY PYROSEQUENCING OF 16S RRNA GENES

Magalí S. Marcos[1], Mariana Lozada[1], Mónica N. Gil[1], Walter D. Di Marzio[2], Hebe M. Dionisi[1]

[1]Centro Nacional Patagónico (CENPAT-CONICET), Puerto Madryn, Chubut [2]Universidad Nacional de Luján, Luján, Buenos Aires (magali@cenpat.edu.ar)

Marine bacteria drive globally important biogeochemical cycles and have a major role determining the environmental quality of marine ecosystems. Marine sediments can act as a trap of hydrophobic contaminants, and they may become sufficiently polluted to disrupt these essential biological processes. The aim of this study was to analyze bacterial community structure in coastal sediments of Patagonia in the context of environmental variation. This project is part of the International Census of Marine Microbes initiative. Composite intertidal sediment samples were retrieved at two sites with a different history of hydrocarbon exposure: Fracasso Beach (FB), located in the protected natural area of Valdes Peninsula, and Cordova Cove (CC), near Comodoro Rivadavia and close to oil exploitation activities. The measured physico-chemical parameters include pH, temperature, oxidation-reduction potential, granulometry, organic matter, as well as ammonia and polycyclic aromatic hydrocarbon (PAH) concentrations. Pyrosequencing of the V6 hypervariable region of 16S rRNA genes from Bacteria was performed at the Marine Biological Laboratory in Woods Hole, USA, using a GS20 system (Roche). Rarefaction curves, species richness indexes (ACE and Chao1) and taxonomical assignment of V6 sequences were performed by using the VAMPS interface (Visualization and Analysis of Microbial Population Structures). Both sediment samples differed markedly in their total PAH concentration (FB: not detected, CC: 758 µg kg^{-1} dry sediment), particle sizes, ammonia concentrations and organic matter content. The polluted sediment contained a lower ammonia concentration, suggesting the existence of a nutrient limitation in this environment. Although almost 60,000 bacterial V6 sequences were obtained from both samples, rarefaction curves still show that additional sampling would be needed to estimate total number of OTUs present in each sample. These results are an evidence of the vast diversity of bacterial communities indigenous of coastal sediments. Species richness indexes ACE and Chao1 were 20% higher in CC than in FB, suggesting that microbial diversity in CC is not being affected by its exposure to anthropogenic pollutants. Community composition was similar in both samples at the Phylum level, although differences were found at lower taxonomic resolution. In both samples *Gammaproteobacteria* was the dominant group within the Phylum *Proteobacteria*, followed by *Alphaproteobacteria* or *Deltaproteobacteria* in the non-polluted and impacted sample, respectively. In this study, we used next-generation sequencing to deeply characterize the bacterial community structure in coastal marine sediments, with an extensive analysis of metadata. The characterization of microbial community structure is the starting point for understanding ecological and environmental processes shaping the microbial communities in the marine environment.

BB-O1

DYNAMICS OF THE PRODUCTION OF CYCLIC LIPOPEPTIDES BY *Bacillus* spp. AND ITS FOLIAR APPLICATION ON SOYBEAN

Florencia Alvarez[1], Pablo Rodríguez[2,3] , Ubaldo Estanga[2], Lucrecia Couretot[4], Jorge Cozzi[2], Gladys Mori[1], Edgardo Jofré[1]

[1]Dpto. de Ciencias Naturales, Universidad Nacional de Río Cuarto. [2]Dpto. de Desarrollo, NITRAP SRL. [3]Cátedra de Terapéutica Vegetal, Dpto. de Tecnología, Universidad Nacional de Luján. [4]Estación Experimental Agropecuaria, INTA Pergamino. (falvarez@exa.unrc.edu.ar)

Plant fungicides based on synthetic chemicals are extensively used in agriculture. In fact, there are now more than 113 active ingredients registered as fungicides worldwide. However, they cause severe and long-term environmental pollution, are highly and acutely toxic, and can even be carcinogenic toward humans and wild animals. Consequently, biological control agents represent an environmentally friendly alternative to chemicals and offer different modes of action for combating pathogens. In this work, strains from native soils belonging to *Bacillus subtilis* group were able to inhibit the growth of several phytopathogenic fungi *in vitro*. Antibiosis played a critical role in the suppression of fungal growth. MALDI-TOF mass spectral analysis revealed the presence of cyclic lipopeptides (CLPs) in the cell-free supernatants of *Bacillus* sp. strains, a group of antibiotic with surface-active properties (biosurfactants) synthesized by non ribosomal peptide synthetases. The mayor lipopeptide isoforms of *Bacillus* sp. $ARP_2$3 and *Bacillus* sp.A7 were surfactins (C13-C16) and fengycins (C14-C18) while the main lipopeptide produced by *Bacillus* sp. A6 was iturin A. These lipopeptides were able to inhibit the germination of sclerotia from *Sclerotinia* spp. and *Sclerotium rolfsii*. Moreover genetic markers associated with CLPs biosynthesis were detected by PCR assays in these strains. In order to determine the dynamics of the antifungal compounds production during the growth of the *Bacillus* sp. strains, the antibiotic activity present in the cell-free supernatants, taken at different time intervals, was assayed. The antifungal compounds were detected by bioassays during exponential growth and showed maximum production at the end of the stationary phase. Based on this observation, stationary cultures of *Bacillus* sp. were used for foliar application on R5.3-stage soybean plants showing typical symptoms of Septoria brown spots (40% of incidence) and Cercospora leaf spot traces. A reduction between 8-12% in symptoms severity and a delayed defoliation were observed after 15 and 20 days post-application, respectively. Moreover, average soybean yield was increased between 101 and 180 kg/ha following *Bacillus* treatments with respect to non-treated plants. These preliminary results suggest that bacterial and/or CLPs foliar application could be a promising strategy against phytopathogens fungi.

BB-O2

ISOLATION, SELECTION AND CHARACTERIZATION OF BACTERIAL STRAINS FOR HIGH ORGANIC MATTER CONTENT WASTEWATER TREATMENT.

Florencia A. Ficarra[1,2] , Martín Espariz[1,2] , Sebastián H. Lagorio[2], Christian Magni[1,2]

[1]Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET) [2]Facultad de Cs. Bioquímicas y Farmacéuticas de Rosario (UNR) (ficarra@ibr.gov.ar)

In Argentina more than 23% of industries are involved in agro-alimentary production. Together with domestic wastes, this activity is the major contributing factor in water pollution with organic matter. For this reason, large investments are required in wastewater treatments to avoid environment deterioration. Bioaugmentation is the use of selected microbial strains isolated from the environment to improve some of the processes implicated in waste treatment, for example, to degrade xenobiotics or to increase BOD removal. The aim of the present work is to isolate and select bacterial strains able to reduce the organic matter content from industrial wastewater. In particular, we look for exoenzyme-producing bacterial strains. Here we report methods for screening, typification and characterization of the isolations. We followed a classical microbiological approach to isolate strains from a wastewater treatment lagoon of a local factory. We performed several strategies of selection including selective and differential media cultures. We studied their growth profiles, colony morphologies, oxygen relationships and biofilm-forming ability in solid and liquid media. We found that twenty-four isolations were spore-forming, twenty-two showed mobility capabilities, and seven were able to aggregate. We tested their fermentative properties as well as sulfate reducing activities. Twelve strains had a fermentative phenotype and two of the isolations were able to reduce sulfate. We selected four bacterial strains for further analysis. For these strains a 1000 bp region of their 16S ribosomal and their *gyrA* genes were amplified by PCR and sequenced. This allows us to establish unambiguously that these strains belong to *Bacillus* genera. In order to distinguish them between Bacilli species we performed RAPDs analysis. This technique allowed us to classify them below species level. With the purpose of finding exoenzymes activities, we analyzed lipase, protease and amylase activities of the cultures. Two of the four strains showed both protease and amylase activities. We complemented the strains characterization with the determination of their nutrient requirements at laboratory scale. Interestingly, our isolations required five times less amount of nitrogen than traditionally recommended for wastewater depuration. This suggested that these strains could be applied to solve low nitrogen operational problems. In conclusion, our results provide insights into isolation and screening methods that contribute to the selection of best candidates for bioaugmentation and improvement of industrial wastewater treatment.

BB-O3

SELECTION OF EPIPHYTIC YEASTS FOR THE BIOCONTROL OF *Penicillium expansum* AND *Botrytis cinerea* IN POSTHARVEST PEARS IN PATAGONIA

Cecilia M. Lutz[1], Christian A. Lopes[1], Cristina Sosa[2], M P. Sangorrín[1]

[1]*Laboratorio de Microbiología y Biotecnología.IDEPA. CONICET-U.N.Comahue* [2]*Laboratorio de Fitopatología. IDEPA. CONICET-U.N.Comahue (sangorrinmarcela@conicet.gov.ar)*

The valleys of Neuquén and Rio Negro, are the main national producer and exporter of apples and pears in the country. The "blue mold" caused by *Penicillium expansum* and the "gray mold" caused by *Botrytis cinerea* are responsible for the major losses of fruit in storage. Synthetic fungicides have long been applied for controlling these moulds; however, its use is restricted because of the rise of new quality standards for food and environmental safety. Biological Control using microorganisms adapted to the post-harvest environment is promising option. With the aim to find potential antagonistic yeasts adapted to the fruits and storage conditions (1-0ºC, 8 months), selective isolation protocols were used. Artificial wounds were caused on pear fruits of cvs Packham's and D'Anjou obtained from organic and transition production process. After 150 days, healthy fruit wounds were extracted and mixed in sterile water. Additionally, whole fruits were used for the obtention of epiphytic yeasts. Fifty microliters of each wash water was co-inoculated with a suspension of 10^3 conidia/mL of *P. expansum* in new healthy fruits wounds. Fruits were then incubated at 4°C for 50 days. Aliquots of the same wash waters were used for yeast isolation in GPY and pear juice agar plates at 0ºC. No differences were observed among the biocontrol percentages obtained with yeast samples from both whole fruit and wounds and from both production processes. Moreover, the same yeast species were detected in most samples: *Aureobasidium pullulans, Crytococcus* sp. and *Cryptococcus laurentii* were the predominant yeasts, while *Rodothorula glutinis,Sporobolomyces roseus,Candida patagonica* and *Pichia capsulatta* were only found in low proportions. One isolate from each wash waters (only from those exhibiting a biocontrol capacity higher than 40%) was selected to be tested individually in biocontrol assays against the more virulent and resistant isolates of both pathogens previously characterized. A total of 34 yeast isolates were selected: 10 from fruit surface and 24 from wounded fruit. Two chemical treatments were also included in the study. In all cases, five fruits with one wound in the equatorial zone were inoculated with 20 ul of a suspension of each isolate 10^6 cells/ml and then 10 ul of 10^3 or 10^4 conidia/ml of the pathogens (minimal infective concentration). Fruits were incubated at 0/-1°C) for 100 days. Fifty-six percent of the total tested isolates showed biocontrol activities of 100% against *P. expansum*; however, only eight isolates belonging to *Cryptococcus sp.,Cr. laurentii,C. patagonica* and *P. capsullatta* were effective against *B. cinerea* (with biocontrol from 45 to 66%). Contrarily, chemical fungicides controlled 100% infection by *B. cinerea* but they were not able to control *P. expansum* infections.

BB-O4

PAH-DEGRADING MICROORGANISMS CAPABLE TO WITHSTAND THE ENVIRONMENTAL STRESS CONDITIONS OF PATAGONIA CENTRAL.

Laura Madueño[1], Héctor M. Alvarez[2], Irma S. Morelli[1]

[1]*CINDEFI (UNLP, CCT-La Plata, CONICET), La Plata, Argentina.* [2]*CRIDECIT (UNPSJB) and CONICET, Comodoro Rivadavia, Chubut, Argentina (lbmh@biol.unlp.edu.ar.)*

Despite its long-term use in bioremediation, bioaugmentation of contaminated sites with microbial cells continues to be a source of controversy within environmental microbiology. Until now the strain selection has been based in a single criterion: degradation ability, with little or no consideration given to other essential features that are required to be functionally active or persistent in target habitats. In our Patagonia several environmental stresses, such as inorganic nutrients, water, pH and temperature, might limit the degradative activity of not adapted bacterial inoculum. The aim of this work was to study the resistance to different stress conditions of four autochthonous isolates (1A, 22A, 22B, and 36), belong to *Sphingomonadaceae* family, capable to degrade polycyclic aromatic hydrocarbons (PAH); and compared their stress response with that a PAH-degrading strain, *Sphingomonas paucimobilis* 20006FA, isolated from La Plata soil and phylogenetically closely related with the Patagonia strains. It was observed that all the strains were capable to grow using phenanthrene as sole carbon and energy source, reaching a degradation of around 90% of phenanthrene supplied after 3 days of incubation. A different behavior was observed in case of fluorene, however none of the strain was capable of growing, the strain 1A and 36 showed a percentage of degradation significantly higher than the another strains. A microscope chemotaxis assay demonstrated that all the strain showed chemotaxis response towards fluorene and phenanthrene. For C-starvation resistance testing, 5 ml of liquid mineral medium was inoculated with $1x10^7$ cfu/ml, in absence of FCE, and incubated at 28°C during 100 days. Periodically number of cfu/ml was determined by counting on R2A medium. Whereas the *S. paucimobilis* 20006FA demonstrated to be the most sensitive strain to C-starvation, showing a reduction in the cfu/ml of two orders of magnitude at the end of the incubation time, the strains 22A and 22B maintained a relatively stable number of cfu/ml during the whole experiment. The strain 1A and 36 showed midway behavior. To measure the survival of the strains under water stress, drops of a cells suspension (DO_{600} 4) were spotted onto Petri plates; the plates were incubated during 14 days at 28 °C and allowed to dry at a relative humidity of 18%.

The survival rate was calculated as $CFU_{after\ drying}/CFU_{before\ drying} \times 100$. Clear differences were found between the strains. The strains 22A and 22B showed a highest survival rate, 72,6±1,29% and 82,2±2,31% respectively, whereas the strains 1A, 36 and *S. paucimobilis* 20006FA exhibited a survival rate below to of 60%.The results suggest the presence of mechanisms of adaptation to the typical environmental local conditions in the strains 22A and 22B, and they might be the most suitable strains for use as bacterial inoculum in PAH-contaminated soils of Central Patagonia.

BB-O5

ANTAGONISM OF *Clostridium difficile* BY *Bifidobacterium bifidum* CIDCA 5310: *IN VIVO* AND *IN VITRO* STUDIES.

Fernando M. Trejo[1], *Pablo F. Pérez*[1,2] , *Graciela L. De Antoni*[1,3]

[1] *CIDCA-UNLP-CONICET CCT La Plata,* [2] *Cátedra de Microbiología, FCE UNLP* [3] *Comisión de Investigaciones Científicas (CIC-PBA). (ftrejo@cidca.org.ar)*

C. difficile is responsible for antibiotic-associated colitis and diarrhea. Two protein toxins, TcdA and TcdB, are related to the virulence of this microorganism. Probiotic bacteria (i.e. bifidobacteria and lactobacilli) constitute an alternative approach to prevent/treat *C. difficile* associated diarrhea (CDAD). However, mechanisms involved in the protective effect remain unknown. The present work aims to gain insight on the underlying mechanisms of the protective effect by using in vitro and in vivo models. *B bifidum* CIDCA 5310 and *C. difficile* 117 were grown in pure or mixed cultures (co-cultures) in BHI at 37 °C for 20 h. Biological activity of the culture supernatants (SN) was evaluated on Vero cells. Cells were incubated with SN from *C. difficile* pure cultures or co-cultures for 16 hours at 37°C. Fraction of detached cells was evaluated by staining remaining cells with crystal violet, further dye extraction and colorimetric determination. Biological activity was defined as the SN concentration that detaches 50 % of cells (D50). D50 is inversely related to the biological activity. Intra and extracellular toxins concentrations were evaluated through immunobloting by using monoclonal antibodies. For in vivo assays, hamsters (4-7 weeks-old) were administered with 108 CFU/ml bifidobacteria or placebo in drinking water starting at day 0. At day 7, a single dose of clindamycin (3 mg/animal) was administered by intragastric gavage. At day 10 all animals were infected intragastrically with 108 CFU of *C. difficile*. Diarrhea, enterocolitis and survival of the animals were assessed. Cecal content was tested for biological activity on Vero cells and samples of cecum were processed for histology. *In vitro*, concentrations of both toxins were reduced around 50 % in co-cultures as compared with pure cultures of strain 117. Intracellular toxin concentration was increased 3 times, compared with pure cultures. There was a significantly ($p=0.05$) higher ratio of survivors in the probiotic-treated group (0/13) as compared with the placebo-treated group (4/13). Ratio of animals showing enterocolitis was also significantly lower in the probiotic group (3/13) than in the placebo group (11/13, $p=0,002$). Histological analysis of cecum showed significant inflammation only in the placebo group and D50 in cecal content was significantly higher in the animals administered with bifidobacteria (2,9 ± 1,2) as compared with those that received only placebo (0,6 ± 0,5). Our findings are compatible with 3 mechanisms: decrease of toxin production/secretion, blockade of the interaction between toxins and receptors or antagonism of signals triggered by toxins. The present work shows for the first time, the correlation between in vitro and in vivo results thus providing a rationale basis for the use of bifidobacteria-based probiotics for the prevention/treatment of intestinal pathologies associated to *C. difficile*

BB-O6

ANALYSIS OF THE INHIBITORY CAPACITY OF *Lactobacillus plantarum* ON BIOFILM AND QUORUM SENSING SIGNALS OF *Pseudomonas aeruginosa*.

Alberto N. Ramos[1,2] , *Diego Noseda*[2], *Alejandra Bosch*[2], *Osvaldo M. Yantorno*[2], *Juan C. Valdez*[1]

[1] *Cat. de Inmunología, Inst. de Microbiol, Fac. de Bioq., Qca., Fcia. y Biotec. Univ. Nac. de Tucumán* [2] *CINDEFI. Conicet. Facultad de Ciencias Exactas. Universidad Nacional de La Plata (anramos@fbqf.unt.edu.ar)*

In previous works we have demonstrated that *Lactobacillus plantarum* (cultures and supernatants) interferes with the pathogenic capacity of *Pseudomonas aeruginosa*, both *in vitro* and *in vivo*. These studies are important because a topical treatment with *L. plantarum* cultures is currently being carried out by our medical team on chronic infected wounds in humans with encouraging results. To deepen the knowledge of *L. plantarum* cultures (SLp) capacity to interfere with *P. aeruginosa*, we analyze the inhibitory behavior of SLp by the following assays: 1) Measure of DL acid lactic concentration, 2) Presence of DNAase, 3) Effect of heat 4) effect of proteases, 4) Effect on *P. aeruginosa* acyl-homoserine lactones (quorum sensing signals) by Thin Layer Chromatography (TLC) , Spectrum Fourier Transform Infrared (FT-IR), Gas Chromatography-Mass Spectrometry (GC-MS) and bioassay using reporter bacteria (*Cromobacterium violaceum* 026, *Cromobacterium violaceum* Vir 07, *Agrobacterium tumefaciens* KYC55). Results: The concentration of LA was 130mm (MIC for *P. aeruginosa* =100 mM). No DNase activity was detected in SLp. The heat removes the inhibitory capacity of SLp. Neutralization decreases the inhibitory capacity of SLp. Proteases inhibit biofilm increasing the inhibitory effect of SLp. The *P. aeruginosa* acyl-homoserine lactones remained unchanged after treatment with SLp. Conclusions: Lactic acid is important in the inhibition of *P. aeruginosa* biofilm. Furthermore, it

adds the effect of a no protein factor in this inhibition, so another non protein factor would be involved. Besides, this inhibition is not related to the removal of acyl-homoserine lactones. The putative factor could be the AI-2, a precursor or a by-product due to presence of *lux S* gene in Lactobacilli. We are conducting studies in this regard.

PM-O1

PHOSPHATIDYLCHOLINE SYNTHASE IS INVOLVED IN THE SYNTHESIS OF PHOSPHATIDYLCHOLINE IN *Pseudomonas putida* ATCC 12633 GROWN WITH TETRADECYLTRIMETHYLAMMONIUM AND AL^{3+}

Paola S. Boeris[1,2] , Andrés S. Liffourrena[1], Mario A. Salvano[1], Isabel M. López-Lara[2], Gloria I. Lucchesi[1]

[1]Dpto. Biología Molecular. FCEFQyN. UNRC. Río Cuarto. Córdoba [2]Centro de Ciencias Genómicas, UNAM, Mexico (pboeris@exa.unrc.edu.ar)

Pseudomonas putida A ATCC 12633 responds to tetradecyltrimethylammonium (TTAB) and Al^{3+} through quantitative changes in membrane phospholipids. The presence of TTAB resulted in an increase in phosphatidylglycerol and phosphatidic acid levels (6 and 20 fold, respectively) with respect to the levels in cells grown without the surfactant, indicating that the negative charges of the headgroups of phospholipids are the primary membrane-associated factors for the response to TTAB. In the presence of Al^{3+}, phosphatidylcholine (PC) increased three fold. In prokaryotes, PC can be synthesized by two pathways, the methylation pathway mediated by phospholipid *N*-methyl-transferase (Pmt) or the CDP-choline pathway that involves phosphatidylcholine synthase (Pcs) that condenses choline directly with CDP-diacylglyceride. Only Pcs activity was detected in cell-free extracts obtained of *P. putida* grown in choline-free medium with TTAB as carbon and nitrogen source. The methylated intermediates of a Pmt pathway were not detected in such extracts. Cell-free extracts obtained from *P. putida* grown with TTAB and exposed to Al^{3+} during 15 or 180 min contained approximately 3 fold more Pcs activity than cell-free extracts from TTAB. When these cell-free extracts were extensively dialyzed, the concentration of Al^{3+} decreased from about 7 to 1 nmol mg $protein^{-1}$ and in these extracts the Pcs activity was similar to the activity detected in extracts from cultures without $AlCl_3$, indicating that Al^{3+} is an activator of the enzyme. Using the *Sinorhizobium meliloti* Pcs sequence as query for BLAST we identify a candidate gene, *pp0731*, that might code for the enzyme Pcs in *P. putida* KT2440. Using specific oligonucleotides, *pp0731* was amplified from genomic DNA and cloned into the expression plasmid pET9a. Expression of *pp0731* from *P. putida* KT2440 in *Escherichia coli* BL21 (DE3) (pLysS) leads to the formation of PC. Based on these results, we suggest that the Pcs pathway might be the only pathway for PC biosynthesis when *P. putida* grow with TTAB. Thus, we expect that a P. putida mutant defective in Pcs can be an useful model to elucidate the synthesis of PC and to clarify the role of PC in Al^{3+} stress responses.

PM-O2

ISOLATION OF A PHOTOSYNTHETIC BACTERIUM FROM EXTREME ENVIRONMENTS IN HIGH-ALTITUDE ANDEAN WETLANDS

Cecilia B. Di Capua[1,2] , María E. Farias[3], Néstor R. Cortez[1,2]

[1] Instituto de Biología Molecular y Celular de Rosario (IBR - CONICET) [2] Fac. de Cs. Bioq. y Farm. - Universidad Nacional de Rosario (UNR) [3] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI- CONICET) (dicapua@ibr.gov.ar)

A photosynthetic bacterium was isolated from high-altitude wetlands (Laguna Azul) as purple colonies using rich media in anaerobiosis under light. After genomic DNA extraction, PCR amplification was performed using 16S 8-27 and 16S 1512-1491 primers. The single 1500bp product was sequenced and blasted against NCBI nucleotide database displaying and 99% similarity with *Rhodopseudomonas palustris* genome. We investigated the antioxidant response of the new isolated strain, in particular the detoxifying enzyme superoxide dismutase (SOD). Crude extracts from both aerobic and photosynthetic cultures were subjected to non-denaturing gel electrophoresis and in situ SOD activity staining. A single SOD band was visualized, which displayed no inhibition by potassium cyanide or hydrogen peroxide indicating that Mn is the metal cofactor of the dismutase. Expression control by oxygen is deduced as a significant increase in SOD activity of cellular lysates was detected in cultures shifted from photosynthetic to respiratory conditions. Exponential cultures were plated onto rich and minimal agar-media and subjected to oxidative challenge using methyl viologen or hydrogen peroxide. When compared to *Rhodopseudomonas palustris* 1e5, a german collection isolate, no significant differences were found between antioxidant responses of both strains.

PM-O3

CHARACTERIZATION OF SUCROSE METABOLISM PROTEINS AND THEIR ENCODING GENES IN A BLOOM-FORMING CYANOBACTERIA.

Maria A. Kolman[1], Laura E. Giarrocco[1], Graciela L. Salerno[1]
[1]CEBB-MdP, CIB, FIBA. Vieytes 3103, CC 1348, 7600 Mar del Plata, Argentina. (mkolman@fiba.org.ar)

Sucrose (Suc) metabolism has been reported in oxygenic photosynthetic organism. Suc is synthesized through a two-step pathway involving Sucrose-Phosphate Synthase (SPS) and Sucrose-Phosphate Phosphatase (SPP), either in unicellular or filamentous cyanobacterial strains. However, to date Suc cleavage by Sucrose Synthase (SuS) has only been reported in filamentous heterocyst-forming cyanobacteria. *Microcystis aeruginosa* is a unicellular non-N2 fixing strain, well known as one of the most common bloom-forming cyanobacteria in fresh water environments. By genome sequence analyses we retrieved three contiguous nucleotide sequences (IPF_1564, IPF_1566 and IPF_1565) corresponding to open reading frames (*orfs*) homologous to Suc metabolism genes. Their deduced amino-acid sequences are 53%, 55% and 72% identical to the protein sequences of *Synechocystis* sp. PCC 6803 SPP, and SPS-A and SuS-A from *Anabaena* sp. PCC 7120, respectively. To carry out their functional characterization, the sequences were cloned and expressed in *Escherichia coli* cells. The recombinant proteins exhibited SPP, SPS and SuS activity, respectively. In addition, cell free extracts from *M. aeruginosa* were chromatographed through an ion exchange column and the enzyme activities were assayed in the eluted fractions. Expression analyses by RT-PCR showed that the three genes are transcribed during standard culture conditions (BG11 medium, 20±1 ºC) and are induced after a salt stress. This is the first report on the presence of Suc enzymes activities in a potential toxic and bloom-forming cyanobacterium. It is worth noting the presence of a SuS protein in a unicellular strain. Suc metabolism role and its relationship with bloom formation remain to be elucidated.

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PM-O4

ROLE OF ALKALINE/NEUTRAL INVERTASES IN *Nostoc sp.* PCC 7120

Carolina N. Nishi[1], Laura E. Giarrocco[1], Walter A. Vargas[2], Graciela L. Salerno[1]
[1]Centro de Estudios de Biodiversidad y Biotecnología de Mar del Plata (CEBB-MdP), CIB, FIBA, MdP [2]The J. Craig Venter Institute (JCVI), Rockville, U.S.A (gsalerno@fiba.org.ar)

Sucrose (Suc), a key sugar in plant life, is also metabolized in cyanobacteria. These photosynthetic oxygen-evolving prokaryotes were shown to present proteins for Suc synthesis and degradation similar to enzymes from plants. Particularly, the hydrolysis of Suc can be performed by Alkaline/Neutral Invertases (A/N-Invs). Two A/N-Inv isoforms were biochemically characterized in *Nostoc* sp. PCC 7120(*Anabaena*), a filamentous nitrogen-fixing strain, and their encoding genes (*invA* and *invB*) were identified and functionally characterized. To elucidate the role of both proteins, insertional mutants lacking either InvA (*invA*$^-$ mutant) or InvB (*invB*$^-$ mutant) activity were generated. The phenotype of the mutants were studied and compared. Whereas *invA*$^-$ cultures were not affected, *invB*$^-$ cells stopped growing under diazotrophic conditions. On the other hand, a differential response to a salt treatment was obtained for both mutant strains. The localization of A/N-Invs in nitrogen-fixing filament cells was evidenced with transcriptional fusions of an optimized version of the green fluorescent protein (GFP) gene (*gfp-mut2*) to putative promoters of *invA* and *invB*. Contrary to previous report that proposed an exclusive heterocyst location (Schilling & Ehrnsperger, 1985), A/N-Invs were shown to locate in both the heterocysts and vegetative cells. Taken together these results show that InvA and InvB play distinct physiological functions, and that Suc hydrolysis by A/N-Inv is important for nitrogen fixation and to cope with sodium chloride stress.

PM-O5

β-CAROTENE IS INVOLVED IN NO PRODUCTION IN *METHYLOBACTERIUM EXTORQUENS* AM1

Martín E. Tagliotti[1], Celeste Molina Favero[1], Cecilia M. Creus[1], Lorenzo Lamattina[2]
[1]Unidad Integrada Balcarce FCA, UNMdP - EEA INTA [2]IIB, FCEyN, UNMdP (tagliottimartin@hotmail.com)

Methylobacterium extorquens AM1 is a well studied pink-pigmented facultative methylotroph (PPFMs), as an organism that derives energy and, in many cases, cell carbon from reduced molecules that have no C–C bond. These bacteria synthesize carotenoids that confer them color and protection against oxidative stress. The phyllosphere is frequently colonized by PPFMs; in this place they can use the methanol released by the stomata as a subproduct of pectin metabolism. On the other side they promote the growth of several plants. In plants, nitric oxide (NO) mediates several process, like root growth, senescence and stomatal closure. It was reported that NO can be produced in vitro by the reaction of β-carotene with nitrogen dioxide (NO_2) in presence of light. The aim of this work was to study if β-carotene synthesis is related to NO production in *M. extorquens* AM1. *M. extorquens* AM1 wild type (Pink) and its mutant in β-carotene synthesis (white; fitotene desaturase null) were grown in AMS liquid media with methanol and nitrate as C-

and N-source, respectively, at 28 °C with orbital agitation (250 rpm). In order to inhibit β-carotene synthesis, AMS media was supplemented with 74 MM diphenylamine (DPA), an inhibitor of lycopene β-cyclase, and incubated with or without light. NO production was quantified in the middle and end of log-phase growth by Electronic Paramagnetic Resonance (EPR). NO was produced by both strains in the middle and end of log-phase growth. Pink strain produced higher NO values in the middle while the white mutant produced higher NO values in the end of log-phase growth. The pink color characteristic of Pink strain became white when bacteria were grown with DPA, showing that β-carotene synthesis was effectively inhibited. In absence of light, DPA had no effect in NO synthesis. In contrast, in presence of light, NO production was inhibited by DPA, particularly in the wild type strain. These results show evidence that *M. extorquens* AM1 produces NO by different mechanisms, one related to nitrate reduction and another involving the presence of β-carotene. *This work was supported by ANPCyT and UNMdP. MET is an undergraduate student of UNMdP*.

MM-O1

THE GLYCANASE PLYB FROM *Rhizobium leguminosarum* BV. *Viciae* IS POLARLY LOCATED ON THE CELL SURFACE AND MODULATES THE LENGTH OF THE EXOPOLYSACCHARIDE AS SHOWN BY ATOMIC FORCE MICROSCOPY.

Patricia L. Abdian[1,2] *, Catalina von Bilderling*[3]*, Nicolás Vozza*[1]*, Daniela M. Russo*[1,2] *, Lía I. Pietrasanta*[3]*, Angeles Zorreguieta*[1,2]

[1]*Fundación Instituto Leloir* [2]*IIBBA, CONICET* [3]*Centro de Microscopías Avanzadas, FCEyN, UBA (pabdian@leloir.org.ar)*

The acidic exopolysaccharide (EPS) is a key component of the biofilm matrix formed by *Rhizobium leguminosarum* bv. *viciae*. The PlyA and PlyB glycanases from *R. leguminosarum* strain A34 cleave the EPS molecules and seem to have synergistic effects. PlyA and PlyB are secreted by a type I secretion system (PrsDE). PlyA remains attached to the cell surface, while PlyB is responsible for most of the diffusible activity. Surprisingly, both glycanases have been shown to be active only on the surface of EPS producing cells. The analysis of biofilm formation in a *plyB* mutant strain or a *plyAplyB* double mutant showed that the increase in EPS length affected normal biofilm development. We have raised a polyclonal antiserum to a truncated form of recombinant PlyB expressed in *Escherichia coli*. The anti-PlyB antiserum was used to analyze by Western blot the localization of PlyB in planktonic cultures of different strains of *R. leguminosarum*. The strains tested were wild type A34; the sequenced strain 3841; a mutant impaired in EPS production, A1077 *pssA*; and a mutant in the type I secretion system, A412 *prsD*. PlyB was differentially located in the extracellular media or in the surface-associated protein fraction, depending on the medium in which cells were grown (rich or minimal medium, the last favoring EPS production). Surface localization of PlyB was analyzed by indirect immunofluorescence with intact A34 cells expressing the green fluorescent protein. Interestingly, PlyB was observed to localize at one pole of the cell. On the other hand, a direct comparison of the EPS produced by wild type A34 and *plyB* mutant strain was performed by atomic force microscopy (AFM) single molecule measurements. As expected, a significant increase in the length of EPS molecules synthesized by the *plyB* mutant compared with those produced by the A34 wild type strain was observed by AFM. We also observed a different association of the EPS molecules produced by the *plyB* mutant, which results in the formation of an open and loose mesh as compared to the wild type. This could in part explain the deficient biofilm formed by the glycanase mutant.

MM-O2

CHARACTERIZATION OF THE PHOP/PHOQ SYSTEM IN *S. marcescens* AND ITS ROLE IN PATHOGENESIS.

Julieta Barchiesi[1,2] *, Maria E. Castelli*[1,2] *, Eleonora García Véscovi*[1,2]

[1]*Instituto de Biología Molecular y Celular de Rosario. CONICET.* [2]*Facultad de Ciencias Bioquimicas y Farmaceuticas .UNR. (barchiesi@ibr.gov.ar)*

The opportunistic human pathogen *Serratia marcescens* is a Gram-negative bacterium that causes disease in a wide range of both invertebrate and vertebrate hosts and in plants. *S. marcescens* is a significant cause of hospital-acquired infection, especially in patients with impaired immunity. As many *S. marcescens* strains are also resistant to multiple antibiotics, it represents a growing problem for public health. However, relatively little is known about the factors that contribute to *S. marcescens* pathogenesis within its host. In our laboratory, with the aim of finding regulatory mechanisms that would participate in *S. marcescens* pathogenesis, we undertook the characterization of PhoP/PhoQ two component system. This system controls transcription of key virulence genes in diverse bacterial pathogens. In order to study the physiological role of *phoP* in *S. marcescens*, we have constructed a *phoP* mutant strain. This mutant exhibited impaired growth in minimal broth limited in Mg^{2+}, in acid pH, and showed increased sensitivity to antimicrobial peptides than the wild type strain. Furthermore, the *phoP* strain was attenuated in the survival inside epithelial cells. These results suggest that the *phoP* gene is required for *S. marcescens* invasion of epithelial cells. In addition, β-galactosidase assays revealed that *phoP* transcription was modulated by the Mg^{2+} and Polimixin B levels, predicted to be environmental signals detected by the system. To further explore the PhoP regulon, we perform a random mutagenesis strategy selecting for Mg^{2+} transcriptional regulated clones. One of the novel PhoP-activated genes identified was *mgtE*. MgtE is a Mg^{2+} transport protein and it has never been reported before as a PhoP regulated gene. Conversely, MgtA and MgtB, members of another Mg^{2+} transport family, have been extensively characterized as PhoP regulon member in *Salmonella*. This result adds a new Mg^{2+} transporter as a PhoP target, reinforcing the importance of PhoP/PhoQ system in Mg^{2+} homeostasis control. On the other hand, we carried out an *in silico* search of PhoP binding sites in the *S. marcescens* genome by MEME/MAST programs. We identified several predicted gene members of the PhoP regulon, such as *phoP*, *mgtA*, *mgtCB* and *pmrG*. The other PhoP binding sites identified corresponded to genes previously uncharacterized as PhoP regulon members in others bacteria. Among them, genes implied in copper resistance (*cueR*), citrate metabolism (*citB* and *citC*), oxidative stress resistance and antibiotics resistance (*ramA*). To our knowledge, this represents the first study of the virulence PhoP/PhoQ system in *S. marcescens*.

MM-O3

THE REGULATION OF *PLCH* GENE OF *Pseudomonas aeruginosa* DEPENDS ON ENVIRONMENTAL CONDITION.

Marina A. Forrellad[1,2] , Maria J. Massimelli[1], Fernando Govantes-Romero[2], Angela T. Lisa[1]
[1]Dpto. Biologia Molecular, FCEFQyN, UNRC. [2]CABD, Universidad Pablo Olavide, Sevilla, España. (mforrellad@exa.unrc.edu.ar)

The *plcH* gene encodes the hemolytic phospholipase C (PlcH), a mayor virulence factor secreted by *P. aeruginosa* in response to choline or phosphate (Pi) starvation. *plcH* is part of an operon with *plcR*, that encodes the $PlcR_{1,2}$ chaperones, which positively modulates the PlcH activity. *plcH* is transcribed as a monocistronic mRNA in response to choline, whereas it is transcribed as *plcHR* biscistronic mRNA, in response to Pi starvation. Our aim is to identify the *plcH* regulatory region and the mechanism involved in its transcription. We constructed several *P. aeruginosa* mutants that carry, in the chromosome, the upstream *plcH*, *plcR* or *plcHR* sequence as transcriptional fusion to the *lacZ* gene. The transcriptional levels observed in these mutants, allowed us to identify the regulatory region that responds to choline or Pi starvation in the 529bp upstream of *plcH* gene. By primer extension experiment, we identified the +1 site that responds to choline, in the G at position -101bp upstream of the ATG start codon. An additional mRNA was observed, its +1 site was located at position -75bp upstream of the ATG. The *"in silico"* analyses of the *plcH* regulatory region allowed us to identify a σ^{70}-type promoter and all elements implicated in a σ^{54} depends transcription, such as: two -12/-24 elements, the IHF regions, the UAS (upstream activator sequence) region and a typically NtrC binding site. With the purpose to know which is the mechanism involved in *plcH* transcription we inserted the P*plcH::lacZ* transcriptional fusion in the *P. aeruginosa* Δ*rpoN*, Δ*ntrC* and Δ*cbrB* background. We observed that the *plcH* transcriptional levels are 60% lower in the Δ*rpoN* background compare with the wild type when choline is present in the medium. The same results were observed in the Δ*ntrC* and Δ*cbrB* background. These data suggest that, in response to choline, the *plcH* is transcribed by a σ^{70} and σ^{54} dependent RNA polymerase and both, NtrC or CbrB are the activators involved in the *plcH* σ^{54}-dependent transcription. On the other hand, we observed that the *plcH* transcriptional levels in Pi starvation medium are twofold lower that those found in choline supplemented medium. Previous studies suggested that, in response to Pi starvation, the *plcH* transcription is not dependent on RpoN and that the Vfr protein is necessary for *plcR* transcription, which is co-transcribed with *plcH*, as *plcHR* mRNA. In conclusion our results suggest that *plcH* is strongly regulated and that the mechanism of *plcH* or *plcHR* transcription depends on the nutritional condition sense by the bacteria in the environment.

MM-O4

THE ABILITY TO FORM BIOFILMS IS NOT ALWAYS CORRELATED WITH THE PRODUCTION OF QUORUM-SENSING SIGNALS IN CLINICAL ISOLATES OF *Acinetobacter baumannii*

Laura E. Friedman[1], Catriel E. Gatto[1], Carlos Vay[2], Mirta A. Franco[1]
[1] Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, UBA [2] Laboratorio de Bacteriología, Dpto de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, UBA (lfriedma@ffyb.uba.ar)

Acinetobacter baumannii (Ab) is a multidrug resistant (MDR) pathogen involved in nosocomial infections. Its ability to grow as biofilms could be central to pathogenicity. However, little is known regarding factors required for biofilm formation. In Gram-negative bacteria cell-to-cell signaling and biofilm development is often mediated by the production of N-acyl-homoserine lactone (AHL) molecules. The gene for an autoinducer synthase, *abaI*, has been cloned in Ab, and AbaI protein is highly similar to members of the LuxI family. Some biosensor strains detect AHLs signals by activation of the reporter genes *tra::G lacZ* in *Agrobacterium tumefaciens* (At) or by production of a purple pigment in *Chromobacterium violaceum* (Cv). The use of both biosensors allows the detection of a wide range of AHL compounds. The resistance nodulation cell division-type (RND) efflux pumps play an important role on the MDR phenotype, and were implicated in virulence mechanisms. RND efflux pumps AdeABC and AdeIJK have been described in Ab. The aim of this study was to know if there is correlation between the ability of Ab to grow as biofilms, the production of AHL molecules, and the presence of the *abaI*, *adeB* and *adeJ* genes. A set of 33 non repetitive Ab clinical isolates and reference strains ATCC19606, RUH134 and RUH504 (genomospecie 3) were investigated. Quantitative biofilm formation assay was performed in microtiter plates. The AHLs production was detected using the biosensors At and Cv. The presence of *adeB*, *adeJ*, and *abaI* was assayed by PCR. Twenty-three Ab clinical isolates and all the reference strains formed biofilms. The *abaI* gene was detected in 30 AB isolates and in ATCC19606, and RUH134. All the *abaI*+ isolates and RUH504 produced AHLs, detected by the At biosensor. None of the strains showed autoinduction activity with the Cv bioreporter. Two *abaI*- isolates formed important amounts of biofilm. On the other hand, the majority of the clinical isolates (31/33) were *adeB/adeJ*+, suggesting the co-existence of the two effux systems; in agreement with their MDR phenotype. According to the REP-PCR fingerprints the isolates *abaI*-/biofilm+ belong to different types, and one of them was *adeB*-. These results suggest that the ability of form biofilms does not correlate with the presence of *adeB* and *adeJ* genes. On the other hand, most of biofilm forming Ab isolates expresses the *abaI* gene, detected by At, but *abaI*-/biofilm+ strains might produce other signals not detected by the biosensors used in this study. *Supported by UBACYT B117*

MM-O5

RAPID IDENTIFICATION AND DIFFERENTIATION OF *Burkholderia cepacia* COMPLEX ISOLATES BY PCR-RFLP PATTERNS OF *gyrB* AND *recA* GENES.

Pablo Martina[1], Gonzalo Sequeira[1], Alejandra Bosch[1], Marisa Bettiol[2], Carlos Vay[3], Laura Galanternik[3], Claudia Hernández[3], José Degrossi[4], Patricia Montanaro[5], Osvaldo Yantorno[1]

[1]Cindefi-Conicet, Facultad De Ciencias Exactas, Unlp, 50 E/115 Y 116, La Plata 1900, Argentina. [2]Hospital de Niños, La Plata. [3]Hospital de Clinicas, Hospital Gutierrez, Hospital Garraham. Bs.As. [4]Facultad de Farmacia y Bioquimica, UBA. [5] Hospital Santisima Trinidad, Cba. (pfmartina@hotmail.com)

The *Burkholderia cepacia* complex (BCC) is a closely related group of Gram-negative bacteria found in many niches of both natural and clinical environments. Members of the BCC are symbionts of plant rhizospheres, contaminants of pharmaceutical and industrial products, inhabitants of soil and surface waters, and opportunistic pathogens, capable of causing disease in plants, invertebrates, animals, and humans. They can be particularly devastating, highly virulent, cystic fibrosis (CF) pathogens that are also able to cause nosocomial infections. BCC taxonomy has undergone considerable changes over the last years, and is known to include at least 15 distinct species. The identification and discrimination of BCC at the species level need multiple diagnostic tests due to misidentification that can easily occur. A relatively new technique that is fast becoming the "gold standard" of bacterial typing methods is multilocus sequence typing which represents the sequencing of 7 different genes. However, this technique is expensive, laborious, time consuming, and considered unattractive for routine application. In this work we report the use of a combination between the PCR-RFLP patterns of *recA* and *gyrB* genes applying *HaeIII* digestion enzyme as a tool for rapid discrimination and identification of BCC isolates. Fifty-seven isolates belonging to different environments and hospitals previously identified by biochemical method and *recA* gene sequencing (as gold standard identification method) were used. PCR-RFLP analyse were performed by amplification of *recA* and *gyrB* genes, subsequent digestion with *HaeIII* enzyme and the program NEBcutter V2.0 was applied. The *recA*-RFLP patterns obtained were the ones described previously for all the species, which do not allow the discrimination among *B. cenocepacia* , *B. stabilis*, and *B. contaminas*. Nevertheless, when these patterns were combined with the ones obtained by *gyrB*-RFLP treated with *HaeIII* restrictive enzyme, the BCC species of incidence in our country could be discriminated. In addition, *gyrB*-RFLP *HaeIII*, which formal patterns for the different species is described here for the first time, revealed an important diversity among *B. contaminans* isolates, the species of major incidence in Argentina, In conclusion, the strategy of combining these two RFLP patterns proved to be adequate, rapid and simple for the proper discrimination among the most closely related BCC species.

MM-O6

SIMPLE SEQUENCE REPEATS AND MUCOID CONVERSION: BIASED *mucA* MUTAGENESIS IN MISMATCH REPAIR-DEFICIENT *Pseudomonas aeruginosa*

Alejandro J. Moyano[1,2] , Andrea M. Smania[1,2]

[1]Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), CONICET. [2] Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. (asmania@mail.fcq.unc.edu.ar)

In *Pseudomonas aeruginosa*, conversion to the mucoid phenotype marks the onset of an irreversible state of the infection in Cystic Fibrosis (CF) patients. The main pathway for mucoid conversion is mutagenesis of the *mucA* gene, frequently due to -1 bp deletions in a simple sequence repeat (SSR) of 5 Gs (G^5-SSR_{426}). We have recently observed that this *mucA* mutation is particularly accentuated in Mismatch Repair System(MRS)-deficient cells grown *in vitro*. Interestingly, previous reports have shown a high prevalence of hypermutable MRS-deficient strains occurring naturally in CF chronic lung infections. Here, we used *mucA* as a forward mutation model to systematically evaluate the role of G^5-SSR_{426} in conversion to mucoidy under a MRS-deficient background, with this being the first deep analysis combining SSR-dependent localized hypermutability and the acquisition of a particular virulence/persistance trait in *P. aeruginosa*. In this study, *mucA* alleles were engineered with different contents of G:C SSRs, and tested for their effect on the mucoid conversion frequency and *mucA* mutational spectra in a *mutS*-deficient strain of *P. aeruginosa*. Importantly, deletion of G^5-SSR_{426} severely reduced the emergence frequency of mucoid variants, with no preferential site of mutagenesis within *mucA*. Moreover, although mutagenesis in *mucA* was not totally removed, this was no longer the main pathway for mucoid conversion, suggesting that G^5-SSR_{426} biased mutations towards *mucA*. Mutagenesis in *mucA* was restored by the addition of a new SSR (C^6-SSR_{431}), and even synergistically increased when G^5-SSR_{426} and C^6-SSR_{431} were present simultaneously, with the *mucA* mutations being restricted to the -1 bp deletion within any of both G:C SSRs. These results confirm a critical role for G^5-SSR_{426} enhancing the mutagenic process of *mucA* in MRS-deficient cells, and shed light on another mechanism, the SSR-dependent hypermutability, contributing to mucoid conversion in *P. aeruginosa*.

MM-O7

NOVEL INSIGHTS ABOUT CLASS 2 INTEGRONS FROM EXPERIMENTAL AND GENOMIC EPIDEMIOLOGY

María Soledad Ramírez[1], *Silvia Piñeiro*[2], *Argentinian Integron Group*[1], *Daniela Centrón*[1]

[1]*Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, UBA, Argentina.* [2]*School Of Medicine, University Of Maryland Allied Health Building, Baltimore, USA. (ramirez.mariasoledad@gmail.com)*

Integrons are genetic elements that contain the components of a site-specific recombination system that recognizes and captures mobile gene cassette. The basic structure of an integron possesses a gene for an integrase (*intI*), a recombination site (*attI*) and a promoter (PC) that permits the expression of the gene cassettes incorporated in the variable region. Class 1 and 2 integrons are both usually associated with antimicrobial resistance gene cassettes in clinical samples. There is scant documentation of the architecture and epidemiology of class 2 integrons. In order to contribute to the knowledge of the architecture, epidemiology and clinical impact of class 2 integrons, we performed a class 2 integron molecular survey analyzing 726 isolates in two bacterial populations, environmental and non-epidemiologically related clinical samples collected during 1982-2007. We recovered the *intI2* gene in 130 out of 726 isolates, most of them detected in clinical isolates, and only one corresponded to a water sample of a psychrophilic *Pseudomonas* spp.. Unlike the widespread distribution of class 1 integrons within gram-negative bacilli, only *Acinetobacter baumannii* and *Enterobacter cloacae* harbored high frequency of class 2 integrons when compared to other genus. In addition, novel arrays of class 2 integrons were documented. As another distinction between the role of both classes of integrons in regards to the multidrug contribution, in the case of class 2 integrons we found that only in one species, *A. baumannii*, the presence of the gene cassette *dfrA1* is relevant from a clinical perspective. When we proceed to characterize the transposition module of Tn*7*, the genetic platform where class 2 integrons were always reported, we observed that the *tns* module harbored a mosaic genetic structure. The bioinformatic analysis performed for *tns* genes, the finding of *intI2* without association to *tns* genes, and the genetic examination of novel *tns*-like genes found in three isolates, indicate that both components associated with horizontal gene transfer, class 2 integrons and the Tn*7* transposons, might have evolved independently

MM-O8

INSIGHT INTO THE SECRETION AND MATURATION PATHWAYS OF THE SUBTILISIN-LIKE EXTRACELLULAR PROTEASE FROM THE HALOALKALIPHILIC ARCHAEON N*atrialba magadii*

Diego M. Ruiz[1], *María I. Giménez*[1], *Rosana E. De Castro*[1]

[1]*Instituto de Investigaciones Biológicas, CONICET-UNMDP (dmruiz@mdp.edu.ar)*

The Twin arginine translocation (Tat) pathway is unique in its ability to translocate folded proteins across and into the cytoplasmic membrane and is extensively used by Haloarchaea. The alkaliphilic haloarchaeon *Natrialba magadii* (optimum growth in 20% NaCl, pH 12) produces an extracellular serine protease (Nep) that has been biochemically characterized in our laboratory. The gene encoding Nep was cloned, sequenced and recombinant active enzyme was produced in *Haloferax volcanii* cells. The polypeptide deduced from the gene showed that Nep is closely related to proteases of the subtilisin family and has a N-terminal prepropeptide containing the Tat consensus motif which is absent in the mature enzyme. Subtilisin-like proteases are synthesized as inactive precursors having at the N-terminus a signal peptide (prepeptide) followed by a propeptide which prevents protease activity. Once translocated by the Sec pathway (general protein secretion pathway), the prepeptide is cleaved by a signal peptidase and the propeptide is processed autocatalytically to render active enzyme. To date, the mechanisms of secretion and maturation of extracellular proteases remains unclear in halophilic archaea. We have shown that the precursor of Nep synthesized in *E. coli* can be trans-activated in vitro by the mature enzyme. The aim of this work was to confirm the mechanism of secretion and get insight into the maturation process of the extracellular protease Nep. The coding sequence of nep was PCR-amplified, cloned into pET24b and used as template for site-directed mutagenesis to generate modified versions of the enzyme: 1. in the signal peptide of Nep (GRRSVL) the arginine residues were substituted by lysines (RR/KK); 2. the serine residue of the active site was replaced by alanine (S/A). Each version of nep gene (wt, RR/KK and S/A) was subcloned into the shuttle vector pJAM and transformed into the neutrophilic haloarchaeon *H. volcanii*. Secretion and maturation of Nep were analysed by Western blotting and protease activity determination on casein contaning-agar plates and by the azocasein assay. Nep(RR/KK), was only immunodetected in the cellular fraction while Nep(wt) and Nep(S/A) were visualized in the cells as well as in the culture medium, although the amount of Nep(wt) accumulated in the extracellular fraction was higher than that for Nep(S/A). Interestingly, Nep(S/A) displayed a higher apparent molecular mass than the two other forms. When the protease activity was measured, cells expressing Nep(wt) produced the highest level of activity in the culture medium while Nep(RR/KK) showed lower levels of total activity which was associated to the cells. As expected, Nep(S/A) did not display protease activity in any fraction. Altogether, these results confirm experimentally that Nep is secreted by a Tat-dependent pathway, and that protease maturation is an autocatalytic process. *Supported by UNMDP, ANPCyT and CONICET.*

MM-O9

ST5-IVA GENOTYPE: AS A MAJOR CAUSE OF INVASIVE INFECTIONS BY CA-MRSA AT A PEDIATRIC HOSPITAL IN CÓRDOBA, ARGENTINA

Claudia Sola[1], Analía Garnero[2], Catalina Culasso[3], Lydia Carvajal[3], Patricia Montanaro[3], Ana L. Egea[1], Eduardo Glatstein[2], José L. Bocco[1]

[1]Departamento de Bioq.Clínica, Fac. Cs. Químicas, UNC-CIBICI-CONICET [2] Servicio de Infectología Hospital de Niños de La Santísima Trinidad [3] Servicio de Microbiología, Hospital de Niños De La Santísima Trinidad (csola@fcq.unc.edu.ar)

Community-Associated Methicillin Resistant Staphylococcus aureus (CA-MRSA) infections are increasing worldwide. In Argentina, the emergence and dissemination of the ST5 lineage among CA-MRSA strains has been reported. The aim of this work was the study of the evolution of the clinical and molecular epidemiology of CA-MRSA infections from a pediatric tertiary care public hospital in Córdoba, during 2003-2008.Prospectively, 64 consecutive single patient CA-MRSA isolates were collected and the corresponding clinical and demographic information was associated during six years (2003-2008) at the "Hospital de Niños de la Santísima Trinidad" from Córdoba. Molecular typing studies were performed. The proportion of CA-MRSA increased significantly between 2003(4%) and 2008(47%)-p=0.0001(CI95%:-0.666 to -0.194) as well as the frequency of invasive infections [0%-2003 vs 36%(9/25)-2008] with higher levels during 2007 [59%(10/17)]. The average age of patients was 4 years old. Twenty-six of them had invasive infections, of which 11(42%) had bone and joint infections with torpid evolution associated. One of these was complicated with sepsis and pulmonary involvement, other with endocarditis and another one with brain abscesses. The remaining patients had: necrotizing pneumonia(4), primary bacteremy(4), necrotizing fasciitis(2) and adenitis(3). No patients died. Molecular analysis of the strains showed that 98% of them belong to the genotype: PulsotypeI-ST5-SCCmecIVa-spat311 that harbor Panton-Valentine-leukocidin and the enterotoxin A genes, which was previously reported as the predominant clone in Argentina during 2005-2006. Only two isolates were characterized as PulsotypeN-ST30-SCCmecIVc, which carried adhesin for bone sialoprotein-(bpp) and pvl genes.The results demonstrate that the dissemination of the clone ST5-IVa, PVL and EntA (+), which caused originally and preferentially mild skin and soft tissue infections, is now the main cause of the significant increase of invasive CA-MRSA infections during 2005-2008 in this hospital in Córdoba.

MM-10

IDENTIFICATION OF A HUTC-MEDIATED REGULATORY NETWORK: THE HISTIDINE UTILIZATION REPRESSOR RECOGNIZES MORE THAN ONE PROMOTER

Gastón M. Arocena[1], Rodrigo Sieira[1]

[1]Instituto de Investigaciones Biotecnológicas - UNSAM (garocena@unsam.edu.ar)

HutC is the transcriptional regulator of the *hut* genes (for histidine-utilization), which confer the ability to use histidine as a sole carbon source to many bacterial species. Until now, the known target of HutC was restricted only to the operator sites present in the *hut* promoters. However, we recently found that in addition to the *hut* promoter, HutC also binds specifically to regulatory sequences of the virB genes of the animal and human pathogen *Brucella abortus*. Such genes code for the Type-IV Secretion System VirB, a multicomponent secretion apparatus that, as in other pathogenic bacteria, is essential for the virulence of *Brucella*. Analyses of activity of the *virB* and *hut* promoters (P_{virB} and P_{hut}, respectively) revealed that HutC modulates expression of both systems. Using electrophoresis mobility shift assays (EMSA), we determined dissociation constants for the binding of HutC to both P_{virB} and P_{hut}, and observed that this regulator binds to each promoter with different affinities. Such differences can be explained by the different architectures of both HutC-binding sites, which were identified by DNase I Footprinting experiments. Following these observations, we hypothesized that in addition to the HutC-binding sites of P_{virB} and P_{hut}, this transcriptional regulator could recognize other operator sequences present in the genome of *B. abortus*. To test this possibility, we searched for additional HutC-binding sites using PATSER software. Neither the dyad symmetric sequence of P_{hut} nor the HutC-recognized sequence of P_{virB} were found twice in the *B. abortus* genome. However, when we performed a search using a degenerate consensus sequence that matches both motifs, two putative additional HutC-binding sites were found. EMSA experiments showed that HutC binds specifically to one of the two identified sequences, which is located upstream of a gene that codes for a type-V autotransporter that shares homology with bacterial adhesins.Our results show that, in contrast to the initial notion that HutC regulates transcription of a single locus, it interacts with at least three different promoters. These observations suggest that histidine catabolism triggers an adaptive response mediated by HutC through modulation of expression of virulence determinants and putative surface-exposed proteins.

EM-O1

METAGENOMIC ANALYSIS OF NITROGEN FIXING BACTERIAL POPULATIONS FROM SOILS WITH DIFFERENT AGRICULTURAL MANAGEMENT FROM THE HUMID PAMPA REGION.

Verónica M. Bergottini[1], *Mónica Collavino*[1], *Daniel H. Grasso*[2], *O. Mario Aguilar*[1]

[1]*Instituto de Biología Molecular y Biotecnología, UNLP, CCT-La Plata CONICET.* [2] *Inta Castelar. (verobergottini@biol.unlp.edu.ar)*

In this study we investigated the nitrogen fixing populations of bacteria from soils with different agricultural management from The Humid Pampa region by metagenomic analysis. Soil samples were collected at two field sites located in Monte Buey (Córdoba) and Viale (Entre Ríos). At each location, we took samples from soils corresponding to different agricultural management such as 1) accepted good agricultural practices with records of no-tillage management within the aim to accomplish sustainable agriculture such as crop rotation and nutrient reposition, 2) contrarily, mono-cropping without reposition, which is definitely unsustainable, and 3) undisturbed natural environment, as reference soil . In the beginning of this study, we tested two degenerated pair of primers, Zf/Zr and 19F/407R (Zehr *et al*., 1989; Ueda *et al*., 1995), to amplify the *nif*H gene of diverse representative nitrogen fixing bacteria. Both pair of primers resulted in successful amplification. Next, DNA was directly extracted from the soils and used as template to amplify *nif*H sequences using the *nif*H primers. In order to reveal diversity among strains and soils, RFLP assays was performed. Several endonucleases were assessed such as *Hae*III, *Sau*3AI and *Mnl*I which resulted in the best profiles to discriminate *nif*H diversity, resulting in differences in strain at the species level. The representative N_2-fixing strains were all discriminated by RFLP, indicating that the technique was suitable to study diversity of environmental samples. Analysis of soil provided a specific profile for each DNA sample. In particular, the restriction patterns obtained from soils samples under the different agricultural management showed distinct restriction patterns in Monte Buey and Viale. These preliminary results confirm that agriculture practices may affect the N_2-fixing community in soils from sites with very different edafic conditions. Considering these results, we approached construction of DNA libraries with the amplified *nif*H fragments of soils samples to determine microbial structure by DNA sequencing clones and use data to perform a phylogenetic analysis to identify some of the N_2-fixing bacteria predominating in each sample. A comparative and limited comparison of some of our sequences will be shown. Our results indicated the capacity of our analysis to reveal differences in the structure of nitrogen fixing population in soils that underwent different agricultural practices.

EM-O2

ASSESSMENT OF MICROBIAL COMMUNITY FUNCTION AND STRUCTURE IN SOIL MICROCOSMS EXPOSED TO GLYPHOSATE

María C. Zabaloy[1,2] , *Jay L. Garland*[3], *Marisa A. Gómez*[1,2]

[1] *Departamento de Agronomía, Universidad Nacional del Sur* [2] *CERZOS-CONICET* [3] *Dynamac Corp., Kennedy Space Center, Nasa (mzabaloy@uns.edu.ar)*

Glyphosate is widely used in Argentina to control weeds in glyphosate-resistant soybean and in no-till management systems. Glyphosate- resistant crops can be treated post-emergence with glyphosate without killing the crop. Thus, these systems are often subjected to repeated use of glyphosate. This herbicide inhibits the shikimic acid pathway which leads to synthesis of aromatic amino acids in plants and microbes. The herbicide may change the soil environment due to toxicity to soil microorganisms or through the influx of carbon (C), phosphorus (P) and nitrogen (N) in the form of glyphosate or plant residues, which would support rapid growth of opportunistic microbes. The aim of this work was to study changes in soil functional and structural diversity due to two different glyphosate doses in laboratory incubations. We also assessed if glyphosate mineralization occurs within the incubation period, and whether this releases N and/or P to the soil nutrient pools. The experiment consisted of soil microcosm exposed to low and high rates of herbicide (LG= 15 mg kg^{-1}, HG= 150 mg kg^{-1}) or un-treated (Control= 0 mg kg^{-1}), and incubated at 28^{o}C for 7 d. The BD Oxygen Biosensor System (BDOBS) is a microplate platform with an oxygen sensitive fluorophore that allows monitoring O_2 consumption in soil slurries amended with the researcher's choice of substrates, nutrients, etc. We used BDOBS as a community-level physiological profiling (CLPP) tool to examine herbicide-derived changes in functional diversity. Microbial community structure was analyzed using quantitative PCR (qPCR) and terminal restriction fragment length polymorphism (T-RFLP), in order to quantify bacterial and fungal numbers as well as to detect shifts in community composition in the assay conditions. CLPP was slightly modified by HG treatment, resulting in decreased use of coumaric acid, while glyphosate itself was not metabolized as a C source in soil slurries. Moreover, there is no indirect evidence that glyphosate mineralization occurs in soil in less than 7 d, as we did not detect significant increases in microbe-available N or P in soil slurries. Cluster analysis of T-RFLP data showed that Control microcosms grouped together but there was no pattern for glyphosate treatment, which appeared to decrease the predictability of community structure. Q-PCR analysis showed that bacterial counts almost doubled in glyphosate-treated microcosms with no change in fungal abundance. Summarizing, the function of the community was slightly impacted, but not due to mineralization of the glyphosate itself. Bacteria biomass responded to glyphosate treatment, but consistent selective enrichment for specific bacteria species did not appear to occur.

IN-O1

RHIZOBIAL NOD FACTORS ARE REQUIRED FOR NODULE PRIMORDIA DEVELOPMENT IN THE CRACK ENTRY INFECTION PROCESS IN PEANUT

Fernando Ibañez[1], *Herminda Reinoso*[1], *Adriana Fabra*[1]

[1] *Departamento de Ciencias Naturales, FCEFQYN, Universidad Nacional de Río Cuarto (fibanez@exa.unrc.edu.ar)*

Plant growth is often limited by the availability of nitrogen. Plants of the Fabaceae family (legumes) have developed a nitrogen-fixing symbiotic association with soil bacteria collectively known as rhizobia to overcome this nutrient limitation. *Arachis hypogaea* L. (peanut) is a widespread leguminous plant of great agricultural and economic significance that can be used as food crop or as source of several food products. Peanut exhibits a particular mode of rhizobial invasion known as crack entry. In this mechanism, infection threads are never formed, not for epidermal or cortical invasion neither for nodular dissemination. The development of the symbiotic association involves a complex molecular signal exchange between the symbiotic partners. Nod factors are the best characterized of these signals, and their role in the symbiosis with legumes where rhizobial entry involves infection thread formation is well known. Recently, our group reported the Nod factor structure from a native bradyrhizobial isolate. However, information regarding the role of Nod factors in peanut-rhizobia symbiosis is scarce, and the question whether these molecules are required for crack entry infection in peanut remains unanswered. In this work, a bradyrhizobial mutant strain unable to produce Nod factors was obtained. Further research revealed that it is not affected in the colonization of peanut roots. In addition, the mutant strain is not able to reinitiate the meristematic activity in cortical cells and, therefore, it is incapable to nodulate peanut. Taken together, the results obtained in this work indicate that rhizobial Nod factors are required for nodulation of peanut, and that they are involved in the induction of the cortical cell division in order to form the nodule primordia. Although this role for Nod factors has been proposed for the root invasion that involves infection thread formation, this work constitutes the first evidence of the link existing between these molecules and the cortical cell division for nodule primordia formation in peanut.

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IN-O2

ADHESION FACTORS AND SIGNALING PATHWAYS INVOLVED IN THE INTERACTION BETWEEN *Bacillus cereus* AND CULTURED HUMAN ENTEROCYTES.

Jessica Minnaard[1,2] , *Ivanna S. Rolny*[1,2] , *Pablo F. Pérez*[1,2]

[1]*CIDCA (CONICET. La Plata)* [2]*Cátedra de Microbiología. Facultad de Ciencias Exactas, UNLP. (jm@biol.unlp.edu.ar)*

In the context of intestinal pathologies, *Bacillus cereus* is involved in two syndromes: emetic and diarrheic. Even though pathogenesis of *B. cereus* was traditionally ascribed to toxin production, recent studies demonstrated that adhesion-invasion onto enterocytes could also contribute to *B. cereus* virulence. The present study sough to gain further insight on the factors involved in the interaction of *B. cereus* with cultured human enterocytes as well as on the signaling pathways responsible for the biological effects. Five strains of *B. cereus* were used: ATCC 10876, T1, M2, 2 and B10502. Cultured human enterocytes (Caco-2 cells) were incubated for 2 h (1 x 10^8 CFU/well) with bacteria from mid-log cultures in DMEM containing 100 µg/ml cloramphenicol at 37°C in a 5 % CO_2 atmosphere. Associated bacteria (adhering + invading) were evaluated by microscopy on stained smears and plate counts. Invasion was evaluated by plate counts after killing of exocellular bacteria by gentamicin. Signaling pathways involved in the interactions were assessed by using specific inhibitors. Cytoskeleton was labeled with FITC-phaloidin. Killing of bacteria by UV irradiation decreases association of *B. cereus* to enterocytes thus demonstrating that bacterial viability is crucial for the interaction with eukaryotic cells. Treatment of bacteria with LiCl (5 M), proteases (trypsin or chemotrypsin) or exhaustive washing with PBS showed that non-covalent bound structures, proteins and loosely associated material are involved in the interaction in a strain-dependent manner. In contrast when bacteria were treated with metaperiodate (50 mM), association values were not modified. Extracellular factors from strain 2 increased its own association and invasion values and also association of strain B10502. Calcium depletion significantly increased the ability of strains T1 and 2 to gain access to the intracellular domain but did not modify association values. In addition, higher association/invasion values were found for cells cultured for 4 days as compared with cells cultured for 9, 15 and 21 days. Dantrolene, wortmannin, U73122 and staurosporin were able to abrogate citoskeleton disruption that follows infection of enterocytes by *B. cereus*. Adhesion of strain T1 decreased in the presence of U73122, wortmannin and when those inhibitors were used together. In contrast, invasion values were diminished by U73122 and not by wortmannin. Results show that different bacterial structures are related to the interaction of *B. cereus* with cultured human enterocytes. In addition, signaling cascades related to phosphorilated lipids are involved in the profound modifications on the cytoskeleton that follows infection of cells by *B. cereus*. These changes would in turn facilitate the invasion of the monolayers through both the apical and the basolateral domains.

IN-O3

ROLE OF EXOPOLYSACCHARIDE SYNTHESIS AND BIOFILM FORMATION DURING THE ESTABLISHMENT OF THE *Sinorhizobium meliloti*-ALFALFA SYMBIOSIS.

Luciana V. Rinaudi[1], Fernando Sorroche[1], Ángeles Zorreguieta[2], Walter Giordano[1]

[1]Dpto. Biología Molecular, Universidad Nacional de Río Cuarto. Río Cuarto, Córdoba. [2]Fundación Instituto Leloir, CONICET, FCEYN, Universidad de Buenos Aires. Buenos Aires. (lrinaudi@exa.unrc.edu.ar)

Sinorhizobium meliloti is a nitrogen-fixing bacterium that establishes a symbiotic relationship with *Medicago sativa* (alfalfa). The synthesis by *S. meliloti* of either succinoglycan (EPS I) or galactoglucan (EPS II) is essential for a successful symbiosis. While exopolysaccharide-deficient mutants induce nodule formation, they fail to invade them, and as a result, no nitrogen fixation occurs. It has been shown that the ExpR/Sin quorum-sensing system controls biofilm formation in *S. meliloti* through the production of the EPS II, which provides the matrix for the development of biofilms, both *in vitro* and *in vivo*. Phosphate concentration constitutes an environmental signal that determines which EPS is produced by *S. meliloti*. Low-phosphate conditions normally found in the soil (typically 1 to 10 µM) stimulate EPS II production, while high-phosphate concentrations inside the plant (10 to 100 mM) block EPS II synthesis and induce the production of EPS I. Here we studied how phosphate availability regulates exopolysaccharide synthesis and the role of EPSs during invasion of the legume plant. We performed nodulation assays using wild-type strains of *S. meliloti* (Rm1021 and Rm8530) as well as mutants in EPS I (*exoY*) and EPS II (*expA*) production. Alfalfa plants were supplied with nitrogen-free Hoagland solution containing different phosphate concentrations (0.1 to 10 mM) as needed and scored for pink and white nodules after 30 days of growth at 25°C, 60% relative humidity, and a 16-h light cycle. Our results suggest that under phosphate limitation (0 and 0.001 mM), EPS II-producing strains are more efficient to nodulate alfalfa than succinoglycan-producing strains. However, when phosphate availability increases (0.1 and 10 mM), EPS I promotes a better nodulation than EPS II-producing strains. These findings allow us to propose a model to explain the role of EPSs during the establishment of the symbiosis between *S. meliloti* and alfalfa. Low-phosphate conditions normally found in soils would induce EPS II production mediating root attachment and colonization. Inside the plant, rhizobia would find higher phosphate concentrations resulting in EPS I production, whose major role seems to be avoiding legume-defense responses. In light of the data presented here, it is tempting to speculate that the main role of EPS II is to let bacteria attach appropriately to the host root by forming biofilms so invasion can occur. The biofilm matrix might provide *S. meliloti* cells a suitable microenvironment for colonization and eventual invasion of the root hairs.

IN-O4

CIRCADIAN VARIATION IN HYDROGEN CYANIDE-MEDIATED PARALYSIS OF *Caenorhabditis elegans* BY *Pseudomonas fluorescens* STRAIN CHA0

Andrés Romanowski[1], María Laura Migliori[1], Claudio Valverde[2], Diego Golombek[1]

[1] Laboratorio de Cronobiología, DCYT, UNQ [2] Programa Interacciones Biológicas, DCYT, UNQ (cvalver@unq.edu.ar)

Pseudomonas fluorescens strain CHA0 is a soil bacterium that produces a set of secondary metabolites that antagonize phytopathogenic fungi and therefore promote healthy growth of several plant species. In addition, the metabolites help strain CHA0 to avoid grazing pressure by different protists. The Gac/Rsm posttranscriptional regulatory system is required for coordinated expression of genes and operons related to the synthesis of secondary metabolites. Here we show that strain CHA0 is able to intoxicate *C. elegans* either under growth-limiting conditions (i.e., slow killing) or by rapid paralysis under conditions of *ad libitum* nutrients (i.e., fast killing). Both types of toxicity require the Gac/Rsm pathway, and the rapid paralytic killing strongly depends on hydrogen cyanide production. The response observed in *C. elegans* nematodes to fast paralytic killing varies along the day and its sensitivity is higher during the night, at ZT12 (lights off). This behavior correlates well with HCN tolerance, which is higher during the day, at ZT0 (lights on). This innate immune response to *P. fluorescens* CHA0 might depend on the stress response pathway of *C. elegans*. The fact that the tolerance varies daily gives further proof of an underlying circadian clock that governs cyclic behavior in *C. elegans*.

IN-O5

ROLE OF MEMBERS OF THE AUTOTRANSPORTER FAMILY IN ADHESION AND INVASION OF *Brucella suis* TO HOST CELLS

Veronica Ruiz[1], *Diana M. Posadas*[1], *Fernando A. Martin*[1], *Angeles Zorreguieta*[1]

[1] *Fundación Instituto Leloir, IIBBA CONICETt, FCEN UBA (vruiz@leloir.org.ar)*

Species belonging to the *Brucella* genera are intracellular pathogens responsible of an endemic disease called brucellosis. Even though the intracellular trafficking of brucellae has been extensively characterized, other stages of the infection process remain unknown. It has been shown that brucellae are able to adhere to model epithelial cells, but the factors involved in the adhesion and/or invasion to these cells remain unexplored. By bioinformatical methods we identified several putative adhesins from the autotransporter family in the genome of *Brucella suis*. In this work we assessed the role of three members of the autotransporter family (AubD, AubE and AubF) in the adhesion to an abiotic surface and HeLa cells by heterologous and mutational approaches. Autotransporter genes cloned with their own promoters into pBRRMCS1 were transferred to non-adhesive non-invasive *Escherichia coli*. The strain of *E. coli* with cloned *aubF* showed an enhanced attachment to polystyrene indicating that AubF increase either the attachment to the abiotic surface or adhesion between bacteria. Besides, AubF conferred to *E. coli* enhanced ability to adhere and invade HeLa cells. A deletion mutant in the *aubD* gene showed a dramatic loss in the ability to attach and invade HeLa cells compared with the wild type isogenic strain. Likewise, deletion of *aubE* significantly reduced adhesion and invasion to HeLa cells, albeit less marked than the effect caused by the *aubD* mutation. Taken together, these results strongly suggest that these members of the autotransporter family are involved in the adhesion and/or invasion of *B. suis* to eukaryotic cells.

BF-O1

A RAPID-BOD BIOSENSOR BASED IN LYOPHILIZED *Klebsiella pneumoniae*. EFFECTS OF OXYGEN, FERRICYANIDE AND MICROBIAL CONCENTRATION

María C. Bonetto[1,2] , *Natalia J. Sacco*[1,2] , *Eduardo Cortón*[1,2]
[1] *QB, FCEYN, UBA.* [2] *CONICET (celinatt@yahoo.com.ar)*

The traditional BOD determination through the BOD_5 test that correlates biodegradable organic matter with dissolved oxygen consumed by microorganism in samples after 5 days incubation requires to be replaced with a faster determination method when active interventions for environmental monitoring and control process are needed. A ferricyanide mediated approach has been proposed to overcome oxygen solubility problematic; O_2 low solubility in water quickly becomes the rate-limiting reagent in catabolism of organic matter, the higher solubility of ferricyanide enables the increase of microbial load reducing determination time to hours. The design of a microbial amperometric biosensor, where *Klebsiella pneumoniae* BO365 strain uses ferricyanide as the last electron acceptor to organic compounds catabolism, is being developed in our lab. BO365 strain has been isolated from a commercial non pathogenic consortium and identified by API 20 E as *Klebsiella pneumoniae*, a facultative aerobe Gram negative rod and would use ferricyanide (Fe^{3+} actually) as a last acceptor in absence of O_2. When a high bacterial load ($2.3\ 10^8$ CFU/mL) was incubated with ferricyanide under aerobic conditions, currents registered (0.61 ± 0.03 μA) were not significantly different to those registered under anaerobic conditions (0.51 ± 0.03 μA); this may be due to a fast depletion of dissolved O_2 when a high bacteria concentration is being employed. These results confirms our hypothesis that there is no need to work in anaerobic conditions when high concentrations of bacteria are being used, simplifying measurement protocols either in the laboratory as on the field. Even though the design of a discarding sensor depends on the way bacteria are disposed in the device. Immobilized living cells as biofilm have been assayed in many works, however living cell biosensors are not viable for commercial mass production. We have assayed lyophilized *Klebsiella pneumoniae* (in concentration of $3.2 * 10^8$ CFU) in presence of ferricyanide and found higher currents (2.82 ± 0.15 μA) than those obtained employing similar concentration from a liquid culture (1.62 ± 0.09 μA; with $4.6 * 10^8$ CFU concentration), this may suggest that proteins required for ferricyanide reduction are still functional after the process even when the cells are not able to form colonies.These results are getting us closer to our ultimate goal, the design of a disposable microbial sensor for rapid BOD determination in wastewaters, treatment waters and water natural sources samples.

BF-O2

MINERAL PHOSPHATE SOLUBILIZATION BY *G. diazotrophicus*

Juan M. Crespo[1], *Verónica Guidi*[1], *Maria L. Molinari*[1], *Jose L. Boiardi*[1], *María F. Luna*[1,2]
[1]*CINDEFI (UNLP; CCT-La Plata, CONICET), Facultad de Ciencias Exactas* [2] *CIC-PBA (mafla@quimica.unlp.edu.ar*)

Phosphorus (P) is one of the major essential macronutrients for biological growth and development. Most agricultural soils contain large reserves of P, a considerable part of which accumulates as a consequence of regular applications of P fertilizers. However, a greater part of soil phosphorus is present in the form of insoluble phosphates and hence cannot be utilized by plants. Phosphate solubilizing microorganisms (PSM) play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers. In the case of calcium phosphates, a significant body of evidence has been developed to show that Gram negative bacteria exhibiting superior mineral phosphate solubilizing capabilities utilize the direct oxidative pathway. This pathway (also called nonphosphorylating oxidation) produces gluconic acid and 2-ketogluconic acid directly in the periplasmic space. These strong organic acids can dissolve poorly soluble calcium phosphates such as hydroxyapatite. *G. diazotrophicus* strains have been reported to posses the ability to solubilize insoluble phosphates. It has been reported that this microorganism posses a pyrroloquinoline quinone (PQQ)-linked glucose dehydrogenase responsible for the periplasmic conversion of glucose into gluconate. However the relationship between poorly soluble mineral phosphates solubilization and gluconic acid production are not yet completely understood. In the present work it was checked the ability of *G. diazotrophicus* to solubilize insoluble inorganic phosphates. Batch cultures of *G. diazotrophicus* were carried out in erlenmeyer flasks using a defined minimal medium with 1.32 $g.l^{-1}$ $(NH_4)_2SO_4$ and glucose (20 $g.l^{-1}$) as C-source. When the organism was grown under N_2-fixing conditions (BNF) the $(NH_4)_2SO_4$ concentration was diminished to 0.132 $g.l^{-1}$. Soluble calcium phosphates, $Ca_3(PO_4)_2$, hydroxyapatite and rock phosphate (20 % P_2O_5) were employed as P sources. It is shown that soluble phosphate concentration in the medium was significantly increased by the presence of organic acids of bacterial origin. These acids solubilized not only insoluble phosphates but also hydroxyapatite. Phosphate solubilization was directly related to the pH decrease caused by growth of *G. diazotrophicus*in the medium containing glucose as carbon source.

BF-O3

GENETIC ENGINEERING OF NITROGEN FIXATION TOWARDS OPTIMIZATION OF SYNTHETIC MICROBIAL COMMUNITIES FOR NEXT GENERATION BIOFUELS

Juan C. Ortiz Marquez[1,2] , *Leonardo Curatti*[1,2]

[1] *CEBB-MDP-CONICET* [2] *CIB-FIBA (lcuratti@fiba.org.ar)*

Dwindling fossil fuel reserves, and an increasing global warming, prompts the prospecting of alternative energy sources. Harvesting solar energy as photosynthetic biomass, and its further processing into different biofuels as energy carriers is one of the most promising strategies for the years to come. Last-generation biofuels technology includes culturing of either cyanobacteria or unicellular Chlorophytes. The most remarkable advantages of this feedstock in comparison to traditional crops are superior yield, no requirement of fertile land (avoiding competition with food production and preventing deforestation) and more efficient management of water and nutrients, among others. Although genetic engineering of microalgae to improve different aspects of biofuels technology is in the pipeline in many laboratories worldwide, transference of major metabolic pathways such as nitrogen fixation seems not to be possible in the near future. To overcome that drawback, we aim at assembling synthetic microbial communities made up of model organisms with complementary metabolic capabilities. In this work we show some properties of mixed cultures of the unicellular non-nitrogen fixing cyanobacteria *Synechocystis* sp. PCC 6803 and the diazotrophic, plant growth-promoting proteobacteria *Azotobacter vinelandii* in a minimal synthetic medium using air as the carbon and nitrogen sources. Addition of *A. vinelandii* cells to a cyanobacterial culture produced a modest but reproducible increase in biomass, cells counting and chlorophyll accumulation. *A. vinelandii* mutants with complete deletion of either the transcription activator NifA or the anti-activator NifL, producing a concerted silencing or constitutive over-expression of nitrogen fixation (Nif) genes, respectively, were obtained. Changing Nif properties of *A. vinelandii* appeared to alter more significantly biomass accumulation and cell countings than chlorophyll accumulation. Strain PCC 6803, had an increased plating efficiency when co-cultured with *A. vinelandii* onto solid medium. Moreover, the cyanobacterium show, in addition to the regular small round colonies, some larger and diffuse colonies, which corresponded to cell patches containing both microorganism. Strain PCC 6803 colonies, show unidirectional gliding motility towards *A. vinelandii* colonies when present at a minimal threshold proximity, suggesting a positive taxism. This study shows: i) a "proof-of-concept" for the synthetic community approach using two genetically characterized microorganisms; ii) the initial genetic dissection of *A. vinelandii* cyanobacterial growth-promoting properties; iii) one of the first steps towards the improvent of photosynthetic-biomass production properties of synthetic microbial communities by genetic engineering. *Supported by ANPCyT & UNMdP.*

Paneles

Posters

BD-P1

MOLECULAR DETECTION OF *PSEUDOMONAS* SPP. IN AGRICULTURAL SOILS

Betina Agaras[1,2] *, Luis G. Wall*[1,2] *, Claudio Valverde*[1,2]

[1] *Programa Interacciones Biológicas, DCyT, UNQ* [2] *BIOSPAS (betina_agaras@yahoo.com.ar)*

Among the so-called plant growth promoting rhizobacteria (PGPR), members of the *Pseudomonas* genus have been isolated and identified worldwide as potential plant probiotic microorganisms based on their properties to antagonize phytopathogens, induce systemic acquired resistance and increase phosphate availability. Little is known about the dynamics of pseudomonads population in agricultural soils under the influence of biotic and abiotic factors. As part of a national consortium to study soil biology and its relationship with productivity (the BIOSPAS project), our lab began to characterize quantitatively and qualitatively both culturable and unculturable pseudomonads in agricultural soils. To this end, molecular methods are rapid, specific and comprehensive enough to approach such ecological issues. Based on bibliography and available gene sequences, we have selected two markers to detect pseudomonads in soil samples, *oprF* and *gacA*, which encode the major non-specific porin and a response regulator involved in post-transcriptional regulation of gene expression, respectively. Oligonucleotides were designed to set up PCR-RFLP protocols and were first validated with pure cultures of type strains, then applied to soil isolates obtained by selective growth on Gould's S1 medium. The results obtained so far with yet uncharacterized soil isolates indicate that *oprF* and *gacA* will be useful targets for studying pseudomonads load and diversity in soils with culture-independent methods as real time PCR and PCR-DGGE.

BD-P2

METAGENOMIC ANALYSIS OF BACTERIAL POPULATIONS IN SOILS FROM THE DEPRESSION AREA OF THE RÍO SALADO

Eugenia Calello[1]*, Monica Collavino*[1]*, Daniel H. Grasso*[2]*, O. Mario Aguilar*[1]

[1] *Instituto de Biotecnología y Biología Molecular* [2] *INTA, Castelar (mecalello@biol.unlp.edu.ar)*

Aiming to exploit the genetic potential of soils microorganisms from extreme environments to retrieve salinity tolerance genes, the diversity of saline soil was examined and soil metagenomic DNA was used. Since the estimated size of the cultivable bacterial population ranges between 0.1 and 1%, limited information about the population diversity is available. On the other hand, a metagenomic approach allows to access to knowledge of a broader range, and to be more representative of the microbial communities. In the first step of our study, the biodiversity of two different soils was assessed in soil samples that were collected from sites in the depression area of the Río Salado (Chascomús, location CH1: S 35 28 12 3 W 58 02 19 6, 40 m height, CH2: S 35 33 11 5, W 57 58 59 3, 14 m height) and have different physical chemistries properties (CH1: elec. cond.: 0.46 mS/cm; pH 1:25 water 6,39; 1.83% organic carbon, 0.18% organic nitrogen, and CH2: elec. cond. 1.6 mS/cm; pH 1:25 water 9.93; 0.67% organic carbon; 0.10% organic nitrogen). Metagenomic DNA from both soil samples was extracted and used to amplify the 16S rRNA gene by using primers deduced from the division bacteria. A library was constructed using the PCR product and clones were randomly sequenced. The collected data was compared against RDP (Ribosomal Database Project II) and Genebank NCBI. Analysis at the level of class, revealed a major representation of Actinobacteria, whereas α-proteobacteria were represented to a lesser extent. The most represented genera in the library were *Conexibacter*, followed by *Xiphinematobacteriaceae*. Furthermore, DGGE (Denaturing Gradient Gel Electrophoresis) technique was used to separate the products of a nested amplification (the variable region of 16S rRNA gene) using bacterial primers, and primers specific for the genera *Burkholderia*. Different patterns were observed in the two different soils.In addition, some clones from a library constructed with the 16S rRNA PCR product using primers for the genera Arquea were sequenced and a phylogenetic tree was built. Taken these results altogether, we conclude that, microbial community in that environment were found to be represented by highly diverse microorganisms, interestingly most of them seems to be uncultured. Actinobacteria was the most represented genera, which were reported by other authors to be also detected in several environmental niches including those similar to our saline soil

BD-P3

FUNGAL DIVERSITY DURING COMPOSTING OF AGRICULTURAL WASTES

Vanina V. Galzenati[2,1] , *Fabiana F. Bressan*[1], *Diana D. Crespo*[1], *Cecilia C. Carmarán*[2]

[1] *IMYZA, INTA Castelar* [2] *DBBE, FCEyN, UBA (vaninagalzenati@yahoo.com.ar)*

Composting is an aerobic biotransformation of solid organic waste in stable products, free of pathogens that can be applied to improve soil characteristics. During this process, physical and chemical changes occur that determine the biodiversity, which the temperature is the major selective factor for determining the composition of microbial community. Fungi, bacteria and actinomycetes constitute the microbial community in the compost. In this work, we studied fungal diversity associated with the composting of agricultural wastes (poultry manure + rice hulls + wood thin shaving). Samples were taken at four times, corresponding to different composting stages. For the screening, four culture media were used: Potato dextrose agar (PDA), Carboxy-methyl cellulose agar (CMC), Minimum medium + rice hulls (RHA) and Minimum medium + shredded paper (SPA) and for the identification, the isolated strains were identified by macro and micromorphologic characteristics. Beside, changes in temperature, electrical conductivity, moisture content, pH and C/N ratio were registered. A total of 300 fungal isolations were obtained. The more represented genera were *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria*, *Epicoccum* and *Trichoderma*. The major number of strains were obtain in the first sampling (mesophilic stage). In the next phase (thermophilic stage), the number of strains declined and in the following stages an increase in number of recovered strains was observed. Physical and chemical data were partially correlated with changes on the fungal diversity. The implications on the process are analyzed.

BD-P4

OCHRATOXIGENIC CAPACITY OF BLACK *Aspergillus* ISOLATED OF SOIL OF VINEYARDS OF DIFFERENT REGIONS FROM ARGENTINA.

Guillermo Giaj-Merlera[1], *Mauricio Barberis*[1], *María M. Reynoso*[1], *Sofía N. Chulze*[1], *Adriana M. Torres*[1]

[1] *Departamento de Microbiologia e Inmunologia, FCEFQ y N, UNRC. (atorres@exa.unrc.edu.ar)*

The different ochratoxin A (OTA)- producing species lives as saprophytes in the superficial cap of the vineyard soil which is the major inoculum source of *Aspergillus* section *Nigri*. There has been postulated that the movement of the air deposits the spores from the soil in the grape surface, thus the risk of contamination with OTA in wines might be related to the presence of toxigenic strains in the soil. The aim of this work was to evaluate the frequency of contamination with OTA- producers species in vineyard soils from different wine-producers regions of Argentina for classic and molecular methodology; and to determine the toxigenic capacity of the strains isolated by thin layer chromatography (TLC). The sampling was done in the wine-producer regions of Mendoza, San Juan, Neuquén, Rio Negro and La Rioja, during the harvest season 2008. We determined the total fungal and *Aspergillus* section *Nigri* count using surface spreading in the media dichloro rose bengal chloramphenicol (DRBC). The strains belonging to the genera *Aspergillus* section *Nigri* isolated were subcultured on agar malt extract, for identification by classical methods, and Czapek yeast extract agar, for subsequent toxin extraction and quantification by TLC. The strains were identified by molecular methods based on PCR. Vineyards with high incidence of *Aspergillus* section *Nigri* were founded the Rio Negro province (General Enrique Godoy and General Roca) with levels of $2{,}9x10^4$ and $2{,}3x10^4$cfu/g, respectively, while the other locations ranged from $9{,}1x10^2$ and $4{,}8x10^3$cfu/g (mean = $2{,}8x10^3$). From a total of 147 strains isolated belonging to *Aspergillus* section *Nigri*, 61 were classified as black *Aspergillus* uniseriated and 81 as biseriated, of which 5 were *A. carbonarius*. Conditions were optimized for PCR amplification for each pair of primers which resulted in the identification of first *Aspergillus* black uniseriate (*A. aculeatus*/*A. japonicus*) *Aspergillus* black biseriate (*A. niger*, *A. tubingensis*) and *A. carbonarius*. CONCLUSIONS:-All soil samples analyzed were contaminated with *Aspergillus* section *Nigri*-The biseriated species were isolated in higher frequency.-The main ochratoxigenic species *A. carbonarius*, was isolated with low-frequency.-Those black *Aspergillus* strains not presenting a distinctive morphological characteristics to be classified by classical taxonomy were possible to identified using PCR with specific primers.-In general, all the strains isolated demonstrated low capacity to produce OTA.

BD-P5

MOLECULAR CHARACTERIZATION OF ENRICHMENT CULTURES OF ANAEROBIC-ARSENIC-RESISTANT AND -REDUCING BACTERIA FROM LAGUNA TURQUESA: A HIGH ALTITUDE SALT FLAT OF NORTHERN CHILE

José A. Lara[1], *Cecilia Demergasso*[2], *Marcela A. Ferrero*[1]

[1] *Lab. Microbiología Ambiental- PROIMI- CCT TUCUMÁN-CONICET* [2] *Centro de Biotecnología, Universidad Catolica de Norte, Antofagasta, CHILE (bioteclara@hotmail.com)*

Contamination with natural arsenic is a well known phenomenon that impacts the environment and is an important issue of current public health. Bio-mining releasing soluble arsenic from rock produces local environments of high and toxic arsenic levels. Arsenic mobility and bioavailability in sediment vary depending on the oxidation state, and this last can be controlled by microbial mediated transformations between the most prevalent types of dissolved arsenic: arsenite and arsenate. Salar de Ascotán (about 234 Km2) is one of the Andean salt marsh of the north eastern Antofagasta region (Chile, 3700 m). This salt marsh shows a very irregular distribution of surficial "evaporitas". Because Laguna Turquesa showed the highest concentration of total arsenic (28 g l-1), sediment samples from this lake were choose for enrichment cultivation with As (V) in anaerobic conditions. The bacteria population composition of arsenic-reducing enrichment cultures obtained was analyzed by 16S rDNA PCR-DGGE. Clone libraries of RAP-PCR-amplified genes fragments were performed using RNA extracted from sediment samples. Bacterial number determined by epifluorescence microscopy was similar (106-107 or 107-108 cells ml-1) between samples from water or sediment, respectively. However, culturable arsenic- reducing bacteria (AsRB) detected by most-probable-number (MPN) ranged around 102 cells g-1. DGGE analysis of 16S rRNA genes amplified from As (V)-reducing culture conditions demonstrated that distinct microbial communities were developed at low and moderated temperature. According to phylogenetic analysis of DGGE bands, the following closet related sequences were found: Alkaliphilus metalliredigens: the representative strain, QYMF, could be grown with Fe(III)-citrate, Fe(III)-EDTA, Co(III)-EDTA, or Cr(VI) as electron acceptors; Bacillus agaradhaerens: an halotolerant-alcalophilic and Fe (III)-reducing bacteria; Clostridiaceae: which metabolize arsenic by producing beta-realgar (arsenic sulfide). Approximately 70 % of DGGE band sequences, recovered from all dependent-culture assays, matched with "uncultured bacteria" sequences. Phylogenetic analysis of cDNA clone library also revealed that the clone LT42 was clustered with Leptospirillum ferriphilum LMT3 (100% in 448 nucleotides). This strain was isolated from an extremely acidic Pb/Zn mine tailings in China. Relevant protein sequences were obtained by using the tblastx program of BLAST: As III S-adenosylmethionine methyltransferase (arsM) gene, responsible for the removal of arsenic as the volatile arsines from the bacteria; S-adenosyl-methyltransferase MraW protein from Salinibacter rubber DSM13855, homolog with ArsM gene; acetyl-CoA acetyltransferase de Mycobacterium gilvum PYR-GCK, which present similarity with a novel arsenate resistance gene, arsN. Our results suggest that novel bacteria with important biotechnological properties could be exploited in this unique and stressed saline environment.

BD-P6

YEAST BIOTA ASSOCIATED WITH TRADITIONAL FERMENTED BEVERAGES IN PATAGONIA

Christian A. Lopes[1], *Julieta S. Saéz*[1], *Marcela P. Sangorrín*[1], *Adriana C. Caballero*[1]

[1] *Laboratorio de Microbiología y Biotecnología, IDEPA (CONICET-U.N.Comahue) (clopes@conicet.gov.ar)*

Several raw sources, including cereals and fruits involved wild fruits such as strawberries (*Fragaria chiloensis*), "maqui" (*Aristotelia chilensis*), "calafate" (*Berberis* spp.), apples (*Malus* sp.), as well as seeds of different plants have been traditionally used by aboriginal communities in Patagonia to prepare different fermented beverages. A particularly interesting fermented beverage is that obtained from seeds of the *Pehuen* tree (*Araucaria araucana*), from which the Mapuche´s communities prepare the fermented beverage called *Mudai*. No works involving the microbial biota present in *Mudai* and other traditional fermented beverages in Patagonia have been published until now.With the aim to investigate this particular yeast biota for the first time, juices obtained from *Pehuen* seeds and wild apples were obtained in the traditional way and fermented in sterile flasks under laboratory conditions (3 fermentations from *Pehuen* seeds and 2 from apple). Yeast sampling in complete medium was carried out during the processes, showing maximum population densities of $1.5x10^8$cfu mL-1. Molecular identification of these colonies by using ITS1-5.8S-ITS2 PCR-RFLP and sequence analyses, revealed the presence of only two yeast species at the beginning of *Mudai* fermentation: *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* in percentages of 80% and 20% of the total biomass respectively. In Apple fermentations, non-*Saccharomyces* yeasts corresponded to 25% and 84% of the total isolates at the begining of the two analyzed fermentations respectively, including isolates belonging to *H. uvarum* and *Metschnikowia pulcherrima*. In subsequent stages of all *Mudai* and apple fermentations, the yeast biota corresponded exclusively to *S. cerevisiae* in all analyzed fermentations.Intraspecific analysis of the *S. cerevisiae* isolates from *Mudai* fermentations by means of mtDNA-RFLP analysis evidenced a single restriction pattern. This kind of biological homogeneity is frequently observed in inoculated fermentations in which the yeast starter selected culture dominate the fermentation process, but it is not expected to occur in natural processes Traditional production of this beverage does

not involve the use of commercial yeasts; however, the environment in which this product is elaborated is in permanent contact with the commercial yeasts used in bread making. MtDNA-RFLP analysis of commercial bakery yeasts showed the same molecular pattern detected in *Mudai* fermentations, evidencing a cross-contamination of yeasts in these traditional fermented products. The intraspecific diversity in *S. cerevisiae* populations from apple fermentation samples are being currently analyzed in order to find indigenous *Saccharomyces* isolates and to evaluate its phylogenetic relationships with regards to wine strains of this species.

BD-P7

METABOLIC AND MOLECULAR DIVERSITY OF BACTERIAL COMMUNITIES INHABITING OLIGOTROPHIC-PSYCHROPHILIC LAKES FROM NORTHERN-PATAGONIA, CHILE.

Roy Mackenzie[1], *Javier A. Barros*[1], *Miguel A. Martínez*[1]

[1] *Laboratorio de Microbiología Básica y Biorremediación, Universidad de Concepción. Concepción, Chile. (rmackenzie@udec.cl)*

Bacteria from oligotrophic-psychrophilic aquatic ecosystems must be able to adapt and survive environmental oscillations. In addition, they possess adaptive strategies that allow them to efficiently compete against other microorganisms for nutrients and substrates. In cold environments on different geographic areas, bacterial species could be phylogenetically related and share physiological properties, being able to metabolize a wide range of substrates. These properties could allow them to survive and maintain metabolic activity at low temperatures. Hence, the characterization of the microbial diversity in the active bacterial proportion allows obtaining information related to the ecosystem functioning, as well as to speculate the ecological role that every group could be playing in it. The aim of this work was to determine bacterial metabolic and genetic diversity in three superficial water samples of oligotrophic and psychrophilic Patagonian lakes. Water samples were obtained from lakes Alto Reino, Las Torres and Venus (XI Región of Aysén, Chile). The genetic and metabolic diversity were evaluated by DGGE and Biolog Ecoplate system respectively, using broth samples enriched and non-enriched with R2A. Additionally, total bacterial counts and live/death proportions were determined by epifluorescence microscopy. The viable bacterial count was performed through the micro-drop method using R2A agar. The results indicated that the three lakes share a high similarity in both 16S rDNA profile (>70%) and metabolic activity (86%). Also, the metabolic diversity index showed an equal use of the available substrates in the Biolog Ecoplate system. Nevertheless, no D-xylose was use as a carbon source by any bacterial population. The enriched water samples showed a less diverse 16s rDNA profiles, indicating a diminished bacterial diversity as compared with non-enriched samples. On the other hand, the proportion of live bacteria in water samples was near to 10%, and less than 1% of CFU were cultivable on R2A medium. These results demonstrate the low representativeness of culture-dependent analysis in microbial ecology studies. The results suggest that under these environmental conditions, the proportion of cells that are actively involved in bacterial production is low in comparison with the total bacteria present in an aquatic ecosystem. Moreover, these results show that bacteria inhabiting oligotrophic-psychrophilic Patagonian lakes are genetic and metabolically similar, probably due to their geographical proximity and phisico-chemical patterns. We conclude that despite the similarity between the evaluated lakes, each one of them has a particular and unique bacterial group that differentiate and characterize each other.

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BD-P8

CAPSULE DETECTION OF *Streptococcus equi subsp. zooepidemicus*

Alejandra J. Muñoz[1], *Solange Gonzalez*[1], *Nora Guida*[1]

[1] *Cátedra de Enfermedades Infecciosas. Facultad de Ciencias Veterinarias. UBA (alejandrajmunoz@gmail.com)*

Streptococcus equi subsp. zooepidemicus is a Lancefield group C, β hemolytic streptococcus that is considered a part-mucosal commensal of the oral cavity, pharynx, and reproductive tract of horses. It causes disease as an opportunistic pathogen of the respiratory tract (rhinitis, bronchitis, pneumonia) and reproductive tract (endometritis, placentitis and abortion) of horses. These bacteria can be isolated from infections in a wide variety of animals including pigs, monkeys, sheep, cows, goats, foxes, birds, rabbits and guinea pigs. The isolation from humans has been reported from cases of endocarditis, meningitis, septic arthritis and cervical lymphadenitis. The hyaluronic acid capsule is thought to be one of the critical virulence factors of *Streptococcus equi subsp. zooepidemicus*. It is a high-molecular weight polymer consisting of alternating residues of N-acetylglucosamine and glucuronic acid. The antiphagocytic capsule greatly reduces the number of streptococci that become associated with the surface of neutrophils and are subsequently ingested and killed. This research was designed to study the presence of hyaluronic acid capsular and growth properties of the bacteria in fluid media and in soft agar. The capsule detection has been correlated with electron microscopy. A total of 46 strains of *Streptococcus equi subsp. zooepidemicus* were investigated in this study. Twenty streptococci were isolated from vagina swabs and other thirteen streptococci samples were taken from clitoral sinus swabs of healthy mares. Thirteen streptococci were isolated from nasopharyngeal swabs from healthy horses. The swabs were cultivated in blood agar plates overnight at 37°C in an atmosphere of 5 % (v/v) CO_2, streptococci were identified by catalase test. The streptococcal group was detected by conducting a latex agglutination test (Oxoid). The Api 20 Strep (Biomerieux) was used to identify the species of strains and a Multiplex Polymerasa Chain Reaction was conducted in order to obtain amplification of internal parts of the genes sodA and seeH. The strains were cultivated in Todd Hewitt Broth with 0.2 % of yeast and 10 % of horse serum overnight al 37°C. For capsule detection we made a smear consisting of a drop of bacterial suspension and a drop of India ink solution. The capsule was observed in the form of an unstained halo around purple-colored cells. The growth properties of the bacteria were studied in fluid media (Todd Hewitt Broth), as a uniformly turbid growth or as a sediment with clear supernatant; and in soft agar (Todd Hewitt Broth with 0.15% agar), as diffuse colony formation or as compact colony formation. We could see that the capsule was present in many strains by staining, with both optical and electronic microscope, and we can relate this to the presence of the capsule and the growth properties of the bacteria. *Suported by UbaCyt V013*.

BD-P9

GENOTYPIC CHARACTERIZATION AND SYMBIOTIC PROPERTIES OF SLOW-GROWING NATIVE ISOLATES OBTAINED FROM PEANUT ROOT NODULES

Vanina Muñoz[1], *Fernando Ibañez*[1], *Adriana Fabra*[1]

[1] *Departamento de Ciencias Naturales, FCEFQyN, Universidad Nacional de Río Cuarto (vaninamunoz@yahoo.com.ar)*

Peanut (*Arachis hypogaea* L.) is a widespread leguminous plant of great economical significance. Argentina is one of the main peanut producer countries in the world and 94% of its production is concentrated in the province of Córdoba. The native bacterial population associated with peanut nodules in Córdoba is highly heterogeneous and includes both slow- and fast-growing rhizobia. In this work, the genetic diversity and the symbiotic effectiveness of a population of slow-growing isolates obtained from nodules of peanut growing in six different locations (Pizarro, Suco, Río Cuarto, Charras, Santa Eufemia and Las Higueras) in Córdoba, Argentina, were determined. The analysis of the symbiotic properties revealed differences among the isolates, even when they were all capable of inducing the formation of several nodules in peanut and of significantly increasing the shoot dry weight of plants. The genotypic characterization was performed by ERIC-PCR, using primers E1 and E2. ERIC-PCR profiles were analyzed with Cross Checker and FAMD softwares, using the Dice similarity coefficient and UPGMA clustering method to construct a dendrogram. The analysis of the tree revealed that, at a 60% similarity, three clusters could be recognized. Cluster I grouped the four isolates from Charras, as well as two isolates from Pizarro. Cluster II included isolates from Pizarro, Suco, Las Higueras, and the reference strain SEMIA 6144, recommended as inoculant. Cluster III grouped isolates obtained from Pizarro, Rio Cuarto and Santa Eufemia. The results obtained in this work indicate that the population of slow-growing native isolates from Córdoba is genetically diverse, and it encompass isolates that exhibit different symbiotic properties. The understanding of the genetic diversity and the symbiotic effectiveness of the slow growing population can result in the selection of rhizobial strains for developing inoculants.

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BD-P10

PHYLOGENETIC ANALYSIS OF *Synechococcus marinus* STRAINS DETERMINED FROM DNA SEQUENCES OF 16S RRNA AND OF THE N-REGULATORY GENE *ntcA*

Macarena Pérez-Cenci[1,2] , Gonzalo Caló[1,2] , Ricardo Silva[3], Rubén Negri[3], Graciela L. Salerno[1,2]
[1] CEBB-MdP-CONICET [2] CIB-FIBA [3] INIDEP (gcalo@fiba.org.ar)

Oxygenic photoautotrophs of the genera *Synechococcus* and *Prochlorococcus* dominate the marine picoplankton over vast tracts of the world's oceans and contribute significantly to primary production. While *Prochlorococcus* are largely confined to a 40ºN-40ºS latitudinal band, *Synechococcus* are distributed ubiquitously throughout oceanic regions, ranging from polar through temperate to tropical waters and are generally more abundant in nutrient-rich surface waters. The wide oceanic distribution of *Synechococcus* is mirrored by high physiological and genetic diversity. To date little is known about the prokaryotic phytoplankton diversity in the Southwest Atlantic Ocean. The aim of this work was to characterize at the molecular genetic level, *Synechococcus* strains from the Argentinean Sea and to carry out phylogenetic analyses based on both the ITS of the 16SrRNA gene and on the *ntcA* gene, which encodes a transcriptional activator (NtcA protein) that mediates the control of nitrogen and carbon metabolism by ammonium. Interestingly, it was shown that expression of *ntcA* is a useful indicator for determining the nitrogen-status of cyanobacteria, and hence that of the marine environment. After microscopic observation, two *Synechococcus* strains were isolated and kept in an appropriate growing medium. Total DNA from isolated strains or from environmental samples was used for PCR amplification of either 16SrRNA of ntcA gene fragments, and environmental libraries were constructed and maintained in *Escherichia coli* cells. Essentially identical tree topologies were obtained for the phylogenetic analysis of *ntcA* sequences supporting the phylogenetic relationships obtained with 16srRNA. Also, amplicons from both gene fragments were obtained from DNA of cultivated *Synechococcus* strains or DNA from environmental samples and analyzed by Denaturing Gradient Gel Electrophoresis (DGGE). After visualization, the gel bans were excised and the purified DNA samples were sequenced. We conclude that *Synechococcus* populations belonging to Clade I dominate in the Argentinean Sea.

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BD-P11

THE FAMILY DIATRYPACEAE (ASCOMYCOTA) IN ARGENTINA: NEW CONTRIBUTIONS TO ITS KNOWLEDGE.

Martín R. Proia[1], Susana Pereira[1], Cecilia Carmarán[1]
[1] PHHIDEB-CONICET, Departamento de Biodiversidad y Biología Experimental, FCEN, UBA (martinproia@hotmail.com)

Since 1898 Spegazzini had studied the biodiversity of the Diatrypaceae in Argentina and described 31 species and several varieties. In recent years, the knowledge of diatrypaceous fungi in this country has increased substantially. The main difficulty in identifying the diatrypaceous fungi is the high variability of many of the characters used for species discrimination. Sometimes, the character that should mark different entities can be observed within the same species. For example, some species have been described as having perithecia with necks emerging either in group or separately, asci with either J+ or J- apical ring, ascospores with overlapping size ranges, and some characters can be very variable, for example the development of stromata or the length of necks. Therefore, it is important to describe the specimens thoroughly and to indicate the most important characters for each species. This study is an additional contribution to the extensive project aiming at describing the diatrypaceous fungi on native and cultivated woody plants in Argentina. The sampling and collecting methods have been previously explained. Specimens were preserved in the Herbarium of Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina (BAFC). Samples for light microscopy were prepared from moistened specimens mounted in distilled water, 5% KOH, phloxine and Melzer's reagent. Samples for epifluorescence light microscopy (EFM) were prepared in 0.05 % calcofluor. In the present contribution we focused on Tucuman´s province biodiversity. Previous reports have registered only two species belongs to this family, in the present work ten new records from Tucuman are provided, and we present the distribution of diatrypaceous fungi in Argentina.

BD-P12

BIOSPAS, A COMPREHENSIVE SOIL BIOLOGY PROJECT IN ARGENTINA

Luis G. Wall;[1], F Acosta;[2], OM Aguilar (2); JC Bedano (2); A Bianchini;[2], M Carmona (2); L Dominguez (2) ; D Ducasse;[2], L Erijman (2); J Galantini (2) ; M Gally;[2], G González Anta (2); G Guerra (2) ; M Iglesias;[2], S Lorenzatti (2); R Marona (2) ;H Morrás;[2], J Romagnoli ; A Romero;[2], C Valverde;[1], A Zorreguieta[2]

[1] Programa Interacciones Biológicas, DCyT, UNQ [2] Affiliations: see web page of the project at www.biospas.org (cvalver@unq.edu.ar)

BIOSPAS is the Spanish acronym for *Soil Biology and Sustainable Agricultural Production*, a Research Project organized under the auspices of the Argentinean Ministry of Science, Technology and Productive Innovation. The project combines a consortium of scientists belonging to twelve public academic institutions, a non-governmental organization and two private agro-companies. The aim of this project is to generate the knowledge that will allow us to understand the dynamics between the biological processes taking place in the soil and productivity in no-till agricultural practices. BIOSPAS proposes that efficient and sustainable production of agricultural commodities can take place in an increasingly environment-friendly manner on the basis of deeper, more detailed and comprehensive knowledge of the systems we manage. BIOSPAS aims to put the creativity of academic institutions into action, and by doing so, to boost our potential for the development and creation of wealth and value. The study of the soil carried out in this Project coordinately monitors several qualitative and quantitative biological and edaphic parameters: organic matter fractions; enzymatic activities; fatty acids profiles; glomalin fraction; microbial diversity on a DNA basis; metagenomic analysis of nitrogen cycle and P mobilization; culturable bacteria with emphasis on pseudomonads, P-solubilizers, free N2-fixers and cellulolytics; meso and macrofauna; biofilm formation; mycorrhizae analysis; pathogens and diseases expressed in the study areas; physics and microorganisation of soil structure; and mathematical analysis of all generated data. Our goal is to understand how these parameters are related to each other in different management systems, and to what extent they explain productivity variation. We will compare at least three systems or uses of soil: 1) very good agricultural management with historically no-till management, which is regarded as a sustainable practice with intensive crop rotation and nutrient reposition, 2) their counterpart, mono-cropping without reposition, which is definitely unsustainable, and 3) natural environment, as baseline or control. Due to its magnitude and complexity, BIOSPAS is organized into six different thematic projects with independent funding instruments, in which several research groups participate. BIOSPAS is interested in cooperation with similar or complementary projects operating in Argentine and in other countries. For further information visit the BIOSPAS Web page: www.biospas.org.

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BB-P1

ISOLATION AND CHARACTERIZATION OF *Escherichia coli* O157:H7 12900 MUTANTS RESISTANT TO PHAGE Φ CEV2, Φ 120, Φ 6 AND Φ 65 INFECTION.

María C. Aristimuño Ficoseco[1], *María J. Olaya Passarell*[1], *Elvira M. Hébert*[1], *Raúl R. Raya*[1]

[1] *CERELA -CONICET (ceciaristi@cerela.org.ar)*

Escherichia coli O157:H7 is an endemic pathogen causing a variety of human diseases including mild diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic which is carried without showing symptoms of infection. The main source of infection in humans is the consumption of contaminated and poorly cooked meat. The reduction or elimination of this pathogen from its host environment could greatly reduce human exposure and as such be key in disease prevention. Our group is exploring the role of a cocktail of bacteriophages as a strategy to eliminate or control the presence of this pathogen in food. In this work, mutants of the strain *E. coli* O157:H7 12900 resistant to four phages (Φ CEV2, Φ 120, Φ 6 and Φ 65) were isolated and characterized. Cross infection experiments showed that the four phages recognized different receptors in the sensitive cells: phages Φ 120, Φ 6 and Φ 65 recognize the same receptor which is distinct to the receptor FhuA recognized by phage Φ CEV2. All the mutants isolated, except those resistant to phage Φ 6, were confirmed to be adsorption mutants. Our results suggest that at least two of the four phages (i.e., Φ CEV2 and Φ 120) could be considered in the design of a cocktail of bacteriophages to be applied in the biocontrol of *E. coli* O157:H7.

BB-P2

CU(II), CD(II), ZN(II) AND CR(VI) EFFECT ON *Pseudomomas veronii* 2E CHEMOTAXIS: HOW CAN WASTEWATER BIOTREATMENT EFFICIENCY BE AFFECTED?

Matias R. Barrionuevo[1], *Diana L. Vullo*[1]

[1] *Área Química, Instituto de Ciencias, Universidad Nacional General Sarmiento, Los Polvorines, Bs. As. (dvullo@ungs.edu.ar)*

Several species of *Pseudomonas* genus are being exploited in the development of innovating technologies for industrial waste treatments. Fixed-bed reactors are usually designed for organic compounds biodegradation or metal retention processes, where the biofilm establishment and maintenance play the most important roles. Chemotaxis is a mechanism which determines the bacterial biofilm final properties, which can be influenced by the conditions of the microenvironment. The aim of our work is to study the effect of Cu(II), Cd(II), Zn(II) and Cr(VI) on *Pseudomonas veronii* 2E chemotaxis, to improve biofilm development for metal loaded wastewater biotreatment in fixed-bed bioreactors. Chemotactic response was evaluated using a modified "chemical in plug" assay, based on the characteristics of the isolated *Pseudomonas veronii* 2E. This strain usually grows forming aggregates which make difficult chemotaxis visualization. For an homogeneous bacterial suspension achievement, SDS 0.1 % was added to PYG broth (casein peptone 2.5 g/L, yeast extract 1.25 g/L, glucose 0.5 g/L) and exponential phase cultures in 0.3 % agarose were spread on plates with a central plug of PYG-0.5% agarose as chemoattractant spiked with 0.3-1.5 mM Cu(II), Cd(II), Zn(II) or Cr(VI). Control experiments were carried out without PYG in presence of 1 mM of each metal. Results were obtained after four hours at 32ºC. Cd(II), Zn(II) and Cr(VI) presence enhanced positive chemotaxis from 0.3 to 1 mM while Cu(II) did not evidence any effect at the assayed concentrations. Interestingly, control chemotaxis experiments performed only with metals demonstrated that at concentrations higher than 1 mM, Cu(II), Zn(II) or Cd(II) were clearly chemorepelents meanwhile no effect was registered for Cr(VI). As a conclusion, the chemotaxis mechanism is not being affected by metal presence at typical industrial effluent concentrations and is enhanced when a chemoattractant is present. From this point of view, and complementary to the results previously obtained by swimming and swarming experiments, biofilm development would not have been directly influenced by Cu(II), Cd(II), Zn(II) or Cr(VI). These results reinforce the suitability of the application of *Pseudomonas veronii* 2E in fixed-bed reactors for wastewater treatments, process which is now in progress.

BB-P3

CADMIUM CAUSES OXIDATIVE DAMAGE IN *Bradyrhizobium* sp. STRAINS AND PEANUT PLANT.

Eliana C. Bianucci[1], *Jésica P. Rivadeneira*[1], *Adriana Fabra*[1], *Stella Castro*[1]

[1] *Dpto. Ciencias Naturales. Fac. Cs. Exactas, Fco-Qcas y Nat. Universidad Nacional de Río Cuarto. (ebianucci@exa.unrc.edu.ar)*

Cadmium is one of the most harmful heavy metals in nature and its high toxicity even at low concentration, represents a serious threat to microorganisms and plants of high agronomic value. Its entry in the agricultural soils arises from commonly used practices such as the application of phosphate fertilizers that contain Cd as a trace element. Some of the deleterious effects induced by Cd have been associated with alterations in the oxidative status of the cell. Microorganisms and plants posses both enzymatic and non-enzymatic defense systems to maintain the cellular redox state and to mitigate the damage caused by oxidative stress. The molecule of glutathione (GSH) and the enzymes related to its metabolism glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione S-transferase (GST) are known to be involved in the defense mechanism against heavy metals. In previous works, we studied the effect of Cd on growth and GSH content in *Bradyrhizobium* sp. strains (peanut microsymbionts). *Bradyrhizobium* sp. SEMIA6144 was sensitive while *Bradyrhizobium* sp. NLH25 was tolerant since they grew up to 10 and 30 µM Cd, respectively. The increase in the GSH content in the tolerant strains suggested that this thiol is involved in tolerance. The objectives of this work were to: 1) elucidate the role of GR, GPx and GST enzymes in Cd tolerance in *Bradyrhizobium* sp. strains; 2) evaluate the effect of Cd on peanut growth. The microorganisms were grown in YEM medium supplemented with the maximum Cd concentration that allows them to grow. Plants were grown in Hoagland medium at different Cd concentrations ranging from 0 to 100 µM. *Bradyrhizobium* sp. strains showed that GR activity increased only in the tolerant strain; meanwhile GPx activity increased in both strains exposed to Cd. In contrast, GST activity decreased in both strains with Cd. The capacity to maintain GSH/GSSG ratio mediated by the increase of GR activity, could be one of the causes of tolerance in *Bradyrhizobium* sp. NLH 25. At 10 µM Cd, peanut growth was affected and an enhanced concentration of 2-thiobarbituric acid reactive material (lipid peroxidation products) in roots was observed, presumably due to Cd-induced oxidative stress.

BB-P4

BIODEGRADATION KINETICS OF A MIXTURE OF CHLOROPHENOLS

Ignacio Durruty[1], *Elena Okada*[1], *Jorge F. González*[1], *Silvia E. Murialdo*[1]

[1] *Grupo de Ingeniería Bioquímica, Fac. de Ingeniería UNMdP (durruty@fi.mdp.edu.ar)*

Chlorophenols (CPs) have been extensively used as a wood preservative, pesticide, and fungicide. CPs are persistent and highly toxic, thus they are rated as priority pollutants by the US EPA. Frequently, these compounds are present in organic chemical mixtures in wastewater from industrial and municipal sources, therefore the study of biodegradation on mixed pollutants is an important aspect of biodegradation and wastewater treatment. In this work, we studied the multisubstrate degradation of CPs by a mixed culture of *Pseudomonas aeruginosa* and a novel *Acromobacter sp.* capable of using pentachlorophenol (PCP), 2,4,6 trichlorophenol (TCP) and 2,3,5,6 tetrachlorophenol (TeCP) as a sole carbon and energy source. The main objective of this work was to study the effect of substrate mixtures on the degradation kinetics of PCP. Batch experiments were conducted with each CP separately and in mixtures of PCP + 2,4,6 TCP, PCP + 2,3,5,6 TeCP and PCP + 2,4,6 TCP + 2,3,5,6 TeCP. The removal efficiency and cell growth yield of each CP was compared to the results obtained in the mixtures. The kinetics values for PCP and 2,4,6 TCP were obtained by fitting of Monod kinetics model. On the other hand, during the first 80 hours of the assay 2,3,5,6 TeCP was not mineralized, when present as the only source of carbon and energy or in a mixture with PCP. In the mixture of PCP + 2,3,5,6 TeCP, the degradation of PCP was also inhibited. In the simultaneous degradation experiment of PCP + 2,4,6 TCP an enhancement on the removal of PCP was observed. In the mixture of all the tested CPs, a greater degradation rate was observed for PCP and TeCP, compared to the results obtained as a single substrate. The values obtained by fitting were used in the multi substrate kinetic model to simulate the binary mixture behavior. The results showed that the model can predict multisubstrate degradation of PCP and 2,4,6 TCP.

BB-P5

LEAD BIOSORPTION BY LACTIC ACID BACTERIA ISOLATED FROM KEFIR

Esteban Gerbino[1], *Emma E. Tymczyszyn*[1], *Pablo Mobili*[1], *Andrea Gómez-Zavaglia*[1]

[1] *Centro de Investigación y Desarrollo en Criotecnología de Alimentos -CIDCA-(UNLP-CIC-CONICET) (egerbino@cidca.org.ar)*

Lead is a toxic heavy metal, that unlike many other toxic compounds is not degradable and tends to accumulate in the exposed organisms (WHO, 1995). Food and water are the primary sources of lead exposure. In spite of the fact that natural concentrations of lead in water rarely exceed the guideline value (10 ug/l) (WHO 2006), different anthropogenic sources, such as fertilizers used in agriculture, metal mines and smelters, can increase the concentration of this metal. For this reason, removal of lead from food and water represents a challenge. Available conventional methods (precipitation, flocculation, ion exchange, and membrane filtration) for removal metals from water at low concentrations are claimed to be expensive and inefficient (Atkinson et. al 1998). In this work, two strains of *Lactobacillus kefir* (*Lactobacillus kefir*JCM 5818 and *Lactobacillus kefir* CIDCA 8348) isolated from kefir grains were assessed for their adsorption properties to remove lead from water. Microorganisms were grown in 10 ml LAPTg (Raibaud, P. *et al*1973) broth at 30 ºC for 48 h, harvested and washed twice with milli Q water. The pellets obtained were suspended in 1 ml of different concentrations of lead (0-50 mg/l) and further incubated for 24 hs at 30 ºC. After that, the suspensions were centrifuged and the lead concentration in the supernatant was measured by atomic absorption spectrometry. Lead removal ability of the studied strains was evaluated using a Langmuir isotherm according to Davis *et al.*, 2003. After that, the maximum binding capacity (*q* max) and the coefficient related to affinity of binding (*b*) were determined for each strain. These parameters (*q* max and *b*) were calculated as 26.9 mg/g and 0.32 mg/l for *L. kefir* 8348 and 44.6 mg/g and 0.36 mg/l for *L. kefir* 5818, respectively. The binding of lead increased with the metal concentration until a saturation point was reached. The maximum lead percentage removal was higher for *L. kefir* 5818 than for *L. kefir* 8348, and this could be assigned to differences in the chemical composition of the bacterial surfaces. Taking into account the high efficiency of both strains in lead removal, they could be considered as an interesting alternative in decontamination processes.

BB-P6

Paecilomyces lilacinus* (ASCOMYCOTA: ANAMORFOS): POTENTIAL AGENT FOR BIOCONTROL OF THE LEAF-CUTTER *Acromymex lundii

Daniela Goffré[1], *Norma B. Gorosito*[1], *Patricia J. Folgarait*[1]

[1] *Ant Laboratory, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, B1876BXD Bernal, Argentina (pfolgarait@unq.edu.ar)*

Leaf cutter ants are well known as pests in Latin America, because they cut leaves to cultivate their symbiotic fungus (Basidiomycetes) from which they feed, causing losses in the yield of several crops. Traditionally these ants have been chemically controlled, but these compounds are known for being toxic to non target organism and sinks. An environmentally friendly strategy to reduce the number of the leaf-cutter ants is the biocontrol. In this work we evaluated for the first time the effectiveness of the entomopathogen *Paecilomyces lilacinus* (Ascomycota: Anamorph) to control the leaf cutter ant *Acromyrmex lundii*. Ants from two *A. lundii* colonies were inoculated with conidia of *P. lilacinus*. Two methodologies were tested: method A) all ants were inoculated in the same container, by bath immersion in a conidial solution; method B) each ant was immersed in a separate conidial solution with the same concentration. Before the inoculation with the entomopathogen, the ants were externally disinfected in a solution of sodium hypochlorite. Afterwards, ants were inoculated with *P. lilacinus*. After the death of each ant, they were again externally disinfected with a higher concentration of sodium hypochlorite solution. We also set-up untreated ants as controls, which were only externally sterilized. Each ant was individually followed to record the date of death, the appearance of hyphae of the entomopathogen and subsequent sporulation. With these data we developed the survivorship curves for the treatment and control for each methodology. The ants treated under the methodology A, reached 100% of mortality at the fifth and eighth day after the inoculation, for colonies 1 and 2, respectively; while the ants treated under the methodology B reached 100% and 90% of mortality on the fourth day for both colonies used. All ants with *P. lilacinus* exhibited a lower survivorship compared with the controls. With method A, we found 50% of co-occurrence of *P. lilacinus* and *Fusarium* spp in the bodies of dead ants, whereas with method B the co-occurrence was much lower (29% and 5% for colonies 1 and 2, respectively). With method A, *P. lilacinus* was found alone in 18% and 13% for colonies 1 and 2, respectively; while the methodology B yielded 59% and 75% *P. lilacinus* alone, for each colony, respectively. Our results showed for the first time the potential of *P. lilacinus* to be use as biocontrol agent for *A. lundii*. Finally, we recommend the use of individual ant bath immersion for the inoculation of the conidia because otherwise the group conidia bath may spread the contamination of non completely disinfected ants during the first sterilization.

BB-P7

COMBINED TREATMENT, SORPTION ON HULLS AND BIOLOGICAL DEGRADATION OF CHLOROPHENOLS IN AQUEOUS SOLUTION.

Sandra S. González[1], *Silvia E. Murialdo*[1], *Jorge F. González*[1]

[1] *Grupo de Ingeniería Bioquímica- Facultad de Ingeniería-Universidad Nacional de Mar del Plata (ssgonzalez@fi.mdp.edu.ar)*

The dumping of toxic aromatic structure such as anthropogenic organochloride pesticides, (e.g. pentachlorophenol PCP) is considered an important environmental risk factor. This report gives an overview of experiences using two combined techniques of wastewater treatment, a subsequent sorption on hulls and biological degradation. Local agricultural residues, whose disposal often creates problems, were used as sorbents, and a properly acclimated mixed culture of bacteria was used as bioremediator agent. Adsorption equilibrium studies were conducted using PCP, depending on the concentration of pollutant load on sunflower seed hulls. Sunflower husks from Pcia. Buenos Aires surroundings, without previous treatment were used and were provided by Cargil SA, Quequén, Pcia Buenos Aires. The solutions of PCP were prepared at the laboratory from a stock solution of 80 mg/l (ppm) of at T = 25 ° C. The solutions were placed in 50 ml erlenmeyer flasks with 1 g per 50 ml of shells, and the equilibrium condition was reached after one hour of contact time. Then, the supernatant and the wet hulls were removed separately from the resulting solution. Mineral salts solution was added to the supernatant fluid and was inoculated with a 10% (v/v) bacteria mixed culture (*Pseudomonas aeruginosa* and a novel *Acromobacter* sp.) On the other hand, the PCP adsorbed on the shells was solubilized with an excess of Na (OH) (20 N). Then, their pH levels were adjusted below about 7 with HCl, and the same procedure as with the supernatant was followed. PCP remaining in the liquid was measured through high pressure liquid chromatography HPLC. It was found that PCP-adsorption could be adjusted by the Temkin isotherm and occurred in a short period (less than 1 hour) which showed to be a rapid method to extract most of the PCP from the solution. We hypothesized that the main feature by which the adsorption occurred was the amount of fatty acids contained in the shells that contributed to the adsorption of PCP, which is lipophilic. Furthermore, the amount of PCP remaining in the supernatant was degraded by biological action. Taking advantage of its low initial PCP concentration, the mixed culture showed to be highly effective. Likewise most of the PCP adsorbed on the shells was degraded completely at a stage biological process. For these reasons, these physico-biological combined techniques showed to be an efficient and economic solution for the treatment of PCP-wastewater. Keywords: Combined treatment, physico-biological treatment, sorption on hulls, biodegradation, pentachlorophenol, bioremediation, bacteria.

BB-P8

FUNGAL AND MYCOTOXIN CONTAMINATION OF BALED CORN SILAGE AND ISOLATION OF AFB_1 BINDING *S. cerevisiae* STRAINS.

María L. González Pereyra[1], *Carina E. Magnoli*[1], *Lilia R. Cavaglieri*[1], *Ana M. Dalcero*[1]

[1]*Fac. de Cs. Exactas, Fisico-Químicas y Naturales. Universidad Nacional de Río Cuarto. (mgonzalezpereyra@exa.unrc.edu.ar)*

Baled silage is a preservation method for corn and other feedstuffs based on lactic fermentation under anaerobic conditions (Miller, 2001). In Argentina, corn silage constitutes a significant energy source in cattle diets. Fungal and mycotoxin contamination reduce nutritional value of ensiled feedstuffs and represent a hazard to human and animal health. The use of natural products to reduce mycotoxin contamination in animal production systems is a matter of increasing interest. The presence of yeasts in ensiled feedstuffs is a constant and they are often isolated in high counts. The aims of this work were a) to determine the natural incidence of toxigenic fungi and the mycotoxins aflatoxin B_1 (AFB_1) and patulin (PAT) present in baled corn silage samples and b) to isolate yeast strains able to bind AFB_1 with biocontrol potential. Thirty-five samples were collected from the province of San Luis, Argentina. Total fungal counts (CFU/g) were evaluated onto DRBC and DG18 culture media. Isolation frequency and relative density of *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. were calculated. Potentially toxigenic isolates were evaluated for their ability to AFB_1 and PAT. Natural AFB_1 and PAT contamination were quantified by HPLC. Yeast isolates were identified and a *S. cerevisiae* strain was selected to evaluate its ability to bind AFB_1 to its cell wall *in vitro*. Ninety percent of the samples showed counts higher than the limit recommended as a quality standard for feeds (1×10^4 UFC/g) (GMP, 2008). The prevalent fungal genera were *Aspergillus* spp. (63%), *Penicillium* spp. (31%) and *Fusarium* spp. (11%). Yeasts isolation frequency was 97%. Main toxigenic species were *A. flavus*, *A. fumigatus*, *P. griseofulvum*, *P. paneum*, *P. roqueforti* y *F. verticillioides*. Seventy percent of *A.flavus* strains were able to produce 3 to 112.5 µg AFB_1 per g of micellium. One *P. griseofulvum* and 1 *P. paneum* isolate produced 366.8 µg/g and 14.2 µg/g PAT, respectively. Seventy-five percent of the samples showed AFB_1 contamination containing 5 to 50 µg/kg. PAT was not detected in any of the samples. Seventy-seven percent of yeast isolates corresponded to *Candida* genera, being *C. kruzeii* (57%) and *C. parapsilopsis* (43%) the most frequent species. Twenty-three percent of the total yeast isolates were identified as *S. cerevisiae*. *Saccharomyces cerevisiae* strain RC008 showed ability to bind 38.2% to 67.6% AFB_1 *in vitro*. Screening for fungi and mycotoxins in corn silage and other animal feedstuffs is essential for preventing mycotoxicosis and

chronic intoxications. Yeasts able to bind AFB_1 such as *S. cerevisiae* could be playing an ecological role in silage as potential detoxifying agents.

BB-P9

EVALUATING THE CONTRIBUTION OF ANTIBIOTIC-PRODUCING BACTERIAL SYMBIONTS OF LEAF-CUTTING ANTS TOWARDS GENERAL ENTOMOPATHOGENIC FUNGI

Norma B. Gorosito[1], Michael Poulsen[2], Cameron Currie[2], Patricia J. Folgarait[1]

[1] Ant Laboratory, Universidad Nacional de Quilmes, Roque Saenz Peña 352, B1876BXD Bernal, Argentina [2] Department of Bacteriology, University of Wisconsin, Madison, 1550 Dr Linden,Madison, WI 53706, USA (ngorosito@unq.edu.ar)

Higher attines in the genus *Atta* and *Acromyrmex* are commonly referred as leaf-cutter ants. These ant species are widely known because they cultivate the fungus *Leucoagaricus* sp. (Basidiomycotina) as a food source. To cultivate the fungus, the ants collect fresh leaves from a variety of different plant species. Leaf-cutter ants have an obligate symbiosis with the basidiomycete, which cannot be found free living. The symbiotic leaf-cutter-*Leucoagaricus* association is threatened by a specific pathogen in the genus *Escovopsis* (Ascomycotina), which is a specialized microparasitic fungus, which can destroy the fungus cultivated by the ants. To avoid this microparasite the leaf-cutters are known for having in their exoesqueleton a filamentous bacterium *Pseudonocardia* spp. (Actinomycetales), which produces antibiotics that inhibit the growth of *Escovopsis*. Our goal was to evaluate whether the *Pseudonocardia* bacteria also play a protective role from other pathogens than *Escovopsis*. To test this we inoculated *Acromyrmex octospinosus* and *A. hispidus fallax* with different strains of *Metharhizium anisopliae* and *Beauveria bassiana*, two generalist entomopathogenic fungi. Both ant species have visible *Pseudonocardia* on their bodies; however, not all ants within a given colony carry a full cover of the filamentous bacteria. We predicted that if the cover with *Pseudonocardia* helped in defending the ants against the entomopathogenic fungi, ants without visible *Pseudonocardia* would die faster than ants with visible *Pseudonocardia*. We inoculated both fully covered and uncovered ants with the entomopathogens and compared their survivorship. We found a high mortality rate under both treatments from both ant species. However, different entomopathogen strains showed different levels of virulence in both ant species. Our results suggest that *Pseudonocardia* does not play a role against generalist insect pathogens. In Argentina, leaf-cutters are considered a pest species in human agriculture, so our results support that *Metarhizium* and *Beauveria* can be used for biocontrol and that the antibiotics produced by the *Pseudonocardia* present on these ant species will not interfere with the effectiveness of the mentioned biological control agents.

BB-P10

MYCOPARASITES ISOLATED FROM *Acromyrmex* spp AND *Atta* spp COLONIES: ANTAGONISM AND MYCOPARASITISM

Norma B. Gorosito[1], Matias Cafaro[2], Deborah Colman[1], Patricia J. Folgarait[1]

[1] Ant Laboratory, Universidad Nacional de Quilmes, Roque Saenz Peña 352, B1876BXD, Bernal, Argentina [2] Department of Biology, University of Puerto Rico – Mayaguez, Call Box 9000, Mayaguez, PR 00681 (ngorosito@unq.edu.ar)

Leaf cutter ants belong to the New World, primarily distributed in the Neotropical region. This ant species have an obligate relationship with the fungus *Leucoagaricus* spp (Basidiomycotina), which the ants cultivate to feed from it the structures called ‘‘gongylidia”. To cultivate the fungus the ants collect fresh material from a variety of plant species. The symbiotic relationship leaf cutters-*Leucoagaricus* is threatened by a specific mycoparasite: *Escovopsis* spp (Ascomycotina: Hypocreales). This mycoparasite can completely destroy the colonies of the leaf cutters by parasitizing their food. The ant species that belong to the genera *Atta* and *Acromyrmex* are considered pests due to the high density they achieve and enormous amount of fresh biomass they cut. Usually this ant species are controlled with pesticides which can kill the ants, but also non target species, besides being toxic to humans and the environment. An alternative to reduce the leaf cutter ant numbers is the biocontrol. Our goals were: 1) to evaluate the performance of different strains of *Escovopsis* and *Trichoderma* against the symbiotic fungus of the leaf-cutter ants, and 2) to study the possible antagonism between two mycoparasites: *Escovopsis* and *Trichoderma*, which might be used for the biocontrol of these ants. To achieve our goals we used *Leucoagaricus*, *Escovopsis* and *Trichoderma* isolated from *Acromyrmex heyeri*, *Escovopsis* and *Trichoderma* isolated from *A. lobicornis*, and *Escovopsis* isolated from *Atta vollenweideri*. We challenged the three *Escovopsis* and two *Trichoderma* strains against the *Leucoagaricus*, and two *Escovopsis* with the two *Trichoderma* strains. For the challenges we use PDA petri dishes, and the organisms were plated on them. In the challenges *Escovopsis-Leugoagaricus*, and *Trichoderma-Leucoagaricus*, *Escovopsis* and *Trichoderma* had overgrown the cultivar completely. In the challenges *Trichoderma-Escovopsis*, *Trichoderma* had a higher rate of growth and overgrown the *Escovopsis* 100% of the times. *Trichoderma* was never negatively affected by the presence of *Escovopsis*, and, in fact, grew as fast as its control. Our results highlighted the negative effect of the mycoparisitism by

Escovopsis and *Trichoderma* against the *Leucoagaricus*. Finally, we can conclude that in biological control programs of leaf cutter ants, it will not be desirable to apply *Escovopsis* and *Trichoderma* simoultaneously, due to the antagonistic effects found in this work.

BB-P11

MICROORGANISMS AS POTENTIAL BIOCONTROL AGENTS OF FIRE ANTS

Alejandra A. Habarta[1], Daniela D. Goffré[1], Patricia P. Folgarait[1]

[1] Centro de Estudios e Investigaciones, Universidad Nacional de Quilmes, Bernal, B1876BXD Buenos Aires (alehabarta@yahoo.com.ar)

Red fire ants (*Solenopsis invicta*) are one of the most widely spread ant species throughout the world. This species is native from Argentina and lacks natural enemies that can regulate them in their exotic habitats where they became pests. Very few microorganisms have been investigated to use against this species. Cultivated fire ants on PDA have been shown to carry an array of microorganisms including bacteria, yeasts, and pluricellular fungi. Among these we have chosen those ones that were cultured more often, selecting one Gram negative bacterium and three fungi, as possible entomopathogens. To test the biocontrol potential of these microorganisms first we had to sterilize the fire ants and second we had to bath them in concentrated bacteria or spore solutions. We daily followed the fate of each ant until its death. Afterwards, we sterilized them again but at a higher concentration and kept each ant individually to assess the cause of death. By the third day after the bacteria bath, more than 90% of ants were dead. *Paecilomyces sp.* scored second, *Fusarium sp.* third, *Trichoderma sp.* fourth, and the control last. By the 15th day, all ants were dead from all treatments. Our results showed, on one hand, that our microorganisms proved to be entomopathogens, and on the other, that microorganisms have a much greater potential as biocontrol agents of fire ants as previously thought. In particular, the bacteria (which is under characterization now),as well as the *Paecilomyces sp.* fungi, seem to be promising candidates which deserve further studies.

BB-P12

GROWTH AND EXOENZYME PRODUCTION BY *Trametes trogii* AND *Trametes versicolor var. antarcticus* ON WET OLIVE CAKE ("ALPERUJO" IN SPANISH)

Ruth M. Kaen[1,2] , Luis A. Diorio[1], Laura N. Levin[1,2] , Damiana Salvatierra[1]

[1] Depto de Biodiversidad y Biología Experimental Facultad de Ciencias Exactas y Naturales UBA [2] PROPLAME - PRHIDEB CONICET (ruthkaen@gmail.com)

Wet olive cake, a by-product from olive-oil extraction is generated in huge amounts in areas were olive is cultivated. It exhibits phytotoxic properties, due to its high content of polyphenols. An interest application of this waste is its use in agriculture as organic amendment. To this purpose its phenols must be degraded. In the study we evaluated growth and exoenzyme production by two white rot fungi: *Trametes trogii* and *Trametes versicolor var. antarcticus* on wet olive cake, under different treatments, in views of applying them to phenol degradation. Olive cake was subject to different treatments before inoculation, to reduce its fatty acids content that may interfere with fungal growth. It was saturated either with water or with HCl 1, 3 or 5N and left for 24 hs or autoclaved. Then the samples were repeatedly washed, rehydrated to 60% water content and autoclaved. Non-treated olive cake was used as control. Samples were collected after 14 days, and sugar content and exoenzyme fungal production was measured. Polymethylgalacturonase, polygalacturonase, laccase, Mn-peroxidase, endoglucanase and endoxylanase activities were determined. Treatment with HCl inhibited the growth of both fungi. Highest lacase and Mn-peroxidase activities were produced by *T. trogii*, after treating olive waste with water, leaving it in rest for 24 hs. *T. trogii* produced higher titers of cellulolytic and xylanolytic enzymes and lower levels of pectinases, when applying this treatment to the waste. Best ligninolytic titers were determined in *T. versicolor* when growing it on non-treated olive cake. It also produced high xylanolytic and pectinolytic activities, but lower levels of cellulases.

BB-P13

STUDY OF A PHENANTHRENE-DEGRADING COMMUNITY IN A SIMPLE SOIL MODEL.

Ana M. López[1], M. A. Bosch[1], Osvaldo M. Yantorno[1], María T. Del Panno[1]

[1] CINDEFI (CCT-UNLP) Calle 50 y 115, (1900) La Plata, Argentina. (anitamisiones@hotmail.com)

The polycyclic aromatics hydrocarbons (PAH) are relevant environmental contaminants due to their toxicity, mutagenesis and carcinogenesis properties. The principal process of PAH degradation in the environment is done by the microorganisms, involving the selection of populations with specifics degrading capabilities. The PAH biodegradation in soil is affected by their low bioavailability, due to their low water solubility and their tendency to be adsorbed to the soil organic matter.With the aim to know the microbial communities growth in soil chronically contaminated with PHA, a simple model solid phase preloaded with phenanthrene was studied. We describe a study using Amberlite XAD2 (Sigma), a porous resin of polystyrene, in bath system with liquid mineral medium (LMM). The adsorption capacity of the model was 10.87 mg of phenanthrene/g of XAD2 and the phenanthrene desorption was less than 7% after 30 days of incubation under abiotic condition. An enrichment culture of phenanthrene degrading bacteria was obtained by using a petrochemical contaminated soil. Duplicated batch systems were prepared by inoculation of 1ml of enrichment culture in 20 ml of LMM containing 2g of XAD2 preloaded with phenanthrene. The systems were incubated during 30 days at 28ºC. A 10% of colonized solid phase was removed, washed and transferred to fresh systems. As a whole four transfers were done. The growth was monitored by phenanthrene degrading bacteria counts and FT-IR from planktonic and sessile populations. An increase of three orders of magnitude was detected after the fourth transfer. The FT-IR analysis from the communities established in the third and fourth transfers showed differences in the carbohydrates region (1200-900cm-1). These differences could be seen in both the relative intensity of the bands respect to proteins (Amida II) and the peaks position. In the fourth transference, after 30days of incubation, an increase in the amida II:carbohydrate relation was detected in the sessile population. That increase was corresponded with an increase of the sessile degrading population.The adhesion to XAD2 without phenanthrene of the cells culture was determined. A 10% of colonized solid phase from fourth transfer was removed, washed and inoculated in fresh systems to study the changes in the community during the solid phase colonization. The colonization was monitored by PCR-DGGE method. The visual inspection of the gel demonstrated changes in the communities during the incubation. Some populations from the inoculum did not colonize the solid phase. While some others populations not detected in the inoculum, were detected during the incubation on the solid phase. This dynamic would be a consequence of the competence between degrading populations, which are expressing different strategies to promote the phenanthrene availability.

BB-P14

***Escovopsis* MICROFUNGI AS A POTENTIAL BIOCONTROL OF *Acromyrmex lundii*'s CULTIVAR: VIRULENCE AND INDUCTION OF THE PATHOGEN**

Jorge A. Marfetán[1], Norma B. Gorosito[1], Patricia J. Folgarait[1]

[1] Ant laboratory. Universidad Nacional de Quilmes. Roque Saenz Peña 352. B1876BXD. Bernal. Argentina (pfolgarait@unq.edu.ar)

Leaf-cutter ants (*Acromyrmex* and *Atta*) are the dominant herbivores of the Neotropics. In Argentina, *Acromyrmex lundii* is one of the most important pests of plantations, horticultural crops, and pastures. The leaves cut by the ants are used as a substrate for the cultivation of *Leucoagaricus* (Basidiomycotina), which represents the main food of the queen and brood. This cultivar is attacked by *Escovopsis* (Ascomycotina: Hypocreales), a specialist microfungi which can kill the cultivar in a short period of time, and as a consequence the colonies. We investigated the effect of one strain of the mentioned pathogen over *Leucoagaricus* isolated from different colonies of *A. lundii* from Corrientes, Argentina. Despite having a clonal propagation through ant generations, we showed -for the first time- that *Leucoagaricus* from different colonies exhibited different growth speed and morphology, in vitro. All cultivars, however, were negatively affected by *Escovopsis* reducing their growth (rate and final area), especially for the cultivar with greatest growth. On the other hand, the response of *Escovopsis* did not differ among cultivars in growth rate and ended up covering completely each of the cultivars within a week. However, the percentage of production of mature spores differed according to which cultivar strain was challenged with. Since we observed a greater mycelium growth rate as well as speed and amount of sporulation of *Escovopsis* in presence of the cultivar we hypothesized that the pathogen was induced by a soluble compound released by *Leucoagaricus*. We made a 3 treatments experiment; one treatment with *Escovopsis* growing in presence of a piece of PDA with *Leucoagaricus*, a second with *Escovopsis* growing by itself, and a third with *Escovopis* growing with a piece of agar coming from a plate with the cultivar. We found that *Escovopsis* growth and sporulation was greater in the first and third treatment in comparison to the control, demonstrating that *Escovopsis* was induced by a soluble compound that can diffuse in agar. These results are encouraging from the biological control perspective for three reasons. First, it is useful that *Escovopsis* exhibits the same performance against cultivars from different colonies assuring its negative effect at least in the same site. Second, it is desirable that the biocontrol agent asexual reproduction is induced when necessary, i.e. in presence of the organism

that needs to be controlled. Finally, the inducible sporulation will probably reduce the dissemination of the biocontrol agent towards other sympatric ants after its application in the field.

BB-P15

DETERMINATION OF THE INCIDENCE OF GREY MOULD ON GRAPES OF SAN JUAN, ARGENTINA AFTER APPLYING DIFFERENT CONCENTRATIONS OF NATURALLY OCCURRING ANTAGONIST YEASTS

María C. Nally[1], *Virginia M. Pesce*[1], *María E. Toro*[1], *Lucía I. Castellanos de Figueroa*[2,3] , *Fabio Vazquez*[1]

[1] *Instituto de Biotecnología. F.I- UNSJ- Av. San Martín 1109 oeste. Capital. San Juan- Argentina* [2] *PROIMI- Av. Belgrano y Pasaje Caseros. Tucumán- Argentina* [3] *FBQyF-UNT Ayacucho 455- Tucumán- Argentina. (cristinanally@yahoo.com.ar)*

Introduction: *Botrytis cinerea* is a major pathogen of grape. At present, control in conventional agriculture is mainly achieved through chemical strategies, which can also have many disadvantages, such as the public's growing concern for the human health conditions and the environmental pollution. One promising alternative to pesticides is the biological control, numerous studies indicated that some yeast species are ideal biocontrol agents, as they are natural plant epiphytic colonizers, nonpathogenic to plants and human beings in most cases and can rapidly proliferate. It has been reported that biological control was only effective when high concentrations of antagonist yeasts were applied. Objective: The aim of the present work was to study the efficacy of different concentrations of antagonistic yeasts in reducing the development of *B.cinerea*. Materials and Methods: A- Microorganisms: The pathogen *B.cinerea* was isolated from infected grapes. All yeast antagonists (15 strains of *Saccharomyces cerevisiae* and 1 of *Schizosaccharomyces pombe*) were originally isolated from grape surfaces and fermenting musts. They were selected because of their ability to control *B.cinerea* on grapes, screening them *in vitro* and *in vivo*. B- Tests on fruit: Biocontrol effectiveness was assessed on Red Globe grapes. The fruits were artificially wounded and inoculated with yeasts (10^5, 10^6 and 10^7 UFC/ml) and conidial suspension of *B.cinerea* (10^4 conidia/ml). Each sample, constituted by 9 berries and reproduced with three replicates for each yeast isolate, was incubated for 5 days at 25°C in a plastic box under high relative humidity (100%). After storage, the incidence of disease was analyzed in percentage and these were arcsin-transformed to angular data prior to ANOVA. Results: There were significant negative relationships between concentration of the antagonists and disease incidence (R^2: range on 0.75 to 0.99). The efficacy was higher when a concentration 10^7 CFU/ml of antagonist was used. When yeast cell suspensions of 8 strains of *S.cerevisiae* (BSc5, BSc49, BSc81, BSc92, BSc121, BSc140, BSc175 and BSc203) and *S.pombe* BSc167 reached a concentration of 10^7 CFU/ml, no infection by *B.cinerea* was found in fruits treated. Two strains of *S.cerevisiae*: BSc49 and BSc140 were able to inhibit mycelial growth of grey mould when a concentration of 10^6 CFU/ml of yeasts was inoculated. Conclusions: The concentrations of antagonist had significant effects on biocontrol effectiveness: the higher the concentration of yeast the better biocontrol activity of the antagonist had. When yeast was at 10^7 CFU/ml, the best control was obtained and this concentration was lower than those reported by other investigators.

BB-P16

BIOCONTROL OF FUNGI FROM SOUR ROT BY VOLATILES PRODUCED BY YEASTS IN TABLE GRAPES

María C. Nally[1], *Virginia M. Pesce*[1], *María E. Toro*[1], *Lucía I. Castellanos de Figueroa*[2,3] , *Fabio Vazquez*[1]

[1] *Instituto de Biotecnología-FI-UNSJ. Av. San Martin 1109 (O). San Juan- Argentina* [2] *PROIMI. Avenida Belgrano y Pasaje Caseros.Tucumán- Argentina* [3] *FBQyF-UNT Ayacucho 455.Tucumán- Argentina (virgi_pesce@yahoo.com.ar)*

Introduction: Sour rot is an important disease of grapes that affects both crop yield and wine quality. It is caused by a number of undesirable yeasts and bacteria, in association with fungi like *Aspergillus*, *Penicillium* and *Rhizopus*. Biocontrol of plant diseases with microbial antagonist has been developed as an alternative to fungicides. Objective: To evaluate the action of volatiles produced by wild enological yeast in biocontrol of fungi associated to sour rot disease of grapes. Material and Methods:a- Antagonist isolation: Yeasts were isolated from different sources such as healthy grapes, fermenting musts and enological environments.b-Fungi isolation: The pathogens were isolated from grapes berries with sour rot symptoms. c- Screening of antagonistic yeasts: *In vitro*, antagonism between fungi and yeasts was observed by placing both on the same Czapeck Agar plate and incubating at 25°C, for 5 days. Then, antagonist yeasts were evaluated *in vivo*: a wound at the equator of grapes berries was made. Aliquots (10 µl) of 10^6 CFU/ml yeast concentration followed by 10µl of fungal conidial suspension (10^4 CFU/ml) were seeded in the hole. d- Production of antifungal volatiles: Interaction tests consisted of the bottom part of a Petri- dish with the seeded yeast inverted on top of another bottom part containing a fungus, were sealed with Parafilm®, and incubated at 25°C. Fungal growth inhibition was determined when the diameter of the fungi decreased in comparison to the negative control. All experiments were repeated three times. Results: *Aspergillus caelatus*, *A.carbonarius*, *A.versicolor*, *A.terreus*, *Penicillium comune*, *Rhizopus stolonifer* and *Ulocladium* sp. were isolated. The screening *in vitro* of 234 isolated

resulted in 95% of yeast with antagonistic activity and 63 isolates showed efficacy to inhibit the 7 grapes pathogens on *in vitro* test. From 63 isolates, 8 antagonist yeasts consistently produced antifungal volatiles, and inhibited mycelial growth of *A.caelatus*, *A.carbonarius*, *A.terreus* and *P.comune*. Volatiles produced by *Kluyveromyces marxianus* BKm153, *Debaryomyces vanrijiae* BDv197, *Pichia guilliermondii* BPg190, *Saccharomyces cerevisiae* BSc44 and BSc78 produced growth inhibition of *A.terreus*, although BKm153 showed the highest inhibition (48.66% ± 2.44). *A.carbonarius* was affected by the presence of volatiles produced by *Candida sake* BCs198, *A.caelatus* by *S.cerevisiae* BSc119 and *P.comune* by *Candida versatilis* BCv223. Conclusion: These results suggest that the production of antifungal volatiles from antagonistic yeasts play a significant role in mechanisms of biocontrol of fungal pathogens of grapes.

BB-P17

USE OF *Aphanocladium* sp., *Acremonium* sp. OR *Aciditiobacilus ferrooxidans* SORBED ON A NATURAL CLAY (MONTMORILLONITE), AS AN URANIUM REMOVAL SYSTEM.

Melisa S. Olivelli[1,2] , *Gustavo A. Curutchet*[2], *Rosa M. Torres Sanchez*[1]
[1] *CETMIC, CONICET* [2] *Laboratorio de Análisis Ambiental, UNSAM (melisaolivelli@hotmail.com)*

Uranium is a radionuclide with noticeable toxicity as heavy metal. It reaches water from natural and man-made sources representing a risk for human health and environment. Generally, conventional sorption techniques for heavy metals removal from wastewaters are not useful due to the great volumes treated and the low concentration of pollutants. Biosorption is an alternative process where different types of biomass allow heavy metals concentration from diluted solutions. Among the most commonly used biosorbents is the fungal biomass, with the advantage of being easily generated at low costs. The main technological drawback in biosorption processes is getting a suitable immobilization of the biomass to obtain efficient biofilters. A methodology to increase biosorption surface and retain biomass is to generate clay biopolymers matrices. Montmorillonite clays are among the possible innocuous sorbents with this characteristic. This type of clay has optimal properties for metal sorption because it is able to complex all kind of organic as well as inorganic compounds on its surface. In this study, we use microorganisms able to grow in the presence of high concentrations of Uranium: *Aphanocladium* sp., *Acremonium* sp., and the acidophilic bacteria *Acidithiobacillus ferrooxidans*. U(VI) adsorption by microbial-natural clay matrices was studied to determine if biomass immobilization on clay could increase the montmorillonites sorption capacity. The different generated matrices were characterized by X- ray power diffraction (XRD), specific surface area and scanning electron microscopy. Uranium sorption capacity was determined by batch systems. Analyzed clays exhibited a great capacity for uranium sorption. This property was remarkably modified by interaction with different microorganisms and culture media. Fungal biofilms that grew on clay with an organic carbon source in the culture medium increased sorption capacity. Furthermore, the separation process was easier as the presence of the biofilm facilitated clay precipitation. The simple sorption of fungal and bacterial biomass on clay matrices also produced an increase in the Uranium sorption capacity. These preliminary studies conclude that these microbial biofilms-clay systems have a great potentiality for uranium biosorption processes given its high cationic exchange capacity and its ability to adapt the process to different situations.

BB-P18

EFFECT OF FUNGICIDES IN THE VIABILITY OF POTENTIAL BIOCONTROL AGENTS AGAINST *Fusarium* HEAD BLIGHT IN WHEAT

Juan M. Palazzini[1], *María L. Ramirez*[1], *Adriana M. Torres*[1], *Sofía N. Chulze*[1]
[1] *Universidad Nacional de Río Cuarto (jpalazzini@exa.unrc.edu.ar)*

Fusarium Head Blight (FHB) caused by *Gibberella zeae* (anamorph = *Fusarium graminearum*) is a devastating disease that causes extensive yield and quality losses to wheat in humid and semi-humid regions of the world. Different strategies have been used to reduce the impact of FHB including crop rotation, tillage practices, fungicide application and the planting of less susceptible cultivars. None of these strategies by themselves are able to substantially reduce the impact of the disease. Biological control offers an additional strategy and can be used as part of an integrated management of FHB. In previous studies three bacteria, *Bacillus subtilis* RC 218, *Brevibacillus* sp. RC 263 and *Streptomyces* sp. RC 87B were selected by their potential to control the growth and deoxynivalenol (DON) production by *F. graminearum*. These microorganisms were able to control FHB and DON production in greenhouse trials. The goal of this work was: - to test the effect of common fungicides, utilized to control *Fusarium* head blight, on the viability of the potential biocontrol agents *Bacillus subtilis* RC 218, *Brevibacillus* sp. RC 263 and *Streptomyces* sp. RC 87B. Three different fungicides were used in the bioassay (prothioconazole, tebuconazole and metconazole) at concentrations ranging from 0.5 to 80 µg/ml. A single colony of each bacterial strain was used to inoculate 100 ml of the basal medium in 250 ml Erlenmeyer flasks and incubated for 12 h (overnight culture) at 28 ºC in a rotatory shaker (150 rpm) in order to obtain mid-log phase cells (approximately 10^6 cells ml^{-1}). Cells counting was done in a

haemocytometer chamber and adjusted to 2 x 10^3 cells ml-1. An aliquot of 0.1 ml of each bacterial strain was inoculated in Petri dishes using the spread plating technique containing the solid basal medium (agar 2%) with the different fungicides. Inoculated plates were incubated at 28 ºC for 48 h. Colony counting were done at 24 and 48h. Controls without fungicides were used for each strain. Differences in the viability of the three bacteria were observed in the presence of the fungicides. *Bacillus subtilis* RC 218 and *Streptomyces* sp. RC 87B showed better tolerance to fungicides than *Brevibacillus* sp. RC 263. Complete inhibition of growth was observed at concentrations of 20 µg/ml for metconazole, 40 µg/ml for tebuconazole and 80 µg/ml for prothioconazole. Results obtained indicate the possibility to use biocontrol agents in combination with fungicides as part of an integrated management to control FHB of wheat.

BB-P19

APPLICATION OF RESPONSE SURFACE METHODOLOGY TO OPTIMIZE BIOMASS PRODUCTION OF THE YEAST *Kluyveromyces thermotolerans*, INTENDING TO BE USED AS BIOCONTROL AGENT FOR OCHRATOXIGENIC *Aspergillus* SPECIES

Román Pelinski[1], Patricia Cerrutti[1], Leopoldo J. Iannone[1,2] , Lorena Ponsone[3], Sofia N. Chulze[3], Miguel A. Galvagno[1,4]
[1] Laboratorio de Microbiología Industrial – Depto. de Ingeniería Química- FI- UBA [2] PROPLAME-PRHIDEP-CONICET [3] Depto. de Microbiología e Inmunología- FCEFN- UNRC [4] IIB-CONICET (mag@di.fcen.uba.ar)

The chlorinated isocoumarin compound, ochratoxin A (OTA), a potent nephrotoxic and carcinogenic compound is produced by several species of *Aspergillus Section Nigri* associated with grapes and it has been reported in wine and grape juice. To study the biocontrol of ochratoxigenic fungi by *K. thermotolerans*, strains isolated from grapes, in vitro tests were carried out in our laboratories and inhibition of fungal growth as well as of OTA production were achieved.Thus, optimization of biomass production of these yeasts becomes a fundamental first step in order to investigate their applicability as bio-controllers of ochratoxigenic fungi in the vineyard. *K. thermotolerans* (strain LB4)was maintained at 4°C in Sabouraud–agar medium. To evaluate vegetative growth, the strain was grown in semisynthetic media contained in shaken flasks (vol flask: vol medium, 5:1). Growth curves were done in Yeast Nitrogen Base (YNB, Difco) medium containing glucose as carbon source at 28°C at 200 rpm. Calculated µmax and td were 0.36 - 0.29 h-1 and 1.9 - 2.4 h, respectively. Glycerol and ethanol at the same molar C concentration as glucose were tested in YNB but only glycerol supported yeast growth aerobically reaching similar biomass production figures, though productivity was (1.5-2.0 fold)higher in glucose. When sugar cane molasses (58 % w/w fermentescible sugars, FS)was employed to replace glucose in the medium, 2 to 3- fold higher biomass concentration was reached. Several categorical fermentation factors studied in aerobic cultures containing molasses incubation temperature and time, inoculum size, vitamin (Yeast Extract)addition and nitrogen source, were assayed. According to the results obtained, we decide to optimize fermentation conditions by means of statistically designed experiments. Various continuous factors were screened using Plackett-Burman and Fractional Factorial Designs. The results obtained showed that the remaining factors significantly affecting biomass concentration were molasses and nitrogen/phosphorous ($(NH4)_2HPO4$, DAP) concentration and inoculum size at the levels chosen and were picked up for the optimization step using Response Surface Modeling (RSM) and a Box-Wilson Central Composite Design was run (28°C; pHi= 4.9; 200 rpm for 24 hrs). The results showed that the optimized biomass concentration achieved (1.1 .10^9 cells.ml^{-1}; OD640nm= 27.98) employing RSM, was obtained with: cane molasses, 21.6 (12.5 FS)% (w/v); DAP, 0.3% (w/v) and inoculum size (OD640nm= 10-12), 2.0 % (v/v). Yield (Yx/FS) values obtained were over 0.26$g.g^{-1}$. After model building and optimization, the predicted biomass optimum was verified. Validation assays in laboratory scale bioreactors are currently carried on.

BB-P20

FIRST RECORD OF *Fusarium verticillioides* (ASCOMYCOTA: HYPOCREALES) AS AN ENTOMOPATHOGENIC FUNGUS OF GRASSHOPPERS (ORTHOPTERA: ACRIDOIDEA)

Sebastian A. Pelizza[1,3] , Sebastián A. Stenglein[2], Marta N. Cabello[3], María I. Dinolfo[2], Carlos E. Lange[1]

[1] Centro de Estudios Parasitológicos y de Vectores (CEPAVE-CCT-La Plata-CONICET-UNLP) [2] BIOLAB-CEBB-CONICET-Facultad de Agronomía de Azul-UNCPBA [3] Instituto de Botánica Carlos Spegazzini, FCNyM-UNLP (pelizza@cepave.edu.ar)

The genus *Fusarium* comprises a large group of species of filamentous fungi widely distributed in soil. More than 13 *Fusarium* species are pathogenic to insects. *Fusarium verticillioides* (Sacc.) Nirenberg is often the most common fungus reported from infected corn kernels and vegetative tissues, but has not been recorded as an entomopathogen. Grasshoppers and locusts are important agricultural pests in different parts of Argentina. *Tropidacris collaris* (Stoll) has become in recent years an increasingly recurrent and extended pest in some of the Northern provinces. Although adults tend to prefer hard-leaf trees and bushes, *T. collaris* is actually a polyphagous species, and the nymphs bands consume virtually all available plant material they find. Currently, chemical insecticides are the only mean of control. Here we report the detection, by morphological and molecular methods, of an isolate of *F. verticillioides* in *T. collaris*, and its pathogenecity in the laboratory against another harmful grasshopper, the Melanoplinae *Ronderosia bergi* (Stal). During February-March 2008-09, nymphs and adults of *T. collaris* were collected in western Chaco Province, Argentina. The samples were immediately taken to the laboratory where grasshoppers were kept in groups in wire-screened cages in a rearing room under controlled conditions (30 ºC, 14L: 10D, 60% RH). Grasshoppers that died within 10 days post-collection were surface sterilized and held in a sterile culture chamber consisting of a Petri dish with a filter-paper disk that was periodically moistened with distilled water and incubated at 25 ºC in the dark. The filamentous fungi emerging from dead individuals were transferred to Petri dishes containing PGA + antibiotics, and incubated 26ºC. The fungal species isolated from *T. collaris* were identified on the basis of the macromorphological appearance of the colonies and molecular techniques were performed to confirm the fungal species. Unfortunately, our efforts to develop a breeding colony of *T. collaris* were not successful, hence the insects used in this study for testing pathogenicity were *Ronderosia bergi*, which are bred in the colony at CEPAVE. Three replicates (on different dates) of fifty third-instar nymphs each of healthy *R. bergi* were sprayed in groups of 10 with 1000 µl of a conidial suspension of 2.8 x 10^6conidia/ml. Three additional replicates of 20 grasshoppers each were used as controls. Mortality caused by *F. verticillioides* on *R. bergi* reached 58 ± 6.53 % by 10 days after inoculation, mortality did not occur among controls. In conclusion, this is the first report of infection of an insect by *F. verticillioides* and was confirmed by laboratory screening.

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BB-P21

APLICATION OF MATHEMATICAL MODELS FOR THE INTERACTION BETWEEN *S. cerevisiae* AND ZEARALENONE.

Carina M. Pereyra[1,3] , Lilia R. Cavaglieri[1,4] , María L. González Pereyra[1,3] , Ana M. Dalcero[1,4] , Stella M. Chiacchiera[2,4]

[1] Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto. [2] Departamento de Química. Universidad Nacional de Río Cuarto. [3] Becario del Consejo Nacional Investigación Científica y Tecnológica (CONICET). [4] Miembro del Consejo Nacional de Investigaciones Científicas y Tecnológicas (CIC-CONICET). (mgonzalezpereyra@exa.unrc.edu.ar)

In the latter years, mycotoxin contamination of animal feeds has turned an issue of global concern. Zearalenone (ZEA) is a toxin frequently detected in feedstuffs and cereals. The use of biological sorbents such as yeast cell walls (YCW) added to feed is an option to diminish bioavailability of the toxin in the gastrointestinal tract and the detrimental effects of ZEA mycotoxicosis on productive parameters (CAST, 2003). The aim of this study was to apply 3 mathematical models (Hill, Langmuir y Frumkin-Fowler-Guggenheim) to explain the interaction between ZEA and a commercial preparation of *S. cerevisiae* YCW by using adsorption isotherms. Interaction assays between ZEA and YCW were performed at pH 2 and pH 6 at 37°C. An aliquot of 500 µL (10 µg/mL) YCW was added to each Eppendorf tube containing 500 µL of 0.5; 5; 10; 20 and 50 µg/mL ZEA solution. Tubes were centrifuged for 30 min at low rpm and 10 min at 1400 rpm to obtain a pellet composed by the toxin which was bound to YCW and a supernatant where free (not bound) toxin was present. The supernatant was separated, evaporated to dryness under N_2 stream and analyzed by HPLC using the methodology described by Cerveró *et al.* (2007). Assays were done in duplicates. At pH 2 the interaction could be explained using the Hill model with an N value of approximately 1. In these conditions, since there was no cooperativity, Langmuir model could also be applied. The adsorption ability of *S. cerevisiae* YCW according to Hill model (R2=0.998) was 0.18 and the adsorption constant was 0.40 x 10^{-6} M^{-1}. For Langmuir model (R_2=0.997) adsorption ability was 0.14 (g/g) and the adsorption constant was 0.74 x 10^{-6} M^{-1}. Since the isotherm showed cooperative effect at pH 6, Hill (R_2=0.997) and Frumkin-Fowler-Guggenheim (R_2=0.996) models were applied. Both models showed identical adsorption ability (0.09 g/g) while ZEA affinity measured by the association constant (β) was

higher when using Hill model (2.00 x 10^{-6} M^{-1}) than when Frumkin-Fowler-Guggenheim model (0.29 x 10^{-6} M^{-1}) was applied. The adjustment of the 3 methods is comparable (R_2). However, Hill model seemed to represent best the adsorption at both pH 2 and 6. Hill model can be applied to sigmoid-shaped as well as to L type isotherms. Its mathematical expression includes the dissociation constant K_D, the maximum adsorption ability (Γ)max and the minimum number of binding sites necessary for cooperative adsorption. The inverse of K_D is precisely the adsorption constant we have stated as (β). The application of this model is the most useful to explain the interaction between ZEA and YCW, since more information on the mechanisms involved in the interaction can be obtained.

BB-P22

SELECTION OF A PAH-DEGRADING CONSORTIUM USING TLP CULTURE SYSTEM.

Jorge Pessacq[1], *Flavia E. Bianchinni*[1], *Maria Teresa Del Panno*[1], *Irma S. Morelli*[1]

[1] *CINDEFI (CCT-UNLP). La Plata Bs.As (jorgepessacq@gmail.com)*

Polycyclic aromatic hydrocarbons (PAH) are environmental pollutants mainly caused by industrial activity. Its low solubility in water and sequestration in soils and sediments contribute to their persistence in the environment. Two liquid phase (TLP) culture systems consist of an aqueous phase and a hydrophobic biocompatible and non-biodegradable phase. They have the potential to improve aqueous mass transfer of the poorly soluble compounds and control the availability of non-polar toxic compounds produced during the microbial development. They are self-regulated systems, so that the availability of the PAH in the aqueous phase is controlled by the partitioning ratio constant between the two phases and by the microbial metabolic activity. The aim of this study was to compare the PAH-degrading activity and bacterial diversity of PAH-degrading consortiums obtained using TLP system (20 ml silicone: 90 ml of mineral medium), with the conventional enrichment in mineral medium (MML). Both systems were supplied with 500 mg/l phenanthrene and pyrene. As initial inoculum a chronically contaminated soil of a petrochemical zone was used. In both systems five successive cultures were done every 30 days of incubation at 28°C. Before each subculture the density of PAH degrading bacteria was determined and predominant strains were isolated. The TLP systems showed an increase in PAH-degrading bacterial counts during the two firsts subculture, after that their kept relatively stable. On the other hand, the MML systems reached the higher PAH-degrading counts during the first subculture, after that the PAH-degrading counts decreased significantly during the successive subcultures. The PAH elimination in the 5th subculture was 89.8% and 55.5% for phenanthrene and pyrene respectively in TLP system and was only 6% for both PAH in MML system. The bacterial compositions of the 5th consortiums were analyzed by PCR-DGGE, showing the TLP system the most diversity composition. These results showed that the selection of PAH-degrading consortium in a TLP system preserve the bacterial diversity with a greater efficiency of PHA elimination.

BB-P23

FIRST REPORT OF RESTING SPORES OF *Pandora neoaphidis* (ENTOMOPHTHOROMYCOTINA: ENTOMOPHTHORALES) AN ENTOMOPATHOGENIC FUNGUS OF APHIDS (HEMIPTERA: APHIDIDAE)

Ana C. Scorsetti[1], *Marta N. Cabello*[1], *Claudia C. López Lastra*[2], *Annette B. Jensen*[3]

[1] *Instituto Spegazzini, Facultad de Ciencias Naturales y Museo, UNLP. La Plata, Argentina.* [2] *CEPAVE, Centro de Estudios Parasitológicos y de Vectores, CONICET, UNLP. La Plata, Argentina.* [3] *Department of Ecology, Zoology Section, Royal Veterinary and Agricultural University, Denmark (anacscorsetti@yahoo.com)*

The entomopathogenic fungus *Pandora neoaphidis* (Remaudière et Hennebert) Humber (Entomophthoromycotina: Entomophthorales), has long been investigated as a potential biological control agent of temperate zone pest aphids (Hemiptera: Aphididae). It is aphid-specific, causing natural epizootics in aphid populations, and interacts favourably with aphid predators and parasitoids. There are several hypotheses in the literature about winter survival of *P. neoaphidis*. One would be that the fungus survives by a continuing infection of anholocyclic populations of aphids. This fact is supported because this fungus is capable of infecting aphids at temperatures up to 5°C. There is evidence that the fungus *P. neoaphidis* has the capacity to survive for long periods outside the host, in soil as conidia, or hyphal bodies. However, it is known that *P. neoaphidis* could survive inside host for long periods, under conditions of low temperatures and low humidity. The objective of this study was to investigate the presence of resting spores of *P. neoaphidis* in aphid at field conditions. Aphids infected by *P. neoaphidis* were incubated for different time periods and examined by TEM. If resting spores were detected, we preserved cadavers in 70% alcohol. We confirm the presence of *P. neoaphidis* resting spores with PCR using fungal universal primers. In order to compare the taxonomic position of the resting spores we blasted the sequences in Genbank and have sequenced few additional Entomophthoralean species. The size of the ITS varied among the different species included with a range of approximate, 1000 to 1500 bp. Amplicons of the resting spores from infected aphids had a length of 1080 bp, as did all the tested *P. neoaphidis* isolates. We were successful in getting sequences from the resting spores of fungal infected aphids. In the present study, the occurrence of resting spores of *P. neoaphidis* at field conditions is reported for first time.

BB-P24

INDUCED SYSTEMIC RESISTANCE IN PEANUT

María Laura Tonelli[1], *Ana Laura Furlan*[1], *Tania Taurian*[1], *Stella Castro*[1], *Adriana Fabra*[1]

[1] *Dpto. de Cs. Naturales.Fac. de Cs. Exactas, Fco-Qcas y Naturales. Universidad Nacional de Río Cuarto (mtonelli@exa.unrc.edu.ar)*

Induced systemic resistance is a state of enhanced defensive capacity developed by a plant when is appropriately stimulated by a PGPB. A sequence of reactions enhance the expression of genes involved in plant defense, such as peroxidases (PO), glucanases, phenylalanine ammonia-liase (PAL), etc. *Pseudomonas* sp. BREN6 and *Bacillus* sp. CHEP5 were selected to evaluate their ability to induce systemic resistance in peanut performing split root experiments. Two weeks peanut plant roots were separated into two tubes with Hoagland solid medium, one of the tubes was inoculated with the bacteria (10^9 ufc/ml) or salicilic acid (1 mM and 10 mM) and, a week later, the other tube was challenged with the rhizoplane phythopathogen *S. rolfsii*. All the plants treated with *S. rolfsii* showed disease signs. However, *P.* sp. BREN6, *B.* sp. CHEP5 and salicilic acid enhanced shoot dry weight and *B.* sp. CHEP5 also enhanced root dry weight. The PO specific activity was increased in those plants inoculated with *P.* sp. BREN6, while PAL specific activity was increased in plants inoculated with *P.* sp. BREN6 and *B.* sp. CHEP5. Specific activity of β-1,3-glucanase was enhanced in all the plants challenged with *S. rolfsii*. With the aim to evaluate the expression of the gen that codified the enzyme PAL in peanut plants, we designed PALf and PALr primers and a 369 pb fragment was amplified by PCR. *Pseudomonas* sp. BREN6 and *Bacillus* sp. CHEP5 did not protect peanut plants from *S. rolfsii* deleterious effect, but as they promoted shoot growth and stimulated the activity of enzymes related to plant defense, we suggest that peanut plants can be systemically induced by these two bacteria.

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BB-P25

ISOLATION AND CHARACTERIZATION OF *Bacillus sphaericus* strains OBTAINED FROM LARVAE OF *Culex pipiens* IN LA PLATA CITY, ARGENTINA.

María C. Tranchida[1], *Pablo M. Riccillo*[1,2], *Juan J. García*[1,2], *María V. Micieli*[1]

[1] *Centro de Estudios Parasitológicos y Vectores (CEPAVE) (CONICET-CCT La Plata- UNLP)* [2] *Comisión de Investigaciones Científicas (CIC) (ctranchida@cepave.edu.ar)*

Bacillus sphaericus is an aerobic, spore–forming bacterium, isolated from the soil and mosquito larvae. Mosquitocida *B. sphaericus* strains have toxicity against mosquito larvae due to binary toxin encoded by genes *bin*. Thus are used in insect control program. The aims of this work were: 1- Isolation, 2- Phenotypic and molecular characterization of native *Bacillus*, with toxicity to immature stages of *Cx. pipiens*. 3- Determination of the toxicity and host range. 4- Identification of genes *bin* and *mtx*. *Cx. pipiens* larvae were collected from natural breeding sites. At laboratory, dead larvae were observed under microscope where the presence of bacteria with spores was detected. The native isolates were grown in NYSM agar media, which showed the presence of three different types of colonies. These isolates were named C107, C207 and C307. Gram and malachite green stain was performed. We evaluated its growth in different carbon sources, in gelatin, ability to utilize casein, acetoin, tyrosine, and lecithin. Resistance to antibiotic (streptomycin, erythromycin, tetracycline and chloramphenicol), growth in different concentrations of sodium chloride and boric acid were evaluated. We studied the catalase activity, the ability to hydrolyze urea and to produce hemolysis. Also sequencing of 16S rRNA genes and the comparison with the sequences available in database BLAST were performed. The sequences from native and references (SPH88 and K7865) strains were aligned using CLUSTAL. The presence of genes encoding toxins in *B. sphaericus* were analyzed by PCR, using primers BSN1/BSN2, BS1/BSN2 to amplify genes *bin* B and BSN3/BSN4, BS3/BS4 to *bin* A. The primers 100.1 and 100.2 were used to detect genes *mtx*. The host range was evaluated according to Institute Pasteur standard protocols. Were tested: *Cx. pipiens*, *Ae. aegypti*, *Cx. dolosus*, *Cx. apicinus*, *Oc. albifasciatus* and *An. albitarsis*. The LC_{50} was obtained through the Probit Analysis. The strains *B. sphaericus* 2362 and SPH88, and strain K7865 of *B. fusiformis* were used as reference strains. The isolates C107 and C207 showed mosquitocida activity. *Cx. pipiens* was the species more susceptible to C107 (LC_{50} $4x10^4$ spore/ml) meanwhile *Oc. albifasciatus* showed more susceptibility to C207 (LC_{50} $3.4x10^6$ spore/ ml). *Ae. aegypti* have not been susceptible to these strains. The presence of genes *bin* A and B, and *mtx* were recorded in both native strains. Analysis of the sequence of 16S rDNA genes, the cytomorphology and biochemical characterization showed that the C107 strain belongs to the group of *B. sphaericus* meanwhile the native strain C207 is more close to the *B. fusiformis* group. The C307 strain was identified as *B. licheniformis* and it did not have mosquitocide activity

BB-P26

MOLECULAR CHARACTERIZATION OF *cry* GENES PRESENT IN A *Bacillus thuringiensis* STRAIN TOXIC AGAINST *Aedes aegypti* LARVAE

María E. Vidal Domínguez[1], *Corina Berón*[1]

[1] *CEBB-CIB-FIBA-Mar del Plata - CONICET. Vieytes 3103 (7600) Mar del Plata, Argentina (cberon@fiba.org.ar)*

The mosquito *Aedes aegypti*, vector of the dengue and yellow fiber viruses, is found in nearly 100 countries worldwide. Current program strategies for the control of dengue and yellow fever have a small impact on the mosquito and are in critical need for a comprehensive, aggressive revamping. The World Health Organization makes special emphasis in the integrated epidemiological and entomological surveillance of the mosquito's population. *Bacillus thuringiensis* comprises a group of aerobic, spore-forming bacteria that produce a toxic crystalline inclusion, delta-endotoxin, or Cry protein, lethal to various insect orders. Although insecticidal proteins from *B. thuringiensis* have been used as spray against *A. aegypti* for the past 20 years, a significant number of disease carrying mosquitos are not affected by the available Cry proteins. Therefore, it is necessary to search for proteins with wider and/or specific toxic spectrum. Additionally, it is important to provide alternatives for coping with the problem of insect resistance that has already appeared against some of the formulates. Recently, we have isolated a new mosquitocidal *B. thuringiensis* strain (FCC 41) native to Argentina. This isolate was partially charaterized as showing higher mosquitocidal effect than the strain *B. thuringiensis israelensis* (HD 567). Described in a previous work, the polypeptide pattern of purified parasporal bodies analyzed by SDS-PAGE, showed two major polypeptides of about *Mr* 70 - 80 kDa, and the *cry* gene sequence named Cry24Ca was isolated. In the present work, a method was used based on PCR-RFLP and Denaturing Gradient Gel Electrophoresis (DGGE) profiling and sequencing of the highly variable *cry* gene to characterize gene contents from *B. thuringiensis* strains. By these methods we found three partial aminoacid sequences that showed identities of about 90 - 95% or less with the Cry24Ca protein previously described.

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PM-P1

COMPARATIVE EXAMINATION OF HEATING ON VEGETATIVE CELLS AND SPORES OF ENTEROTOXIGENIC AND NOT ENTEROTOXIGENIC *Clostridium perfringens*.

Cinthia C. Abbona[1], Patricia V. Stagnitta[1]

[1] *Area Microbiología, Universidad Nacional de San Luis. Argentina. (pvstag@unsl.edu.ar)*

Clostridium perfringens enterotoxin (CPE) is an important virulence factor for both *C. perfringens* type A food poisoning and several non-food-borne human gastrointestinal diseases. Recent studies have indicated that *C. perfringens* isolates associated with food poisoning carry a chromosomal *cpe* gene, while non-food-borne human gastrointestinal disease isolates carry a plasmid *cpe* gene. However, no explanation has been provided for the strong associations between certain *cpe* genotypes and particular CPE-associated diseases. Since *C. perfringens* food poisoning usually involves cooked meat products. The aim of this work was to evaluate comparatively the heat resistance of five enterotoxigenic strains carring a chromosomal *cpe* gene and five not enterotoxigenic strains. The strains used in this study were isolated for foods in San Luis, Argentina. Starter vegetative cultures of each isolate were prepared by overnight growth at 37°C in fluid thioglycolate medium (FTG). Sportulating cultures were preparated by inoculating 0.2 ml of the starter FTG medium culture into 10 ml of Tortora sporulation medium (Tm), and incubated for 72 h at 37°C. The diluted samples were plated onto brain heart infusion agar (BHI) to determinate the total number of vegetative cells at the start of heating (To). For spores Tm cultures were shocked at 75°C for 20 min before the To recount. The temperature used were 55°C during 1,2,4,6,8 and 10 min and 61°C during 5,10,15,20 and 30 min for vegetative cells and 100°C during 5, 30 and 60 min for spores. New recounts were done in every step. At 55°C five enterotoxigenic isolated survive even 40 min, whereas that the not enterotoxigenic isolates die at 30 min. At 61°C the not enterotoxigenic strains died at 10 min. The spores survived over 30 min at 100°C whereas that the not enterotoxigenic isolated die before 5 min. This study was to provide evidence which suggests that vegetative cells and spores of *C. perfringens* CPE-positive isolates carrying a chromosomal *cpe* gene are significantly more heat resistant than vegetative cells and spores of not enterotoxigenic *C. perfringens* isolates. How could possession of heat resistance explain, at least in part, the strong association between chromosomal *cpe* isolates and *C. perfringens* type A food poisoning. Enhanced survival under inadequate warming or incomplete cooking conditions would be a highly desirable trait for a *C. perfringens* food poisoning isolate given that (i) cooked meat and poultry products, as well as cooked meat stews, are the major food vehicles for *C. perfringens* type A food poisoning and (ii) improper holding temperatures and incomplete cooking of foods are recognized as major contributing factors for the development of 75 to 100 and 20 to 50% of *C. perfringens*

PM-P2

GROWTH AND DEPOLYMERIZATING ENZYME ACTIVITY ON AGAR CULTURES AT TWO PH LEVELS IN FUNGI FROM SOILS OF *Celtis tala* AND *Scutia buxifolia* FORESTS AND *Distichlis spicata* GRASSLAND IN THE EASTERN BUENOS AIRES PROVINCE (ARGENTINA).

Lorena A. Elíades[1], Mario C. Saparrat[2,1] , Marta N. Cabello[1], Claudio Voget[4]

[1] *1Instituto de Botánica Spegazzini, Fac de Cs Naturales y Museo, UNLP* [2] *2 Instituto de Fisiología Vegetal (INFIVE), UNLP- CCT-La Plata CONICET* [3] *3 Cátedra de Microbiología Agrícola, Facultad de Ciencias Agrarias y Forestales, UNLP.* [4] *4 (CINDEFI), Fac de Cs Exactas, UNLP (lorenaeliades@yahoo.com)*

The fungal degradation of polymers such as proteins, starch and cell wall-associated polysaccharides involve a battery of extracellular enzymes, whose production and properties depend upon the fungal species, strain, and culture conditions. The pH level in the medium is a key factor key in the process. There are relatively few reports on the pH effect on the growth and levels of depolymerizating enzyme activity in fungi associated with extreme environments under stressful conditions. An example of environments like this is a native xerophilic forest dominated by two tree species *Celtis tala* and *Scutia buxifolia* and its *Distichlis spicata* (L.) GREENE grassland associated located on the eastern part of the Buenos Aires province (Argentina). This area is characterized by diferent soil types including alkaline-calcareous, neutral and alkaline-sodic soils. These soils and their associated plants might be an isolation source for fungi with enzymes tolerant to different pH range and/or highly active at extreme pH, which might be used as biotechnological tools. The alkaline enzymes have potential applications in several industries such as in ones processing leather, food and pharmacological products. The aim of this study was to analyze the ability of several fungal strains, isolated from soils of *C. tala* and *S. buxifolia* forests and *D. spicata* grassland in the eastern Buenos Aires province (Argentina), to grow and produce enzymes with amylase, cellulase, protease and chitinase activities in agar cultures at pH 6.0 and 9.0. Most of the fungal strains grew better on agar cultures at pH 6.0 than ones at pH 9.0. However, *Fusarium* and *Cylindrocarpon* species showed similar growth diameters at both pH tested. All the strains tested revealed proteolytic activity on agar cultures at pH 9.0. However, amilolytic and cellulolytic activities at pH 9.0 were only detected in 12 and 10 isolates respectively. *A. murorum* showed high proteolytic activity as well as amilolytic one. However, any *Acremonium* species tested revealed cellulolytic activity under the assayed conditions. Except *Aspergillus ustus*, which did not present any enzyme activity tested, the other *Aspergillus* species showed ability for producing

amilases, cellulases and proteases on agar-cultures at both pH levels. Although the most of the *Fusarium* species tested revealed only proteolytic activity at both pH, *F. solani* produced also cellulases at pH 6.0 and 9.0. *Paecilomyces lilacinus* and *Penicillium chrysogenum* presented only proteolytic activity at both pH levels. *Trichoderma harzianum* and *T. saturnisporum* showed also proteolytic activity at both pH levels. Only *Acremonium murorum*, *Humicola grisea*, *Metarrhizium anisopliae*, *Scopulariopsis brevicaulis* and *Stachybotrys chartarum* showed chitinase activity at pH 6.0 and 9.0.

PM-P3

TOLERANCE OF LACTIC ACID BACTERIA ISOLATED FROM KEFIR TO HEAVY METALS

Esteban Gerbino[1], *Pablo Mobili*[1], *Emma E. Tymczyszyn*[1], *Andrea Gómez-Zavaglia*[1]
[1] *Centro de Investigación y Desarrollo en Criotecnología de Alimentos -CIDCA-(UNLP-CIC-CONICET) (egerbino@cidca.org.ar)*

Interaction of different organisms with metal ions can be beneficial or harmful according to the metals concentration to which the organisms are exposed. In this sense, some metals as Zn are trace elements essential to carry out many biological processes but they are toxic at high concentrations. On the other hand, there are metals that are not essential to biological processes and can be accumulate into the organisms becoming toxic even at low concentration. According to USA Environmental Protection Agency, Pb, Cd, Zn, Ni and Al are the most toxic heavy metals. These metals can be often found in high concentrations in different aquatic environments or in soils, representing a potential hazard to health. Some bacteria are able to grow in the presence of heavy metals producing at the same time its bioaccumulation or bioadsorption. This capacity transforms these microorganisms in potential bioremediation agents. The objective of this work is to evaluate the capacity of strains of *Lactobacillus kefir* to grow in the presence of different environmentally relevant metal ions (Pb^{+2}, Cd^{+2}, Al^{+3}, Zn^{+2} y Ni^{+2}). *L. kefir* strains CIDCA 8348 (aggregating) and JCM 5818 (non-aggregating) were used. Bacteria were activated in LAPTg broth (Raibaud, P. *et al* 1973) and then grown in the same medium in the presence of different concentrations of metal ions at 30ºC for 48 h. The bacterial growth was determined by measuring the absorbance at 550 nm. Both *L. kefir* strains were able to grow in the presence of all the metal ions assayed, although the tolerated levels were different for each one. The tolerated concentrations were: 5 mM Zn^{+2} and Pb^{+2} and 1 mM Al^{+3} and Ni^{+2}. Cd^{+2} was the most toxic metal. Microorganisms tolerated it in concentrations within the 0.01-0.5 mM range only if they had been previously pre-adapted and further incubated 20-days. Microscopic observation of the Gram stained microorganisms allowed us to detect morphological changes induced by Pb^{+2} or Al^{+3}. The aggregating strain CIDCA 8348 lost the capacity to agglutinate when grown in the presence of Pb. In the case of Al, bacilli continued to form agglomerates but they were smaller than the ones observed among bacteria grown in the absence of this metal. On the other hand, the non-aggregating strain JCM 5818 appeared wider in the presence of these metals. In this work the capacity of two strains of *L. kefir* to grow in the presence of heavy metals was demonstrated even at high concentrations. This capacity is of great importance for the utilization of these bacteria in processes of bioadsorption or bioremediation.

PM-P4

INSIGHT INTO THE CARBOHYDRATE METABOLISM IN MEMBERS OF THE GENUS *Rhodococcus*

Martín A. Hernández[1,2] , *María L. Flores*[1], *Héctor M. Alvarez*[1,2]
[1] *CRIDECIT, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco.* [2] *CONICET (mahernandez@unpata.edu.ar)*

The genus *Rhodococcus*, which belongs to the actinomycetes group, are highly versatile microorganisms with the physiological capability to adapt to a diversity of natural environments such as soil, water and marine sediments. Despite the ecological and biotechnological importance of this genus, the core metabolic strategy that permits cells to be successful in the environment has been poorly studied. The purpose of this study was to examine some aspects of carbohydrate metabolism in species of *Rhodococcus* during both replicative and non-replicative stages. We explored the response of cells to diverse environmental conditions and performed a genome-wide bioinformatic analysis of genes involved in carbohydrate metabolism using the genome database of *R. jostii* RHA1. Moreover, we examined the ability of *R. opacus* and *R. jostii* strains to synthesize and accumulate polysaccharides under different conditions. The RHA1 genome contains all necessary genes/enzymes for both glycolytic pathways, the EMP- and ED-pathways, and for the pentose phosphate pathway. This suggests that cells rely on different alternatives for carbohydrates catabolism for sustained periods, depending on the fluctuating nutritional conditions of the environment. In addition, we identified all key genes for the biosynthesis and mobilization of glycogen in the *R. jostii* genome. In this context, *R. jostii* RHA1 and *R. opacus* PD630 were able to accumulate an intracellular polysaccharide after cultivation of cells on both, nutrient broth and minimal salts medium with gluconate as sole carbon source. The complete analysis of this polysaccharide revealed to be a glucose polymer, such as glycogen. We found that other species of the genus, such as *R. fascians* and *R. erythropolis*, were also able to accumulate glycogen. The studied strains accumulated glycogen during exponential

growth phase and the content decreased during stationary growth phase. This result suggests that glycogen biosynthesis may deal with the sugar excess during exponential growth phase in these bacteria, and probably serve as a pool of sugars for using when necessary. When cells of *R. opacus* and *R. jostii* were incubated under desiccation conditions (non-replicative stage), we observed the production of an extracellular polymeric substance (EPS). Chemical analyses of the EPS from strain PD630 by diverse chromatographic methods and 1H-RMN revealed the occurrence of a polysaccharide polymer as main compound plus proteins and probably glycolipids. The carbohydrate fraction was composed by β-D-glucuronic acid, 2,3-α-D-mannose and minor amounts of glucose and xylose. The results of this study suggest that *Rhodococcus* bacteria are endowed with a robust, flexible and versatile carbohydrate metabolism, which is necessary for metabolically adapting to environments with fluctuating nutritional conditions.

PM-P5

INVESTIGATIONS OF Azospirillum brasilense BIOFILM FORMATION

Fiorela L. Nievas[1], *Fernando G. Sorroche*[1], *Luciana V. Rinaudi*[1], *Pablo C. Bogino*[1], *Erika Banchio*[1], *Walter F. Giordano*[1]

[1] *Departamento de Biología Molecular, F.C.E, F-Q y N. Universidad Nacional de Río Cuarto. Ruta 36 Km 6 (fnievas@exa.unrc.edu.ar)*

Azospirillum brasilense is considered to be an important plant growth promoting rhizobacteria that can improve the growth and yield of many agriculturally important crops, including wheat, corn, and rice. Azospirilla colonize the root surface and may significantly promote plant growth and crop yield, properties that make them attractive candidates for the development of biological fertilizers for these crops. The ability of *Azospirillum* to attain significant populations on the root surfaces of the host is essential for its beneficial effect on plant growth and requires that the bacteria come in close contact with the roots. Initial attachment of soil bacteria to plant cells have been shown to depend on superficial or surface associated polymers, such as lipopolysaccharide (LPS), exopolysaccharide (EPS), capsular polysaccharide (CPS) as well as flagella and outer membrane proteins. Some of these molecules are involved in biofilm formation, which are defined as structured communities surrounded by a self-produced polymeric matrix and adhered to a living or inert surface. In this work we investigated the influence of nutritional factors and the effect of mutations in genes encoding for surface components such as LPS, EPS and CPS, in the biofilm formation ability of *A. brasilense*. We found that three wild-type strains of *A. brasilense* (Az39, Cd and Sp7), are able to form biofilms on a hydrophobic inert surface. In particular, *A. brasilense* Sp7 was able to form relatively more robust biofilms compared with the other strains studied. The effect of the media composition on biofilm formation was also investigated in the Sp7 strain. In a nutritional-limited minimal medium, biofilm biomass was increased, whereas in a complex rich one, biofilm formation was strongly inhibited, indicating that *A. brasilense* is able to sense and differentially respond to the nutritional status of the medium. Three mutants of *A. brasilense* Sp7 showed alterations in biofilm formation. The *exoC* and *exoB* mutants have an altered composition of LPS and produce only the low-molecular-weight fraction of EPS. These mutants were severely affected in the adhesion to PVC, as compared to the wild type. In contrast a *phbC* mutant, unable to produce PHB, (an intracellular storage compound), overproduces EPS, CPS, and shows an increased motility, revealed an increased sessile-biomass accumulation in comparison with the wild type.Interestingly, *exoB* and *exoC* mutants, deficient in biofilm formation, have previously been shown to be impaired in the attachment to roots. In contrast, the biofilm-overproducing mutant *phbC*, was found to be more proficient in the adhesion to the plant host. These results suggest a correlation between root surface attachment and biofilm formation.

PM-P6

ACTIVITY OF DIFFERENT LOCK SOLUTIONS ON PRE-FORMED BIOFILMS OF *Stenotrophomonas maltophilia* ISOLATES FROM PATIENTS WITH DEVICE-ASSOCIATED BLOODSTREAM INFECTIONS

Beatriz N. Passerini de Rossi[1], Laureana C. Feldman[1], Maria V. Saliba Pineda[1], Carlos Vay[2], Mirta A. Franco[1]
[1] Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, UBA [2] Laboratorio de Bacteriología Clínica, Departamento de Bioquímica Clínica, Hospital de Clínicas UBA (bpasseri@ffyb.uba.ar)

Stenotrophomonas maltophilia (Sm) has become a nosocomial pathogen of increasing importance, principally in patients exposed to invasive devices. With its multidrug-resistance phenotype and its ability to form biofilms, the management of Sm infections presents a challenge. Biofilms are highly resistant to antimicrobial compounds. The minimum regrowth concentration values established in our previous publication indicate that currently used concentrations of levofloxacin cannot be used in monotherapy for an efficacious eradication of Sm biofilms. Several studies have demonstrated that antimicrobial lock solutions are useful in the prevention and the adjunct treatment of catheter-related bloodstream infections in hemodialysis and cancer patients, reducing catheter removal rates. The lock technique involves the instillation of a concentrated antimicrobial solution into a catheter. Different combinations of antibiotics and anticoagulants are active in eradicating biofilms from catheters, but require a prolonged dwell time (16-24h). In contrast, ethanol (60 to 25%) has broad-spectrum antimicrobial activity against biofilms after only 1-2h. The aim of this work was to compare the in vitro activity of different lock solutions on pre-formed biofilms of Sm isolates recovered from 10 patients with device-associated bacteremia (6 with vascular catheters and 4 with haemodialysis catheters), between 2004-2008, at a university hospital in Argentina. Sm K279a was used as a reference strain. Isolates were cultured for 24h in 96-well polystyrene microtiter plates. Biofilms were challenged with ethanol 25% and 40% in sterile saline for 1h. The viability of the biofilms was determined by the regrowth technique and by plate counts. Biofilms from all isolates were eradicated after both treatments. Sm13, the highest biofilm producer among the studied isolates, was selected for challenge biofilms established in silicone catheter segments with different lock solutions for 1h, 3h and 24h. For the non-treated biofilms, the mean of viable counts recovered from 3 segments was $1.70x10^6$CFU/ml. Ethanol 25% alone or in the presence of EDTA (30mg/ml) eradicated biofilms after 1h-treatment. In contrast, killing of biofilms by levofloxacin (2.5 mg/ml) was gradual, the percentages of survivals after 1h, 3h, and 24h were 2.52%, 0.23% and 0.02% respectively. EDTA showed antimicrobial activity, but failed to eradicate biofilms after 24h-treatment (1.91% of survivals). The combination levofloxacin-EDTA was not synergistic against Sm biofilms. In conclusion, the ethanol 25%-EDTA catheter lock solution was more effective than any other agent in eradicating Sm biofilms in a short time. EDTA is a chelator with anticoagulant activity equivalent to that of heparin but with the advantage that it has antimicrobial activity. The use of ethanol instead of antibiotics excludes the concern about potential selection of highly resistant bacteria.

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PM-P7

TRANSFER OF NATURAL MICROORGANISMS FROM ORANGE FRUIT TO FRESH JUICE DURING EXTRACTION. EVOLUTION OF LACTIC ACID BACTERIA IN THE NATURAL MEDIUM

María B. Perez[1], Fabiana M. Saguir[1]
[1] Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán (fabianasaguir@fbqf.unt.edu.ar)

In a previous work we compared the indigenous microflora on oranges peel purchased from local packinghouse and market. The microbial load on orange surfaces differed significantly, detecting the presence of lactic acid bacteria (LAB) only on the market fruits. In this work we investigated the capacity of transference of indigenous microflora from orange peel to fresh juice during extraction and the lactic acid microflora evolution in fresh juice during storage at 30°C under microaerophilic conditions. Oranges without any washing procedure were squeezed to obtain the juice under sterile conditions. Microbial flora transferred and the LAB evolution was analyzed by plating aliquots (diluted if necessary) on PCA, and MRS-agar pH 5 added with 10 µg/ml of pimaricin to inhibit yeasts growth (MRS-P) at different times during storage. MRS-P and PCA plates were incubated anaerobically and aerobically at 30 °C for 7 days before enumeration, respectively. LAB colonies by random selection were picked up from PCA and MRS-P plates and further characterized by phenotypic and genotypic methods.The microbial load on orange surfaces determined on PCA and MRS-P plates was 5.2 and 3.0 log cfu/ml, respectively. Majority of colonies on the PCA plates were small, corresponding to microorganism catalase positive as well as colonies belonging to Enterobacteriaceae and yeasts. On MRS-P, the colonies were mainly gram positive and catalase negative cells presumably identified as LAB. After extraction, the initial microbial counts in the fresh juice obtained in PCA and MRS-P media corresponded to 4,07 and 2,99 Log cfu/ml, respectively. At this time although LAB showed the highest transfer capacity, gram and catalase positive bacteria and yeasts were predominant in similar way as on fruit surface. At 72 h of fresh juice storage the counts became maximum increasing by about 4 log cycles, LAB mainly being isolated. From 191 colonies random selected LAB were identified accounting for 65% on the basis that they were Gram positive, catalase negative, non-spore forming, non-motile cells and exhibited a fermentative catabolism from glucose. The majority of them (64%)

were homofermentative while only a few isolates were obligatory heterofermentative . Among homofermentative LAB isolates that did not produce ammonia from arginine the *Lactobacillus plantarum* species was mainly identified by specific PCR of specie. The results demonstrate the high capacity of transference of natural microflora of orange surfaces to fresh juice, especially of bacteria presumably identified as LAB. LAB became predominant microflora during fresh juice storage at abusive temperature under microaerophilic conditions. This fact, might be related with a better adaptation to natural juice and storage conditions and be able to initiate growth more rapidly than the predominant non-LAB microflora from oranges peel.

PM-P8

INCREASED CHEMOTAXIS TOWARDS XYLAN AND HEMICELLULOSE FROM *Paenibacillaceae* ISOLATES

Alicia L. Ragout[1], María J. Dávila-Costa[1], Daniel H. Valdeón[1], Nora I. Perotti[1,2] , Carlos M. Abate[1,3] , María A. Martínez[1]

[1] PROIMI- CONICET [2] Fac.de Cs. Exactas y Tecnología [3] Fac. de Bqca, Qca. y Fcia., [4] Fac. de Cs. Nat e IML UNT (aliciaragout@yahoo.com.ar)

Bacterial chemotaxis is a primitive mechanism by which bacteria reponds to high concentrations of attractants and is activated by changes in pH, temperature, osmolarity, viscosity and chemical compounds, some of which are nutrients and secondary metabolites. Most motile bacteria can sense and respond to low concentration by this process. There is evidence that chemotaxis can enhance biodegradation, presumably by rapidly bringing cells into close contact with degradable substrates. Isolates AR92 and AR 109, related to *Paenibacillaceae* were obtained from bagasse liquor and the batteries in active fermentation for the production of paper, according to their ability to produce xylanases at pH conditions between 5 and 6 at a temperature of 25 to 30º C. Both isolates were Gram + bacillus, mobile, catalase positive. Scanning electron microscopy showed the presence of polar flagella in both strains, as a physiological property related to bacterial motility. Swimming ability related with chemotaxis of both isolates was evaluated in minimal media with birchwood xylan and hemicellulose extracted from sugar cane bagasse with the addition of Tween 80 at two concentrations: 0.02% and 0.2% and minerals salts. Plates were solidified by using agarose 0.3% and incubated at 30 º C for 48h. Results showed that AR 109 exhibited higher swimming, apparently masking clarification of medium due to xylan hydrolysis. This effect was significantly enhanced when calcium and magnesium salts as well as Tween 80 were added to final concentrations of 20 mM and 0.2%, respectively. Isolated AR92 behavior was different, showing smaller colonies with clarification halos of xylan hydrolysis and bigger hydrolytic halos when Tween 80 was added. As a whole, behavior of both isolates tested was for the most part modified by salts and Tween 80 additions to the media. Regarding the carbon source both, colony size and halos were higher when birchwood xylan was used.

PM-P9

EFFECT OF NUTRITIONAL CONDITIONS ON THE ANTIMICROBIAL ACTIVITY OF A LOCAL STRAIN OF *Bacillus* sp. (SAN LUIS-ARGENTINA)

Patricia G. Silva[1], Laura B. Parra Morales[2], Lucía E. Alcaráz[1], Humberto J. Silva[1]

[1] Área Microbiología, [2] Área de Biología Molecular, Universidad Nacional de San Luis (pgsilva@unsl.edu.ar)

Members of the genus *Bacillus* are able to produce a large number of antibiotics, which are mainly of polypeptide nature and synthesized as ribosomal and non-ribosomal peptides. However in recent years, substances containing amino acids plus other constituents have been detected with a wide variety of other applications such as alternative therapeutics in animal production, biocontrol of phytopathogens, biosurfactants with applications in environmental protection and as potential agents in health care and food processing industries. The present study reports the effect of nutritional conditions on the antimicrobial activity displayed by a local strain of *Bacillus* isolated from an aquatic environment of San Luis. The initial screening for antimicrobial activity on solid media was performed inoculating the producer strain (streak) on a preincubated lawn of each sensitive microorganism using agar plates of different media (Mueller-Hinton and Peptone Agar supplemented with Dextrose (PAD). The antimicrobial action was tested against *Escherichia coli*,*Staphylococcus aureus* ATCC 29213, *S. aureus* ATTC 43300 and *Saccharomyces cerevisiae*. For the production of active metabolites *Bacillus* sp. SL-6 was cultured on Peptone Dextrose and Synthetic Mineral Broths (SMB) with orbital shaking at 200 rpm during 24 h at 30ºC. After growth cultures were centrifuged and filtrated to obtain cell-free supernatants, followed by the addition of HCl to pH 2. The acidified supernatant was then kept at 4ºC overnight for complete precipitation. After centrifugation the pellet was resuspended in Phosphate Buffered Saline (PBS); lyophilized and solvent-extracted with chloroform:methanol (3:1). Another fraction of the acídic precipitation was lyophilized and resuspended en PBS at pH= 6.5. The antimicrobial activity in the processed *Bacillus* sp. SL-6 supernatant was tested against *E. coli*,*S. aureus* ATCC 29213 and *Saccharomyces cerevisiae* by the agar well diffusion method. In the initial screening the antimicrobial activity was evident when PAD was used, while in Mueller Hinton agar it was scarcely observed and with poor growth of the yeast. The active metabolites were excreted to the culture

supernatant only when SMB was used, showing inhibition against all the microorganisms assayed. The antagonistic activity against *E. coli* was obtained in the hydrofobic fraction while the hidrophylic one was active against *S. aureus* ATCC 29213 and *Saccharomyces cerevisiae*. The results obtained show that the inhibition spectrum of *Bacillus* sp. SL-6 was strongly influenced by the nutritional conditions of the growth phase, suggesting that this microorganism could secrete more than one active metabolite in the culture medium, which can be detected depending on the separation procedure applied.

PM-P10

THE LOW-MOLECULAR-WEIGHT FRACTION OF THE EXOPOLYSACCHARIDE II FROM *Sinorhizobium meliloti* IS INVOLVED IN AUTOAGGREGATION OF PLANKTONIC CELLS.

Fernando G. Sorroche[1], Luciana V. Rinaudi[1], Erika Banchio[1], Ángeles Zorreguieta[2,3], Walter F. Giordano[1]

[1] Departamento de Biología Molecular, FCEFQyN, Universidad Nacional de Río Cuarto [2] Fundación Instituto Leloir [3] FCEyN, UBA (fsorroche@exa.unrc.edu.ar)

Physical cell-cell interactions determine the aggregative behavior in many bacteria, and leads to the formation of multicellular conglomerates that show an increased resistance to unfavorable conditions. *Sinorhizobium meliloti*, a Gram negative soil bacterium, has the potential to establish a symbiotic chronic infection in *Medicago*, *Melilotus* and *Trigonella* species, inducing the formation of nitrogen-fixing root nodules. Succinoglycan (EPS I) and galactoglucan (EPS II) are two extracellular polysaccharides that play an important role in the symbiotic association. Specifically, the low molecular weight (LMW) fractions of both EPS have been shown to have a symbiotic relevance. ExpR is a LuxR-type regulator that plays multiple roles in *S. meliloti* physiology, including EPS II synthesis. Rm1021, a sequenced wild type reference strain, has an altered, non-functional *expR* locus. Although it is able to produce EPS I, it does not synthesize EPS II under normal conditions. The autoaggregation of Rm1021 was greatly reduced as compared to Rm8530, an Rm1021-derived strain which carries a functional allele of the *expR* gene and produces EPS II. Moreover, Rm2011 and 102F34, two wild type reference strains that carry deleterious *expR* alleles, also showed poorly autoaggregative phenotypes. These observations indicate that an intact, functional copy of the *expR* locus is required for an optimal expression of the autoaggregative phenotype in *S. meliloti*. Autoaggregation of washed cells of Rm8530, was drastically inhibited, indicating that an extracellular factor may be responsible for the aggregative behavior in this strain. The introduction of the mutant allele *exoY210*::Tn5 in Rm8530 abolished the production of EPS I, but had no significant effect on the autoaggregation phenotype. However, Rm8530 *expA*::Tn5-233, which does not synthesize EPS II, showed a strongly reduced autoaggregation, suggesting that EPS II is the polymer responsible for the aggregative phenotype of *S. meliloti*. The autoaggregation of the regulatory mutant Rm1021 *mucR31*::Tn5, which synthesizes HMW EPS II and traces of EPS I, showed a typical poorly aggregative phenotype, indicating that HMW EPS II is not mediating the cell-cell interactions leading to autoaggregation. In fact, complementation of autoaggregation was demonstrated when cell-free culture supernatants from strains Rm8530 and Rm8530 *exoY* (containing LMW EPS II) were able to promote a significant aggregation of Rm1021, Rm8530 *expA*, Rm8530 *expA exoY* and Rm1021 *mucR* (unable to produce LMW EPS II). These results show that the low molecular weight fraction of EPS II is a significant determinant in the establishment of adhesive interactions between planktonic *S. meliloti* cells.

PM-P11

PHOSPHATE SOLUBILIZING ABILITY OF PEANUT ASSOCIATED BACTERIA.

Tania Taurian[1], *Adriana Fabra*[1]

[1] *Universidad Nacional de Río Cuarto (ttaurian@exa.unrc.edu.ar)*

Peanut is an economically important crop in Cordoba being 92 percent of its production concentrated in this area. Nevertheless, intensity of agricultural practices has led to a reduction of important nutrients such as phosphorus. Phosphate solubilizing bacteria (PSB) are beneficial microorganisms that improve plant growth by releasing phosphorus to plants in an available form. The main mechanism by which bacteria release P is by the production of organic acids which quelate cations of insoluble phosphates and thus releasing Pi. The aim of this study was to determine phosphate solubilizing ability of 9 native peanut associated bacteria in liquid media and analyze buffer media effect in this plant growth promoting trait. The isolates were selected from a pool of 109 phosphate solubilizers previously described in the laboratory as producers of larger halos of phosphate solubilization in solid media ranking from 10 to 20 mm. In this study quantification of phosphate solubilization was done in buffered and unbuffered liquid NBRIP-BPB media. Amount of phosphate solubilized (µg/ml) and pH of the media were determined at 24, 48, 72 hours and 7 days of growth. A *Pseudomonas* strain isolated from a commercial biofertilizer was included in this study for comparison. Inoculation of PSB isolates into NBRIP liquid medium resulted in a gradual increase in the amount of soluble phosphate in the medium until 72 h but it decreased by day 7. In all test cultures a corresponding decrease in the pH of the medium was observed indicating acid production. Most of the cultures showed a maximum decrease in pH from 7 to 4 but the quantities of solubilized phosphate were not the same in all the cultures. This could be caused by differences in the phosphate-dissolving strengths of different organic acids released by the bacteria. Isolates J49, J33, L176 and the *Pseudomonas* strain were the best phosphate solubilizers with a maximum amount of released phosphate ranking from 360-420 µg/ml. Although isolate J157 showed one of the largest solubilizing halos it solubilized the smallest amount of phosphate. No differences were observed in their phosphate solubilizing ability in unbuffered and buffered media, except for isolate J260. From these results it is possible to conclude that there is not correlation between diameter of solubilizing halo and phosphate solubilized or with pH reduction of culture media. Adding to that, buffer concentration used in this work did not have effect on phosphate solubilizing ability.

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PM-P12

***Helicobacter pylori* BIOFILM FORMATION AND EXPRESSION OF *luxS* and *omp18* GENES**

Alba E. Vega[1], *Fabio A. Persia*[1], *Teresa I. Cortiñas*[1], *María C. Dalfovo*[1], *Humberto J. Silva*[1]

[1] *Área Microbiología. Fac. de Química, Bioquímica y Farmacia. UNSL. (aevega@unsl.edu.ar)*

Biofilms are communities of bacteria able to survive in unfavorable conditions where they often choose a sessile biofilm lifestyle as a strategy to overcome environmental stress. The expression of *luxS* gene represents an indicator of biofilm production, in which bacteria migrate and adhere to surfaces forming micro-colonies. *H. pylori* have the capacity to attach to different abiotic surfaces and the production of biofilm may be an important step for growth and survival during transmission. The intercellular communication, quorum sensing, is encoded in *H. pylori* by the *luxS* gene that is essential for colonization of the human stomach. Bacterial outer membrane proteins are important for ion transport, bacterial virulence and adherence. In *H. pylori omp18* is a peptidoglycan-associated lipoprotein precursor that is involved in the adherence to gastric cells. Therefore, it is possible that the *luxS* and *omp18* genes can be involved in biofilm formation. We analyzed the *luxS* and *omp18* gene expression in *H. pylori* during biofilm formation in glass and polystyrene surfaces. *H. pylori*, NCTC11638 reference strain and HP796 clarithromicyn and metronidazole resistant strain, were grown in Mueller-Hinton broth supplemented with: a) 5% fetal calf serum (reference) and b) 0.5% alternative supplement of microbial origin. The cultures were incubated under microaerophylic conditions for 196 h at 37ºC. The viability of adhered *H. pylori* bacteria to abiotic surfaces was determined by plate counting and fluorescence microscopy using the LIVE/DEAD BacLight viability kit. The *luxS* and *omp18* genes together with the 16SRNA, as housekeeping gene, were analyzed. For RNA extraction, the confluent growth on the two abiotic surfaces was treated with TRIzol reagent. The results showed that both strains have the capacity of biofilm formation in the surfaces assayed. The resistant strain showed a higher ability of biofilm formation ($p \leq 0.005$), maintaining the viability for 196 h. In relation to bacteria viability, differences of 2 to 3 logarithmic units were detected in the total and viable counts ($p \leq 0.05$) at 196h, regardless of nutritional conditions. Morphological changes were observed by fluorescence microscopy, spiral to cocoid forms and live and dead bacteria were clearly scored in samples processed at 96 h and 196 h. Both strains showed a higher expression of *luxS* and *omp18* genes in bacteria present in the biofilm. The study found no statistical differences in gene expression for conditions assayed. The increase of *luxS* and *omp18* gene expression and the changes in morphology and viability observed during biofilm formation in both surfaces and nutritional conditions, could indicate that *H. pylori* prefers a community-based surface-bound lifestyle, regulating gene expression to optimize the survival in hostile environments.

PM-P13

EFFECT OF SUPERNATANT OF *Lactobacillus plantarum* CULTURES AND LACTIC ACID ON STRUCTURE, BIOMASS, VIABILITY AND CHEMICAL COMPOSITION OF *Pseudomonas aeruginosa* BIOFILMS.

Ramos, AN[1]; Bosch, A[2]; Yantorno, O[2]; Valdez, JC[1]

[1]Cátedra de Inmunología, Instituto de Microbiología, Fac. de Bioq., Qca., Fcia. y Biotec. Univ. Nac. de Tucumán.

[2]Centro de Investigación y Desarrollo de Fermentaciones Industriales (CINDEFI). Facultad de Ciencias Exactas. Universidad Nacional de La Plata. (anramos@fbqf.unt.edu.ar)

In previous work, we demonstrated that supernatants of *Lactobacillus plantarum* cultures (SLp), inhibit quorum sensing, biofilm, virulence factors and growth of *Pseudomonas aeruginosa*. In this work, we investigate comparatively the reasons by which SLp, SLp neutralized (SLpN) and lactic acid (LA) are able to inhibit the biofilm of *P. aeruginosa*. For this, we designed various systems of batch and continuous cultures in which we let form at different times, biofilm of *P. aeruginosa* over: 1) Polypropylene pearls (used to measure biomass by Violet crystal (VC) and live-dead bacteria by Syto 9-propidium iodide stains) 2) Slides (used to determine structure by VC) and 3) ZnSe optical plates (used to study the chemical composition by Fourier Transforms-Infrared (FT-IR). Also in the supernatants of these cultures we measured pH and CFU/ml. The mentioned batch and continuous cultures were carried out in the presence of 0.85% NaCl (control), SLp, SLpN or LA. The biofilm obtained in presence of SLp, SLpN and LA, preserves the chemical composition (detected by FT-IR) obtained with control (NaCl 0.85%). However the amount of biofilm formed was lower in the presence of these three substances compared to control. This means that at different times, SLp SLpN and LA inhibit biofilm formation. SLp showed greater inhibitory capacity than SLpN and LA. LA induced a higher bacterial mortality (within the biofilm) than SLp and SLpN. Apparently this would be its inhibitory mechanism. We do not know exactly the reasons by which SLp inhibit the biofilm of *P. aeruginosa* but appears to be related to its pH and lactic acid composition. However, these two causes do not fully explain its great inhibitory capacity. Conclusions: The biofilm would be inhibited by acid pH, lactic acid and additional elements which are present in the SLp. FT-IR do not determine the qualitative differences between inhibited and not inhibited biofilm, so it must be carried out a deeper analysis.

PM-P14

EXOENZYMES PRODUCTION BY *RIZHOCTONIA SOLANI* IN DIFFERENT GROWTH MEDIUM.

Lis M. Rios [1] , Marta C. Rivera[2], Luis A Diorio[1]

[1] DBBE, FCEyN, UBA, [2] FAGR, UBA (lismrios@bg.fcen.uba.ar)

The presence of plant pathogens in the soil is a severe problem for diverse crops. *Rizhoctonia solani* particularly causes infections by penetrating plant tissues directly. The distinctive characteristics of this species are: a rapid growth, light to dark chestnut-brown mycelia and hyphae of a relatively large diameter. In the absence of hosts or in adverse environmental conditions it can survive in the soil by forming sclerotia. These can be found free or inside vegetable remains. The mycelium that develops form this resistance structures can initiate new infections. To increase the knowledge of this infection strategy, the physiological characteristics of the fungi were evaluated by measuring the enzymatic behaviour in three different growth media, without agitation. These were potato agar, potato agar supplemented with dextrose, and synthetic medium with xylane as the only source of carbon. In all media $Ca(CO_3)_2$ was added due to its natural presence in *Rizhoctonia solani* natural habitat. The mycelium was inoculated with cubes of 3 mm side of potato agar with active growing mycelia. It was incubated at 28 °C of temperature in the dark. The growth was optimum in all three media, and mycelia were detectable from the second day of incubation. The enzymatic concentration was evaluated from the day of inocula to the thirteenth day of growth. To do this, samples were filtrated daily, to separate mycelia from the liquid media. On the latter, essays were performed to determinate the activities xylenase, pectinase and cellulase using xylane, apple pectin or CMC as substrates respectively, and determining the degradation product by Somoyi–Nelson. The results were: maximums concentration of xylenase for the first two media in day 1, with a value of 0.0139 nmol/min*ml for potato agar media, and 0.0137 for potato agar supplemented with dextrose. Lastly, for the synthetic medium with xylane, the maximum was detected on day 6 and the concentration was 0.0152 nmol/min*ml. For pectinase, the maximums were also found on day 1 for the first two media, 0.0133 nmol/min*ml for potato agar and 0.0142 nmol/min*ml for potato agar supplemented with dextrose and on day 6 for synthetic media with xylane with a value of 0.0301 nmol/min*ml. On the contrary, for cellulase the maximums were recorded on day 2 for the first two media, with a concentration of 0.002 nmol/min*ml, and on day 5 for the last one, with a value of 0.0140 nmol/min*ml. *Rizhoctonia solani* cultivated in laboratory conditions is capable of producing the group of exoenzymes necessary to attack vegetable tissues and become a pathogen. The values of enzymatic activity observed would also confirm this assumption.

MM-P1

ARE *Mycobacterium bovis spoligotypes* FROM PIGS IDENTICAL TO CATTLE?

Soledad Barandiaran[1], Marcela Martínez Vivot[1], Eduardo Vicente Moras[1], Angel Cataldi[2], Martín José Zumárraga[2].
[1]*Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina*. [2]*Instituto de Biotecnología, CNIA-INTA, Castelar, Buenos Aires, Argentina (sbaran@fvet.uba.ar)*

Tuberculosis (TB) is a chronic disease affecting domestic and wild animals as well as humans. *Mycobacterium tuberculosis* and *Mycobacterium bovis* are the most important etiologic agents of tuberculosis because of the economic impact and their relevance in human and animal health. These agents belong to *M. tuberculosis* complex, along with *M. bovis subsp. caprae*, *M. africanum* subtype I and II, *M. microti*, *M. canetti* and *M. pinnipedii sp. nov*. Pigs are more susceptible to *M. bovis* although they can also be infected by *M. tuberculosis*. In countries where bovine tuberculosis (BTB) has not been eradicated, the prevalence of *M. bovis* in pigs usually shows the rate registered in the local cattle bovine population. The aim of this study was to type *M. bovis* isolates from pigs and to compare the spoligotypes with the ones found in cattle from Cordoba province. Twenty five tuberculosis lesions were found during the veterinary inspection in slaughterhouse between 2007 and 2008. The samples were processed by the Petroff method, cultured in Lowenstein-Jensen and Stonebrink media and incubated at 37ºC during 60 days. The molecular typing of the *M. bovis* isolates were carried out using the reverse hybridization technique of Spoligotyping. This method is based on PCR amplification of a highly polymorphic DR locus in the *M. tuberculosis* complex. Eleven different spoligotypes were detected among 25 *M. bovis* isolates from Cordoba province. Nine of eleven spoligotypes were previously found in cattle from Argentina. The major one involved 12 isolates (48%) and showed the spoligotype SB 0140. Moreover, it also represents the most frequently observed spoligotype in cattle from Córdoba province and Argentina. These results could explain the source of infection of pigs. Conversely, there were two spoligotypes not detected previously neither in cattle nor other hosts from Argentina. This interesting finding could be due to the partial screening of the bovines with BTB in Argentina or to the existence of *M. bovis* clones circulating exclusively among pigs.

MM-P2

IDENTIFICATION AND CHARACTERIZATION OF A TYPE II POLYHYDROXYALKANOATE SYNTHASE IN *Pseudomonas extremaustralis*

Mariela V. Catone[1], Nancy I. López[1]
[1]*Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA (mcatone@qb.fcen.uba.ar)*

Polyhydroxyalkanoates (PHAs) are polyesters produced by many bacterial species as intracellular storage compounds under unbalanced growth conditions. According to the number of carbon atoms that form the monomer units, PHA can be classified into two groups: short-chain-length (scl) with C3 to C5 monomer units and medium-chain-length (mcl) PHA with C6 to C14 monomer units. Both kinds of polymers have different characteristics: scl-PHAs have properties close to conventional plastics while the mcl-PHAs are regarded as elastomers and rubbers.Type I and type II synthases are the key enzymes that catalyze the biosynthesis of scl and mcl PHA, respectively. *Pseudomonas* species usually have a mcl-PHA cluster that involves two type II synthase genes (*phaC1* and *phaC2*) separated by the gene encoding the depolymerase (*phaZ*). However, *Pseudomonas extremaustralis* has a complete cluster for polyhydroxybutyrate (PHB) production, the most common PHA, including a type I synthase. While the wild type strain produces only PHB, the $phaC_{scl}$ mutant showed the ability to produce PHA_{mcl}. We used a PCR cloning strategy, based on the design of degenerated primers of the neighbour gene sequences of *phaC2* synthase, *phaZ* and *phaD*, of complete genome sequenced *Pseudomonas*, to screen for a type II synthase in *P. extremaustralis*. The resulting amplicon was sequenced and two open reading frames (ORF) were detected, one of 1683 nt and other of 170 nt belonging to a putative complete *phaC2* and a putative incomplete *phaZ*, respectively. Sequence showed a 90% of similarity with *phaC2* of *P. fluorescens* SBW25 and 92% with *phaZ* of *Comamonas testosteroni*. In order to prove its functionality, the putative *phaC2* gene was subcloned into the plasmid pSJ33 and introduced by transformation in *P. putida* GPp104, a mutant strain unable to produce PHA. *phaC2* was able to complement the PHA negative phenotype. PHA granules were observed by microscopy after staining with Nile blue when the strain was grown on sodium octanoate. PHA was not accumulated when glucose or gluconate were used as carbon source. In a previous work we observed that the complementation of *P. putida* KT2440, a mcl-PHA producer, with type I synthase (*phaC*) of *P. extremoaustralis* leads to the production of PHB instead of PHAmcl, suggesting mainly a cynetic control, more than regulatory, between type I and II synthases. The presence of a type II PHA synthase in the genome of *P.extremaustralis* that is expressed when type I synthase is inactive, constitute the first report of this feature in *Pseudomonas* spp. One interesting result of this work is that genetic manipulation allowed the production of polymers with different properties in the same strain, which could be useful for biotechnological applications.

MM-P3

ENDONUCLEASE ACTIVITY OF *Pseudomonas aeruginosa* MUTL PROTEIN

E. M. Eugenia Correa[1], *Carlos E. Argaraña*[1], *José L. Barra*[1]

[1]*CIQUIBIC–CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, UNC. (eecorrea@fcq.unc.edu.ar)*

Replication errors can be corrected by the mismatch repair system found in most organisms. In *Escherichia coli*, and a reduced number of bacteria, MutH protein nicks the non-methylated DNA at a hemi-methylated GATC site, providing the signal to distinguish the parental strand from the newly synthesized strand. Most genomes do not contain a MutH homologue. Therefore, most organisms must use a different nicking endonuclease. It was recently described that some MutL proteins have an endonuclease activity. The endonuclease active site probably involve a DQHA(X)2E(X)4E and a C(P/N)HGRP motif. We started the analysis of the first conserved motif of *P. aeruginosa* MutL protein by mutating its D and first E amino acids. Both mutant proteins are inactive *in vivo*. We also analyzed the endonuclease activity of the C-terminal region (250 aa) of the wild type and mutant proteins. *In vitro* analysis, using supercoiled plasmid DNA as substrate, showed that the wild type and mutant proteins display an endonuclease activity. These results suggest that although the D and the first E amino acids of the conserved region are essential for *in vivo* mismatch repair, they are not essential for the MutL endonuclease activity, suggesting that structural modifications of the mutant proteins affecting some other function of MutL may be responsible for their in vivo lack of function.

MM-P4

IMPROVEMENTS IN THE DESIGN OF NON-HYDROLYZABLE LNA/DNA ANTISENSE OLIGONUCLEOTIDES THAT INTERFERE WITH AMIKACIN RESISTANCE IN VIVO

Carol G. Davies Sala[1], *Alfonso J. Soler Bistué*[1,2] , *Fernando A. Martín*[1], *Nicolás F. Vozza*[1], *Doreen E. Carpio*[2], *Angeles Zorreguieta*[1], *Marcelo E. Tolmasky*[2]

[1]*Fundación Instituto Leloir-IIBBA, CONICET y Dto. Qca. Biológica FCEyN, UBA, Argentina* [2]*Center for Applied Biotechnology, California State University Fullerton, USA (cdavies@leloir.org.ar)*

The increase in antibiotic resistance among pathogenic bacteria is a topic of growing concern. The aac(6´)-Ib gene, which encodes an acetyltransferase that catalyzes the inactivation of several aminoglycosides of clinical relevance including amikacin (Ak), is rapidly spreading in the clinical setting among a variety of gram negative pathogens. Inhibition of aac(6´)-Ib expression could help extending the life of Ak as a viable treatment. EGS technology consists of the use of RNA oligonucleotides, known as external guide sequences (EGSs), that are complementary to a target RNA molecule and elicit RNAse P-mediated cleavage of the target mRNA. EGSC3 is an EGS that induces *aac(6')-Ib* mRNA cleavage by RNase P in vitro and reduces levels of expression in vivo leading to a decrease in the MIC of Ak. Since oligoribonucleotides are rapidly degraded by nucleases, a practical utilization of EGS technology requires the design of isosequential non-hydrolyzable analogs that mimic the effect of the RNA EGSs. Co-oligomers with combinations of locked nucleotides and deoxynucleotides (LNA/DNA) showed EGS activity in vitro. In this work we assessed these EGSs in vivo for their ability to interfere with Ak resistance. In vitro experiments were also carried out to test whether replacements of DNA for LNA in different positions of the EGSs could further improve RNAse P cleavage efficiency. The EGSs were designed to include a 13-nucleotide segment antisense to the target mRNA followed at the 3' end by the ACCA sequence that interacts with the UGG sequence within the RNA component of the *E. coli* RNase P. *E. coli* AS19 is a hyperpermeable strain and pFC9 is a plasmid that includes *aac(6´)-Ib*. To determine the effects of LNA/DNA EGSs on Ak resistance in vivo *E. coli* AS19(pFC19) cells were incubated in the presence of LNA/DNA EGSs for an hour before addition of Ak. In vitro RNase P cleavage experiments were carried out with EGSs with a variety of LNA/DNA configurations to identify those that induced RNAse P cleavage at highest efficiency. EGSs assayed included compounds in which all three or four deoxynucleotides at the ACCA 3'-end were replaced in addition to other substitutions in the antisense region. It was established that four replacements at the 3' terminal ACCA showed higher efficiency of cleavage compared with those with three replacements. Likewise replacements on the 5´-end of EGSs were also tested while keeping 3'end constant. Some particular LNA substitutions were able to enhance in vitro RNAse P cleavage of the *aac(6´)-Ib* mRNA. Cultures exposed to selected antisense LNA/DNA EGSs showed a significant reduction in cell counts when compared to those that had been exposed to sense controls or random LNA/DNA EGSs. These results indicate that non-hydrolyzable DNA/LNA EGSs with the appropriate design interfere with Ak resistance expression in vivo.

MM-P5

STUDY OF THE INVOLVEMENT OF POLIV, IMUB AND DNAE2 ERROR-PRONE DNA POLYMERASES IN UV-INDUCED AND STATIONARY-PHASE MUTAGENESIS IN *Pseudomonas aeruginosa*

Sofía Feliziani[1], *Adela M. Luján*[1], *Alejandro J. Moyano*[1], *Andrea M. Smania*[1]

[1]*CIQUIBIC-CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, UNC. (sfeliziani@mail.fcq.unc.edu.ar)*

Stress-induced mutagenesis is an active process employed by bacteria to adapt and survive to different stresses. This mechanism requires SOS-regulated error-prone DNA polymerases which are able to bypass lesions allowing the continuation of DNA replication. It has been reported that several bacterial species possess these specialized DNA polymerases such as PolIV and ImuB, which belong to the Y-family, and DnaE2 which is related to the catalytic subunit DnaE of Pol III. However, in the opportunistic pathogen *Pseudomonas aeruginosa* these polymerases remain poorly investigated. In this study, we raised a question about the involvement of PolIV, ImuB and DnaE2 in *P. aeruginosa* stress-induced mutagenesis. For this purpose, we constructed *P. aeruginosa* PAO1 *dinB*, *imuB* and *dnaE2* single deletion mutants and investigated their role in UV-induced mutagenesis. After UV irradiation, PAO1 strain showed a 2.6 fold increase *(P=0.038)* in the mutation frequency compared to untreated controls, confirming that *P. aeruginosa* displays a subtle UV-induced mutator phenotype. PolIV deficiency resulted in levels of UV-induced mutagenesis similar to PAO1 (ratio 3.6; *P=0.019*), suggesting that this polymerase was not involved in the mutagenic repair of UV lesions. In contrast, *imuB* and *dnaE2* strains did not show a statistically significant increment in their mutation frequencies (ratio 0.83, *P=0.09*; ratio 0.72, *P=0.29* respectively), indicating that in *P. aeruginosa* UV-induced mutagenesis might depend on both ImuB and DnaE2. We further investigated the role of these polymerases in the stationary-phase mutagenesis. For this, we employed a test system to study starvation-induced mutagenesis which was previously characterized in *P. putida*. This system consists in a set of plasmids containing the reporter gene *pheA*, which encodes for a phenol monooxygenase and whose expression allows phenol degradation. These plasmids were engineered to harbor loss-of-function mutations in the coding region of *pheA*. Thus, this system allows the detection of -1 deletions and base substitutions that revert the engineered alterations and permit bacteria to grow using phenol as the sole carbon source. Results obtained from incubating PAO1 cells that carried the test-plasmids on minimal phenol selective plates, confirmed that, as previously observed for *P. putida*, *P. aeruginosa* displays stationary-phase mutagenesis. We are currently testing the *dinB*, *imuB* and *dnaE2* strains in order to study the role of PolIV, ImuB and DnaE2 in stationary-phase mutagenesis and consequently to contribute to a better understanding of the mutational mechanisms operating in *P. aeruginosa*.

MM-P6

ISOLATION AND CHARACTERIZATION OF *Pseudomonas* sp. SF4C MUTANTS IMPAIRED IN BACTERIOCIN PRODUCTION

Sonia E. Fischer[1], *Edgardo C. Jofre*[1], *Agustina Godino*[1], *Gladys B. Mori*[1]

[1]*FCEFQyN, Universidad Nacional de Río Cuarto,(sfischer@exa.unrc.edu.ar)*

Bacteria living in a competitive environment are able to secrete proteinaceous toxins, known as bacteriocins, which can kill closely related bacterial competitors while causing little harm to the bacteriocinogenic cells. On the other hand, bacteriocins have potential as biological control agents that can be used against bacterial pathogens. *Pseudomonas* sp. SF4C (native strain isolated from wheat rhizosphere) produces a high-molecular-weight bacteriocin (resistant to proteinase K) that is active against *Pseudomonas fluorescens* CTR 212. To obtain mutants impaired in bacteriocin production, random transposon mutagenesis of *Pseudomonas* sp SF4c was carried out. Kanamycin-resistant Tn5-B20 mutants of strain SF4C that had lost the capacity to inhibit the growth of strain CTR 212 were isolated. The presence of a single Tn5-B20 insertion in some mutants (496, 581, 634) was confirmed by Southern blot analysis. All the transconjugants analyzed showed hybridizing fragments of similar sizes. Therefore, it is possible that the transposon has been inserted at identical positions in the genomes of these mutants. An *Eco*RI fragment containing the Tn5-B20 insertion present in the mutant strain 634 was cloned into vector pBluescript to generate the plasmid pSF16. DNA sequence analysis of pSF16 revealed that the transposon was inserted into a gene encoding a putative phage tape-measure protein involved in the determination of tail length in phage. This gene belongs to Prophage 01 cluster from *Pseudomonas fluorescens* Pf0-1. Closely related prophages 01 exist in the genomes of *P. fluorescens* Pf-5, *P. fluorescens* Q8r1-96 and *P. fluorescens* SBW25. The homologous prophage elements from Pf-5 and Q8r1-96 are similar to F-type pyocins. These pyocins were first discovered in *P. aeruginosa* and represent a class of high-molecular weight protease- and nuclease-resistant bacteriocins that resemble flexible and non-contractile tails of bacteriophages. A prophage element found in the identical spot (between *mut*S and *cin*A) in the genome of *P. fluorescens* SBW25 has a similar overall organization but resembling another class of phage tail-like bacteriocins, the R-type pyocins of *P. aeruginosa*. Furthermore, a homologous region from *P. fluorescens* Pf0-1 contains two clusters, similar to the hybrid R2/F2 pyocin locus from *P. aeruginosa* PAO1. The differences in organization of the putative phage tail-like pyocins among these prophages clearly indicate that the corresponding loci are subject to extensive recombination.

MM-P7

IN SILICO ANALYSIS OF HALOARCHAEAL INTRAMEMBRANE PROTEASES OF THE RHOMBOID FAMILY

María I. Giménez[1], Rosana E. De Castro[1]

[1]Instituto de Investigaciones Biológicas, UNMdP-CONICET, Mar del Plata, Argentina (migimen@mdp.edu.ar)

Rhomboids are intramembrane proteases which are conserved in the three domains of life. Even though they share topological traits, their roles in different organisms, when known, are very diverse. Particularly, nothing is known on the biology of rhomboid proteases in archaea. Haloarchaea belong to the *Archaea* domain and thrive in high salt (1.5 M to saturation NaCl) environments. As a first approach to study rhomboid proteases in this group of extremophilic microorganisms, we performed a bioinformatics survey for haloarchaeal intramembrane proteases, focusing on the rhomboid family, in publicly available haloarchaeal genome sequences. Haloarchaeal genomes encode two or three rhomboid proteases, depending the organism. Predicted protein sequence analysis showed that some rhomboid proteases of halophilic archaea show the canonical 6 transmembrane segment (TMS) topology whereas others have unique traits, including extra TMSs for one group of proteases and an N-terminal AN-1 Zn-finger motif for another. The domain combination of the Zn-finger-rhomboid proteases is unique and suggests a novel function for these proteases in haloarchaea. Zn-fingers are protein motifs initially described as DNA interacting domains, which can also interact with other molecules as RNA, lipids and proteins. Interestingly, in all studied genomes, we found that the gene encoding the Zn-finger motif-containing rhomboid is in the same operon with a predicted endonuclease V gene, suggesting the participation of rhomboid proteases in DNA related mechanisms.In an attempt to search for potential substrates of rhomboid proteases in archaea, we looked for an N-terminal extension on the TatA protein, a component of the Tat protein translocation system, which was previously reported to be processed by a rhomboid protease in the bacterium *Providencia stuartii*. We observed that all haloarchaeal TatA homologs show an extension of 5-11 aminoacid residues followed by a predicted serine protease cleavage site, anticipating that the TatA component is also processed by rhomboid proteases in haloarchaea. All the data compiled in this work suggest that haloarchaeal rhomboid proteases may have unique as well as common roles and features compared to their non-haloarchaeal counterparts. Current experimental work in our laboratory is aimed to confirm this hypothesis.

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MM-P8

COMPARISON OF DNA RESTRICTION PROFILES OF *Salmonella enteritidis* STRAINS ISOLATED FROM CHICKEN IN SAN LUIS, ARGENTINA

Valeria S. Lazarte Otero[1], Celina S. Ciacera[2], Gabriela I. Favier[1], Cecilia S. Lucero Estrada[1], Lidia C. Velázquez[1], Maria E. Escudero[1], María E. Placci[2], Ana M. Stefanini De Guzmán[1]

[1]Microbiología General, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis [2]Análisis Clínicos, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis (mescude@unsl.edu.ar)

Pulsed field gel electrophoresis (PFGE) is a powerful tool applied to investigate the heterogeneity of the genomic restriction profiles of bacteria belonging to the same species and establish relations of epidemiological relevance. In the present work, this technique was used for subtyping *Salmonella* Enteritidis strains isolated from 113 chicken intended for consumption in San Luis, Argentina. The strains were subjected to the PFGE protocol standardized by PulseNet (Centers for Disease Control and Prevention, CDC, USA) with minor modifications. Bacterial strains were isolated on Mueller Hinton agar and suspensions of each strain were done in TE buffer (10 mM Tris, 1 mM EDTA, pH 8). The initial concentration was adjusted at OD_{610} 1.35. A volume of 200 µl of each suspension was mixed with an equal amount of 2% (w/v) low melting temperature agarose and poured in moulds. The agarose plugs corresponding to each *S.* Enteritidis strain were separately lysed in 5 ml of lysis solution (20 mg/ml Proteinase K, 50 mM Tris-EDTA, pH 8, 1% sodium lauroyl sarcosine) with gentle shaking at 37° C for 18 h. Plugs were washed two times in 5 ml of ultrapure distilled water and then, four times in TE buffer. Before restriction, plugs were cut in 2 – 2.5 mm thickness slices, and restriction was performed by adding slices of a strain to a Eppendorf tube containing 200 µl restriction solution (1X enzyme buffer and 10 U XbaI restriction enzyme) for 18 h at 37°C. Slices were washed in TBE buffer (0.045 M Tris-borate, 0.001 M EDTA) and loaded in a gel prepared by dissolving 1% high melting temperature agarose in 0.5X TBE. Electrophoresis was carried out using a CHEF-DR III system (BioRad) at 6 V/cm at 14°C for 20 h with the following pulse time: initial time 2.2 s and final time 63.8 s. The gel was stained with ethidium bromide (1 µg/ml in water) for 30 min, destained in water for 10 min, and observed with a UV transilluminator. Restriction fragments of the *Salmonella* Braenderup H9812 reference strain obtained by the same procedure, were used as DNA size standards (fragment sizes ranging from 20.5 to 1135 bp). Identical DNA restriction patterns were observed for the three local *S.* Enteritidis strains. The similarity of DNA profiles of these strains suggested a common contamination source which might be located in the farm, the slaughterhouse or the retail store.

MM-P9

EFFECT OF HYPERMUTATION IN *Pseudomonas aeruginosa* BIOFILMS.

Adela M. Luján[1], María D. Maciá[2], Søren Molin[3], Antonio Oliver[2], Andrea M. Smania[1]

[1]CIQUIBIC-CONICET, Facultad de Ciencias Químicas, UNC, Córdoba, Argentina. [2]Servicio de Microbiología y Unidad de Investigación. Hospital Son Dureta, Palma de Mallorca, España [3]Infection Microbiology Group. Center for Systems Microbiology. Department of Systems Biology. DTU, (alujan@mail.fcq.unc.edu.ar)

In spite of aggressive treatment with antibiotics and the immune host defense, once established *Pseudomonas aeruginosa* persists in the Cystic Fibrosis (CF) airways for years or even decades. One cause of its persistence is the capacity to grow as biofilms that provide increased survival ability under a variety of stress conditions. Contributing to this, high prevalence of hypermutable Mismatch Repair System deficient isolates have been observed in CF chronic lung infection. It has been proposed that hypermutators play an important role in the acquisition of adaptative mutations needed for long-term survival of this bacterium in the CF airways. Despite the potential importance of an increased mutagenesis in the persistence of *P. aeruginosa* CF infections, the effect of hypermutation in biofilm formation has been poorly investigated. In this sense, the aim of the present study was to investigate the effects of hypermutator phenotype on the architecture and phenotypic diversification of *P. aeruginosa* biofilms. For this purpose, the reference strain PAO1, environmental strain Hex1T and their respective *mutS* mutants, PAOMS and Hex1TMS, were used. Biofilms were grown at 30°C in three-channel flow cells and pictures were taken at 2, 4 and 6 days. To study the phenotypic diversification, 6 days-biofilms were harvested, plated on *P. aeruginosa* isolation agar and the morphologies of approx. 2.000 colonies per strain were examined. All microscopic observations were performed by a confocal laser scanning microscope (CLSM). For structural analysis and processing of images software packages COMSTAT and IMARIS were used. Structural data were compared using a Student t test. In this work, we show that biofilms formed by PAOMS were thicker and showed more biomass than those formed by PAO1 ($P < 0.05$ at 4d). However, although no significant differences were found at days 2 and 4, Hex1TMS biofilms were thinner and showed less biomass than Hex1T at 6d ($P < 0.05$). CLSM images of biofilms formed by hypermutable strains showed irregular biofilms structures, randomly smooth, rough and filamentous. Empty mushroom-like structures were characteristically observed in Hex1TMS biofilms. Examination of colony morphology after 6d-biofilm revealed up to 6 different morphotypes in hypermutable and only 2 within wild-type strains. Normal, giant, mini, wrinkly, mucoid, brown and orange pigmented were among the different morphotypes found. Total percentages of colonies with different morphotypes were 8.3 and 0.05 % for PAOMS and PAO1, respectively, and 2.3 and 0.8% for Hex1TMS and Hex1T, respectively. The results presented here demonstrated that Mismatch Repair System deficiency increases the structural diversity of *P. aeruginosa* biofilms and this could probably be a microscopically visible evidence of the acceleration of phenotypic diversification.

MM-P10

IDENTIFICATION AND ANALYSIS OF THE EXPRESSION OF GROWTH-DEPENDENT TRANSCRIPTS DETECTED BY RAP-PCR IN THE HALOALKALIPHILIC ARCHAEON *Natrialba magadii*

Enrique A. Madrid[1], Micaela Cerletti[1], Roberto A. Paggi[1], Rosana E. De Castro[1]

[1]IIB. CONICET. UNMdP (eamadrid@mdp.edu.ar)

Extremophiles live in environments with conditions that are lethal for most life forms. Particularly, haloarchaea have developed strategies to survive in the presence of high salt concentrations (>2M NaCl) and high pH values (alkaliphilic haloarchaea). The transition to the stationary phase induces changes in the morphology and physiology of bacteria. Often, such changes are regulated by a cell to cell based-communication mechanism denoted as quorum sensing. The changes induced during the transition from exponential growth to the stationary phase as well as the occurrence of quorum sensing-like mechanisms have been poorly explored in archaea and in organisms thriving in extreme environments. The *R*NA *a*rbitrarily *p*rimed PCR (RAP-PCR) is a powerful method that can be applied to identify genes that are differentially expressed. Differences in gene expression can be detected using this methodology, as each sample generates its own unique RNA fingerprint for each condition examined. The aim of the present work was to identify those genes that are differentially regulated in response to the transition from the exponential to the stationary phase in the alkaliphilic haloarchaeon *Natrialba magadii*. The screening was also aimed to those genes whose induction responded to the high cell density and thus, may be regulated by quorum sensing. By using RAP-PCR we have previously obtained 9 differentially expressed cDNAs which were cloned, sequenced and analyzed by bioinformatics search. Two of these cDNAs, 500 bp and 350 bp, were further characterized in this work. The 500 bp sequence (L1500) was identified as a HIT protein (*h*istidine *t*riad *p*rotein), while the 350 bp sequence (L6350) showed identity to an oligopeptide ABC transporter solute-binding protein. Specific primers were designed based on sequence analysis of these cDNAs and used to validate the differential expression pattern by RT-PCR. The relative mRNA levels of these transcripts were normalized to the 7S RNA transcript level. The HIT protein mRNA was highly expressed in the exponential phase and decreased 5-fold as the cells entered the stationary phase. On the contrary, the abundance of the ABC transporter mRNA was low in exponentially growing cells and increased 4-fold in the early stationary phase. In addition, the expression of this transcript was also analyzed in response to the presence of stationary phase conditioned

medium (CM) showing a 2.5-fold increase in the exponentially growing cells supplemented with CM compared to the control cells. The identification of genes that respond to different growth stages and the characterization of signal molecules involved in this process will help to understand the nature of the molecular mechanism of cellular communication used by the haloarchaea in extreme environmental conditions. Supported by UNMDP, ANPCyT and CONICET. *Enrique Madrid y Micaela Cerletti contribuyeron en igual proporcion.*

MM-P11

EFFECTS OF GENOMIC CONTEXT ON *Escherichia coli* DNA MUTATION RATE

Mariana A. Martina[1] , *Carlos E. Argaraña*[1] , *José L. Barra*[1]

[1]*CIQUIBIC–CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, UNC (mamartina@fcq.unc.edu.ar)*

In *Escherichia coli* the transitory hemimethylated state of adenines in GATC sequences provide the strand discrimination signal to direct the DNA mismatch repair system (MRS). Strains deficient in any of the principal components of the MRS (MutS, -L, -H, and Dam proteins) are mutators. It has been reported using plasmid heteroduplexes that a decrease in the number of GATC sequences within these vectors lowered the efficiency of mismatch repair *in vitro* and *in vivo*. We analyzed the effect of genomic GATC density on mutation rate in *E. coli*. We introduced a mutated copy of a gene able to confer antibiotic resistance within a genome region with a high or low density of GATC sequences and analyzed the reversion rate of the mutation. Our results show, unexpectedly, that the reversion rate was lower in strains containing the mutated gene in a genomic region with a low density of GATC sequences, than those located in a high density context. Moreover, the reversion rate of the mutated gene located in the low GATC density region of a MRS deficient strain was lower than the reversion rate of the mutated gene located in the GATC high density region of a wild type strain. Two possibilities are discussed: 1- Other(s) *cis* or *trans* acting factor(s) influence the mutation rate; 2- The high and low GATC density genomic regions differently affect the transcription rate of the reporter gene.

MM-P12

CONDITIONED MEDIUMS MODULATE EXPRESSION OF THE RCSB-REGULON GENES IN *S. typhimurium*

María de las Mercedes Pescaretti[1,2] , *Fabián E. López*[1,2] , *Roberto Morero*[1,2] , *Mónica A. Delgado*[1,2]

[1]*Dpto Bqca de la Nutrición-INSIBIO/Inst. Qca Biológica (UNT) Tucumán* [2] *CCT-Tucumán-CONICET (mpescaretti@fbqf.unt.edu.ar)*

The Rcs is an unusual phosphorelay system composed of the inner membrane proteins RcsC and RcsD as sensors, and the response regulator RcsB. At the present, the signals that lead to activate the Rcs phosphorelay system remain unknown. Even though, a wide range of conditions have been described as Rcs system activation state. The growth at low temperature or on solid surface; the polymyxin B exposition; the DjlA overproduction, the *rcsC11* constitutive mutation; mutations that affect cell envelope like *tolB* and *pmrA*, or *rcsB* overexpression are some examples of this activation states. Previously, we reported that the *rcsB* gene is transcribed from two promoters: i) P_{rcsDB} located upstream of *rcsD*, and ii) P_{rcsB} located within the *rcsD* coding region. The first promoter is induced during the exponential growth phase while the last one it does at lower levels in stationary phase. We also reported that the RcsB overproduction repressed the *rcsD* expression by directly binding to the P_{rcsDB} promoter. The repression, resulting in a differential rate of *rcsD* and *rcsB* genes expression levels, was also observed using *rcsC11* mutant or polymyxin B to induce the system. Under these conditions an increased level of RcsB was observed when the bacteria reach the stationary growth phase and the regulator began to be also transcribed from P_{rcsB}. In addition, the P_{rcsB} is physiologically required to maintain the repression on swarming behavior. The subject of the present work was to determine if an Rcs stimulation factor is excreted to the supernatant of *tolB* and *pmrA* mutant culture. This finding would allow us to identify the Rcs system signal. Here, the supernatant obtained from the above mutants' cultures, as "conditioned mediums", was used to determine the reporter expression levels. The *cps* and *flhDC* operons were the reporter of the factor presence in the conditioned mediums. We demonstrated that the *cps* and *flhDC* operons were modulated under the growth on these mediums. As RcsB regulator is expressed exponential and stationary phase and the above reporters are exponentially controlled, we looking for reporter RcsB-depended gene that are transcribed in stationary phase like *bapA* gene. Here we report that *asr* is a new member of the RcsB regulon, which was reported as an stationary phase expressed gene. Additionally, we studied the expression modulation of *asr*, *bap*, *cps* and *flhDC* using conditioned medium harvested from both different growth phase in order to relate with expression of RcsB regulator. Under this condition we observed a growth phase-dependent expression mediated by RcsB. These results increase the Rcs system knowledge on regulon and ligand identification issues.

MM-P13

INTERACTION OF THE C-TERMINAL REGION OF *Pseudomonas aeruginosa* MUTS WITH THE DNAK CHAPERONE

Mariela R. Monti[1], *Virginia Miguel*[1], *Carlos E. Argaraña*[1]

[1]*CIQUIBIC-CONICET. Departamento. de Química Biológica. Facultad de Ciencias Químicas, UNC, Argentina (mmonti@mail.fcq.unc.edu.ar)*

MutS recognizes misspaired bases and it is required to activate downstream events in the postreplicative DNA Mismatch Repair (MRS). In a previous work, we determined that the C-terminal region of *P. aeruginosa* MutS is essential for MRS activity. Different evidences indicated that this region may be involved in the interaction with other factor necessary for the correct function of the MRS. To analyze if the C-terminal region of MutS (Cter) interacts with other protein, the DNA encoding this domain was fused in frame to the maltose binding protein (MBP) gene, and expressed in *P. aeruginosa*. The purified MBP-Cter protein consistently copurified with a 70 kDa protein, which was absent in MBP purified preparations. This protein was identified as DnaK by mass spectrometry analysis. The *P. aeruginosa* cells expressing the fusion MBP-Cter acquired a phenotype similar to that previously described for *dnaK* mutants in other bacteria: diminished viability, cell filamentation and high temperature sensitivity. Similar phenotypes were detected by overexpressing the full length MutS protein. On the contrary, overexpression of a C-terminal deletion MutS mutant did not produce phenotypic changes compared to the wild type strain. We propose that DnaK interacts with the C-terminal region of MutS and the overexpression of the repair protein produces a complete sequestration of DnaK. We are currently analyzing the interaction MutS-DnaK by biochemical assays and generating *P. aeruginosa dnaK* mutant strains to analyze in more detail its phenotype.

MM-P14

MOLECULAR MECHANISMS OF CIPROFLOXACIN RESISTANCE IN HYPERMUTATOR STRAINS OF *Pseudomonas aeruginosa*

Natalia Rosalía N. Morero[1], *Carlos Enrique C. Argaraña*[1]

[1]*CIQUIBIC-CONICET, Dpto. de Química Biológica, Facultad de Cs. Químicas, UNC. (nmorero@mail.fcq.unc.edu.ar)*

Pseudomonas aeruginosa is a major opportunistic pathogen causing human infections and represents the main cause of morbidity and mortality in cystic fibrosis patients. The fluoroquinolone Ciprofloxacin is one the antibiotics most frequently used in the treatment of nosocomial infections with this bacterium. However, once established, *P. aeruginosa* is extremely difficult to eradicate due to the pathogen's ability to progress through a series of genetic and physiological changes that facilitate infection and persistence. Clinical resistance to ciprofloxacin (often accompanied with cross-resistance to other non-related antibiotics) has been reported to emerge quickly among clinical strains of *P. aeruginosa*. Thus, the antibiotic therapy must be carefully selected in terms of drug combination, concentration and time of exposure in order to prevent the selection and establishment of multidrug resistance strains. In this work we analyze the molecular nature of ciprofloxacin resistance for the PAO1 wild type strain and the hypermutators *mutS* and *mutT*, defective in the mismatch repair and the 8-oxoguanine repair systems respectively. To this purpuse, we sequenced the main genes known to be involved in ciprofloxacin resistance (*gyrA* and *parC* genes coding the topoisomerases target of ciprofloxacin, and *nfxB* coding for the represor of a multidrug efflux pump) for groups of resistant cells selected at different ciprofloxacin concentrations. Although we found some differences between the analyzed strains in terms of the resistance mechanisms and the mutational spectra, all of them showed a high percentage of *gyrA* mutations at high ciprofloxacin concentration, while the *nfxB* gene was mainly mutated in the groups of cells selected at low drug concentration. These *nfxB* mutants showed cross-resistance to other antibiotics such as erythromycin, zwitterionic cephems and trimethoprim, in contrast to those solely mutated in the topoisomerase genes. In this context, we discuss the influence of the exposure to low and high doses of ciprofloxacin on the emergence of multidrug resistant strains, both in a wild type and hypermutator background.

MM-P15

STRUCTURAL FEATURES OF A POLYPEPTIDE RELATED TO UBIQUITIN FROM *Natrialba magadii*

Maria V. Ordóñez[1], *Jaime Guillen Casas*[2], *Debora Nercessian*[1], *José Villalain Boullón*[2], *Rubén D. Conde*[1]

[1]*Instituto de Investigaciones Biológicas, UE UNMdP. CONICET. Argentina.* [2] *Instituto de Biología Molecular y Celular - Universidad Miguel Hernández. España (mvordone@mdp.edu.ar)*

Ubiquitin is a highly conserved protein in eukaryotes. Also, many ubiquitin-like proteins (Ubls) and ubiquitin like domain containing proteins (UbDs) have been described in both prokaryotes and eukaryotes. Usually these proteins share high structural homology but little sequence identity with ubiquitin. Also, because they are part of homologous conjugation pathways as that of ubiquitin, prokaryotic Ubls like ThiS and MoaD are functionally related to ubiquitin. All Ubls and UbDs proteins have a β-grasp fold comprised mainly by a β-strand with 5 antiparallel β-sheets with one α-

helix region. We are involved in studying the presence of Ubls and UbDs proteins in halophilic archaea and their evolutionary relationship with ubiquitin. Previously, we isolated by PCR a 400bps (p400) DNA fragment from *Natrialba magadii* genome whose aminoacidic sequence presents a 3D structure similar to ubiquitin by bioinformatic tests. Here we describe the biochemical-structural characterization of this polypeptide after expressing the construction pET24b::p400 in *E. coli* cells rosetta strain. As previously shown, the recombinant P400 polypeptide (P400r) appears in inclusion bodies from where can be partially purified by Ni-NTA affinity chromatography. In this work we show that it can be better purified after SDS-PAGE separation of total *E. coli* rosetta proteins followed by Zinc-Imidazol reverse staining. P400r was eluted from the gel slice with a Tris-glycine buffer with 90% yield. After renaturalization in presence of a buffer containing 1.5M KCl, the purified protein was recognized by the anti-ubiquitin antibody. Then, the secondary structure of P400r was analyzed by Fourier Transformed Infrared Spectroscopy. The resulting amide I spectrum showed that its conformation is mainly composed by β-sheets with a low level of α-helix. This preliminary result is in good agreement with both the predicted 3D model obtained and that expected for proteins with ubiquitin like fold. Finally, the recent publication of the complete genome sequence of *Nab. magadii* allowed us to gain information about the complete protein to which P400 belongs. The pBlast with P400 sequence against this genome revealed that it suits to a major protein of 262 amino acids. P400 is near the N-t region that also has a predicted signal peptide site at the N-teminus that would direct the protein to its secretion. This signal peptide is similar to that described for lipoproteins from bacteria, which may be anchored to the cell membrane in the periplasm. Given these results we hypothesize that P400 is an ubiquitin like domain of a major protein that is secreted and anchored to the membrane in *Nab. magadii* cells. The physiological role of this protein is still to be determined. *Supported by UNMdP and CONICET*

MM-P16

CLPL AND CLPP ARE INVOLVED IN THE DEGRADATION OF SSRA-TAGGED PROTEINS OF *Streptococcus pneumoniae*

German E. Piñas[1], Andrea G. Albarracin Orio[1], Melina B. Cian[1], Paulo R. Cortes[1], Jose R. Echenique[1]
[1]CIBICI-CONICET, Depto. de Bioq. Clínica, Fac. de Cs. Químicas, Universidad Nacional de Córdoba. (jeche@fcq.unc.edu.ar)

S. pneumoniae is a major human pathogen that causes several diseases such as pneumonia, meningitis and bacteraemia. During the infection process, this bacterium is subjected to a number of hassle conditions, such as temperature, oxidative stress and low pH. In our laboratory, we found that acidic stress induces autolysis of *S. pneumoniae*. This phenomenon requires the participation of the acid-induced molecular chaperone, ClpL, the serine protease ClpP and the SsrA-tagging system, suggesting a direct interaction between them. It is known that SsrA-tagged proteins are sent to degradation by proteolytic complexes formed by Clp ATPases and ClpP in other bacteria. We decided to investigate if the removal of SsrA-tagged proteins is mediated by ClpL and ClpP in *S. pneumoniae*. For this purpose, we fused the gfp-mut3 and ssrA genes and we cloned this construction into a replicative vector for *S. pneumoniae*. These plasmids were used to transform the wt strain and the clpL and clpP mutants. The analysis of GFP expression by fluorescence microscopy, fluorometric quantification and western blot, revealed presence of GFP in clpL and clpP mutants harboring the plasmid pAT29-gfpmut3-ssrA, while it was almost undetectable in the wt strain (R801). To confirm these results and to rule out the possibility of a reporter-dependent effect, we fused the ssrA and gus genes, and glucuronidase activity was measured in the wt, clpL and clpP strains transformed with the plasmid pAT18-gusA-ssrA, and similar evidence was obtained. These experiments were performed in another genetic background, strain Cp1015, and we obtained the same results as with the R801 strain. The data presented in this work indicate that ClpL and ClpP are involved in the degradation in vivo of SsrA-tagged proteins in *S. pneumoniae*, and suggest that ClpL and ClpP are able to associate into a proteolytic complex that recognizes SsrA-tagged proteins.

MM-P17

REGULATION OF THE *PHA* GENES IN *Pseudomonas putida* KT2440: INVOLVEMENT OF PHAD AND THE STATIONARY PHASE SIGMA FACTOR, Σ^S

Laura J. Raiger Iustman[1,3], Paula Tribelli[1,3], Martiniano Ricardi[2], Jimena A. Ruiz[1,3]
[1]Laboratorio de Ecología y Genética Bacteriana. Dpto de Química Biológica-FCEyN- UBA- [2] IFIByNE-CONICET. Facultad de Ciencias Exactas y Naturales [3] CONICET (lri@qb.fcen.uba.ar)

Pseudomonas putida KT2440 synthesizes and accumulates medium-chain-length polyhydroxyalkanoates (PHAs) under conditions of nutrient limitation and carbon excess. The *pha* locus of *P. putida* KT2440 consists of seven genes: two PHA polymerases: *phaC1* and phaC2, a PHA depolymerase: *phaZ*, a putative transcriptional regulator of the TetR family: *phaD* and two PHA granule- associated proteins: *phaI* and *phaF*. Previous results from our group demonstrated that the global regulator σ^S negatively affect the expression of the promoter that drives the expression of the genes *phaC1* and *phaZ* (pC1) during the stationary phase. Since RpoS is a sigma factor, and a negative regulation by a direct action of a sigma factor is not a common mechanism, we suggested that there must be other regulator acting on the pC1

promoter. The putative TetR-type transcriptional regulator, PhaD, was a good candidate, because a mutation in the gene that codes for PhaD had the same effect on PHA metabolism that a mutation in *rpoS*. In this work we showed, by means of real time PCR experiments, that in the *rpoS* mutant the expression of *phaD* is greatly reduced (about 11 fold), indicating that σ^S has a positive effect on the expression of *phaD*. On the other hand, the over-expression of *phaD* in *P. putida* KT2440 pC1::*lacZ*, significantly reduced the expression driven by the pC1 promoter. These results would indicate that σ^S and the still not characterized regulator PhaD form part of a cascade that regulates pha genes expression in P. putida. The role of PhaD as a specific- negative regulator of the *pha* genes is currently under research.

MM-P18

***Acinetobacter baumannii* A118, A CLINICAL ISOLATE SUITABLE AS MODEL FOR GENETIC STUDIES**

María Soledad Ramírez[1,2] *, Michelle Don*[1]*, Andrea K. Merkier*[2]*, Daniela Centrón*[2]*, Marcelo E. Tolmasky*[1]

[1]*Department of Biological Science, California State University Fullerton, Fullerton, CA, USA* [2] *Departamento de Microbiología, Facultad de Medicina, UBA, Buenos Aires, Argentina (ramirez.mariasoledad@gmail.com)*

Acinetobacter baumannii (Ab) is an emerging opportunistic human pathogen responsible for a growing number of community and nosocomial infections that can be life threatening or leave permanent sequel in the patients. Clinical Ab strains are often multiresistant, which complicates treatment in the clinical setting as well as performance of genetic experiments in the laboratory. For this latter purpose it would be desirable to have a strain that is susceptible to antibiotics suitable for selection, that is naturally competent, and that supports stable maintenance of plasmids used as cloning vehicles. While data on natural competence of *A. calcoaceticus* and *A. baylyi* abound, studies on natural competence as well as stability of plasmid replicons in Ab are scarce. We have isolated a clinical Ab strain that is susceptible to a group of antibiotics, is naturally competent, and supports propagation of several plasmid replicons. Susceptibility to antibiotics was determined using the agar dilution method. Natural competence was tested incubating untreated cells with plasmid DNA (pJHCMW1, pMET1, pAADA1KN, pAADB, pVK102) or a 10-mer phosphorothioate (PS) DNA analog labeled with Oregon Green 48 at its 5' end. Plasmid stability was tested calculating the proportion of cells that lost the plasmid after 40 generations of growth in the absence of selection. Transformation with the labeled oligomer was tested by fluorescence microscopy. Ab A118 was isolated from the blood culture of a patient and is susceptible to ceftazidime, minocycline, cefepime, piperacillin, gentamicin, kanamycin, trimethoprime-sulfamethoxazole, ciprofloxacin, and amikacin. All five plasmids transformed A118 at high frequency. A118 was also able to take up a fluorophore-labeled PS analog. With the exception of pAADA1KN, a derivative of pACYC184, plasmids were stably maintained after 40 generations. It was not surprise that pAADA1KN was not stable since it is well known that pACYC184 is unstable. Molecular genetic studies of Ab clinical strains have often been limited because they are usually resistant to most antibiotics. The ability of Ab A118 to be transformed and stably support the replication of several plasmids together with its susceptibility profile make this strain a useful model for genetic analyses and studies on virulence and antibiotic resistance.

MM-P19

PULSE FIELD GEL ELECTROPHORESIS OF *Streptococcus uberis* STRAINS ISOLATED FROM BOVINE MASTITIS IN ARGENTINA

Elina B. Reinoso[1]*, Mirta C. Lasagno*[1]*, Liliana Tirante*[2]*, Miriam P. Ferrari*[1]*, Liliana M. Odierno*[1]

[1]*Universidad Nacional Río Cuarto* [2] *Laboratorio LactoDiagnósticoSur (lodierno@exa.unrc.edu.ar)*

Streptococcus uberis is one of the more important environmental pathogens implicated in bovine mastitis. Despite that an increasing prevalence of *S. uberis* mastitis has been reported throughout the world, the epidemiology of *S. uberis* mastitis is incompletely understood. In an attempt to differentiate *S. uberis* strains, a number of typing methods have been developed. DNA macrorestriction analysis by pulsed-field gel electrophoresis appears to be a simple, reliable, and highly discriminatory method to type *S. uberis* strains. It produces distinct patterns that are easy to interpret and is highly reproducible. Strain typing data show that a large variety of strains can cause mastitis, supporting the idea that *S. uberis* is an opportunistic pathogen of environmental origin. The aim of this study was to evaluated genotypic relationships between *S. uberis* isolates from milk of bovines with mastitis from herds located in the east-central region of Argentina. Forty five *S. uberis* were characterized by pulse field gel electrophoresis (PFGE). The analysis showed a total of 19 profiles found by *SmaI*. A great degree of genetic variation was found in S. uberis within the same herd and among herds. Each farm had different PFGE types. However a low number of strains within the same herd shared an identical PFGE type, suggesting that possible transmission between cows or acquisition from a common source did occur presumably during the milking process, but these were uncommon events. This study shows that no predominant PFGE type was identified in any herd. Genomic typing of *S. uberis* was done on a number of samples from localized regions in the United States and Australia. To our knowledge, this is the first report that documents molecular typing studies of bovine isolates of *S. uberis* by PFGE from Argentina, which represents genomic backgrounds of this pathogen. It is concluded that the unrelated pulse field types of isolates from different herds strongly suggesting

environmental sources of *S. uberis* infection. These findings are in agreement with other *S. uberis* epidemiological studies. However, the presence of particular strains in some herds indicates that *S. uberis* infections are epidemiologically complex and further studies might help to elucidate the biology of this bovine pathogen.

MM-P20

***IN VITRO* EVOLUTION OF A BETA-LACTAMASE FOLD INTO A CHORISMATE MUTASE FOLD.**

Alejandro Sánchez[1], *Raúl G. Ferreyra*[1,2] , *Mario R. Ermácora*[1,2]
[1]*Universidad Nacional de Quilmes* [2] *CONICET (alsan2313@yahoo.com.ar)*

It is generally assumed that protein conformation is encoded by the amino acid sequence, but the correspondence between primary and three dimensional structures is not obvious. We hypothesize that the architecture of a protein is determined in such way that any conformation can be generated by any sequence, being the native state the most energetically favorable structure. Consequently, it should be possible to engineer a desired fold by changing a limited number of residues of any sequence. Chorismate mutase catalyzes the rearrangement of chorismate into prephenate, and this is the first step of the tyrosine and phenylalanine synthesis in bacteria and yeast. By *in vitro* evolution, we intend to rebuild a chorismate mutase fold and activity from the sequence of an unrelated hidrolase, the beta-lactamase from *Bacillus licheniformis*. We have developed a selection system that should allow us to direct the evolution of the beta-lactamase toward the chorismate mutase. First, the *Yarrowia lipolytica* chorismate mutase gene was cloned from genomic DNA of the yeast, expressed in *Escherichia coli*, and used to complement strains lacking chorismate mutase gene. Several assays demonstrated the success of the heterologous complementation and the effectiveness of our selection system. Second, by genetic engineering, we placed the catalytic residues of the *E. coli* chorismate mutase in the *B. licheniformis* beta-lactamase sequence. Thus, if the fold of chorismate mutase arose, we should be able to notice it through the chorismate mutase activity. We are carrying out *in vitro* evolution experiments, using DNA shuffling and error-prone PCR. The results of the first round of evolution will be presented.

MM-P21

THE PA5378 GENE OF *Pseudomonas aeruginosa* IS REGULATED BY THE PRESENCE OF CHOLINE AND ITS DERIVATIVES IN THE CULTURE MEDIUM.

Diego G. Sánchez, Agustina Llanos, Angela T. Lisa
Dpto. Biología Molecular, FCEFQyN, UNRC. (dsanchez@exa.unrc.edu.ar)

In P. aeruginosa the PA5378 gene encodes the periplasmic component of a complex system of transport that belongs to an ABC transporter (ATP binding cassette). In our group it was observed that PA5378 has high affinity for choline and its derivatives and could participate in the substrate uptake into the bacterial cytoplasm. Inside the microorganism, choline (or its derivatives) can be utilized as carbon (C), nitrogen (N) or C and N source, and is implicated in pathogenesis. The aim of this work is to identify the regulatory region of PA5378 gene and determine how this gene is regulated by choline under different environmental conditions, as i) nitrogen starvation, ii) carbon starvation, iii)carbon and nitrogen starvation, and iv) hyperosmolarity with choline or betaína as osmoprotectant. The regulatory region of the PA5378 gene was located; and the minimal region with promoter activity includes 312bp upstream from the ATG start codon. PA5378 is expressed differentially, when choline or its metabolic derivatives are used as C, N or C and N source, being the highest transcription observed when choline is used as C/N or C and N source. The transcription levels under hyperosmotic conditions are similar to that found in isoosmolar conditions. Also, we analyzed if the PA5378 expression depends on the choline metabolism regulator, GbdR. For that, it was constructed a deletion mutant ΔgbdR, that carries the transcriptional fusion PA5378::lacZ inserted in the bacterial chromosome. The β-galactosidase activity in this strain decreases 4-fold times, compared with the wild-type strain. In conclusion, the gene expression: a) is highest in response to choline as C/N source; b) is not affected by hyperosmolarity; c) and the GbdR protein participates in its regulation.

MM-P22

PA5378 FROM *Pseudomonas aeruginosa* CODIFIES FOR A HIGH AFFINITY CHOLINE-BINDING PROTEIN

Ana L. Serra[1], *Paola R. Beassoni*[1], *Agustina Llanos*[1], *Sonia Bertolotti*[2], *Ángela T. Lisa*[1]

[1]*Departamento de Biología Molecular, FCEF-QyN, UNRC.* [2]*Departamento de Química, FCEF-QyN, UNRC.* (*aserra@exa.unrc.edu.ar*)

To identify the alkylammonium compounds-binding site in proteins of Gram-negative bacteria, we proposed to use as a model study the choline-binding site of periplasmic proteins involved in the transport of this compound in *Pseudomonas aeruginosa*. Using BLASTp algorithm with ChoX, the periplasmic component of the ABC choline uptake system from *Sinorhizobium meliloti*, we found a protein from *P. aeruginosa* with 60% of identity. Fluorescence-based ligand assays were used to quantify substrate binding by PA5378. These data shown that PA5378 recognizes choline and glycine betaine with high and medium affinity, with Kd values 6,5 and 115,5 nM respectively. Based on atomic coordinates of ChoX (PDB accession code: 2RF1) a three-dimensional model of PA5378 was obtained by homology modelling using ICM-Pro (Molsoft LLC) software. With base on this model, we proposed that the active site of PA5378 would be composed of Asp-13, Trp-16, Asp-18, Trp-63, Tyr-92, Asn-129 and Trp-178 residues. To determine the individual contribution of the tryptophan and tyrosine residues to the stability of the PA5378-choline complex, the corresponding codons in the pa5378 gene were substituted by the codon for alanine. We found that any individual mutation in those aminoacidic residues modify the affinity of PA5378 for choline. This finding shows that individual tryptophan and tyrosine residues found within the hypothetical binding site of PA5378 would not be critical for choline binding. Adittional double and triple site-directed mutagenesis experiments will allow us to know the involvement of proposal residues in the active site.

MM-P23

A PUTATIVE AMINOACID CHEMORECEPTOR FROM *P. PUTIDA*: CLONING, EXPRESSION AND *FUNCTIONAL ANALYSIS IN E.coli*

Debora Soto[1], *Karina Herrera Seitz*[1], *Claudia Studdert*[1]

[1]*IIB-FCEyN-CONICET/Universidad Nacional de Mar del Plata (soy_deby_@hotmail.com)*

The signal transduction pathway involved in chemotaxis is present with little variations along bacteria and archaea. Chemoreceptor genes, coding for proteins that sense environmental stimuli and transduce the signal to the flagellar motors, are easily recognized in genomic studies due to a highly conserved signaling domain. This domain is implicated in the interaction with the histidin kinase whose activity is modulated in response to stimuli. In *E.coli*, chemoreceptors of same or different specificities are organized in trimers of dimers. Dimer-to-dimer interactions involve a region of the signaling domain. It is not well established, however, whether this organization is a conserved feature that might be central to chemoreceptor function. As a first approach to analyze structure and function of non-*E.coli* chemoreceptors, cloning and expression of a putative amino acid sensor from P.putida (pctApp) was carried out. The cloned gene was nicely expressed and recognized by antibodies raised against the conserved cytoplasmatic domain from *E.coli* receptors. Although pctApp expression did not restore chemotaxis to serine in an *E.coli* strain lacking its native receptor, as assessed in soft agar plates, it did restore the ability to respond to serine in rotational assays. This result indicates that pctApp is able to control the *E.coli* kinase, presumably through interactions and conformational changes shared with the native transducers. In vivo crosslinking studies indicate that pctApp is not stably incorporated into mixed trimers of dimers with *E.coli* transducers, but might be able to form trimers of dimers by itself. Results presented here and future studies of heterologous chemoreceptors will contribute to elucidate whether the spatial organization of receptors is a conserved feature and a requisite for chemotaxis signaling.

MM-P24

THE TWO-COMPONENT BACTERIOCIN LAC705 INDUCES THE AMPLIFICATION AND REARRANGEMENT OF PLASMID PRC18 AND REVERTS THE LAC705-NEGATIVE PHENOTYPE OF THE *Lactobacillus curvatus* SAC7 MUTANT.

Tatiana M. Stepanenko[1], *María C. Aristimuño Ficoseco*[1], *Raúl R. Raya*[1]

[1]*CERELA-CONICET (tatula17@hotmail.com)*

Lactobacillus curvatus CRL705 produces two bacteriocins (antimicrobial peptide): Lactocin 705 (Lac705) and Antilisteria 705 (AL705), and harbors the plasmids pRC12 (12 Kb) and pRC18 (18,66 Kb), being the last plasmid associated with production of Lac705. Sac7, a mutant derived from CRL705, is negative in the production of both bacteriocins, in spite of containing the plasmid pRC18. When Sac7 cells were incubated in the presence of a free-cell supernatan of the wild-type *Lactobacillus curvatus* CRL705, the mutant phenotype of Sac7($Lac705^{-/S}$-$AL705^{-/R}$) reverted to the wild-type phenotype ($Lac705^{+/R}$-$AL705^{+/R}$), and kept stable after several generations. The induction factor was identified as the bacteriocin Lac705 itself. PCR studies, using specific primers of pRC18, suggested that the bacteriocin-negative phenotype of strain Sac7 could be due both to the low copy-number and to structural rearrangement of plasmid pRC18.

MM-P25

MUTATIONS IN THE C-TERMINAL REGION OF NADH DEHYDROGENASE-2 FROM *Escherichia coli* AFFECT MEMBRANE ANCHORING

Josefina M. Villegas[1,2] , *María R. Rintoul*[1,2] , *Sabrina I. Volentini*[1,2] , *Viviana A. Rapisarda*[1,2]

[1]*Departamento Bioquímica de la Nutrición, INSIBIO (CONICET-UNT)* [2] *Instituto de Química Biológica "Dr. Bernabé Bloj" -Facultad Bioquímica, Química y Farmacia- UNT (josefinamvillegas@gmail.com)*

Respiratory NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is a membrane-bound flavoprotein, codified by *ndh* gene. It has been proposed that NDH-2 membrane anchorage could occur by multiple amphipatic turns along the enzyme. However, by bioinformatics studies performed in our laboratory, it has been identified a putative transmembrane C-terminal region. In order to validate whether this region could be responsible for NDH-2 membrane anchorage, several mutants were constructed, in which the enzyme C-terminal region was progressively deleted. They were called Trun-1 to Trun-4, lacking 13, 28, 43, and 57 aminoacids, respectively. A NADH dehydrogenases deficient strain complemented with either the wild-type or the mutant *ndh* genes was used for all the experiments. For a preliminar screening of NDH-2 activity, an *in vivo* test was performed in minimal medium supplemented with mannitol as a sole carbon source at 30ºC, where it was described that NADH dehydrogenases null mutants are not able to grow. This phenotype was reverted only by the wild type and Trun-1 proteins. The rest of the truncated versions were not able to grow under these conditions. To check protein localization and enzymatic activities, the different cellular fractions were separated by ultracentrifugation. Trun-1 was localized in the membrane fraction as the wild type protein. The rest of the truncated forms were located only in cytosol, except for Trun-2 which was present in both fractions. NDH-2 enzymatic activities were measured, obtaining equivalent results to the *in vivo* experiment. Wild type and Trun-1 samples were active, while the rest of the truncated analogues showed no activity. However, with inactive mutants, activity was restored by the addition of FAD in the corresponding fractions. Taking together, we demonstrated that NDH-2 C-terminal region is implicated in the protein anchorage to the membrane. For the first time, we obtained an active and water-soluble NDH-2, which would be useful for detergent-free purification and further characterizations.

MM-P26

INTERACTION OF THE RAPA1 ADHESIN WITH THE ACIDIC EXOPOLYSACCHARIDE IN *Rhizobium leguminosarum*

Nicolás F. Vozza[1], *Patricia L. Abdián*[1], *Elías J. Mongiardini*[2], *Daniela M. Russo*[1], *Aníbal R. Lodeiro*[2], *Angeles Zorreguieta*[1]

[1]*Fundación Instituto Leloir, IIBBA CONICET, FCEyN -UBA* [2]*Inst. de Biotecnología y Biología Molecular CONICET, Facultad de Ciencias Exactas-UNL, (nvozza@leloir.org.ar)*

We have shown that the acidic exopolysaccharide (EPS), the O-antigen of the LPS and a functional PrsDE type I secretion system are important for the formation of a structured and typical biofilm in *Rhizobium leguminosarum* bv *viciae* A34. The PrsDE system is responsible for the secretion of several proteins, such as two EPS-glycanases and the Rap (*Rhizobium* adhering proteins) family of proteins. It was previously shown that RapA1 adhesin from *R. leguminosarum* bv *trifolii* R200 localizes at one cell pole and that addition of recombinant RapA1 to a planktonic culture of R200 promote cell agglutination. In addition, it was suggested that attachment of RapA1 to the cell surface would be mediated by the EPS. Although inactivation of the *rapA1* gene in the A34 background had not show a clear biofilm phenotype, overexpression of the *R. l.* bv *trifolii rapA1* in R200 and *R. l.* bv *viciae* A34 and 3841 wild type backgrounds resulted in disruption of tight and typical cell-cell interactions within the biofilm microcolonies as observed by Confocal Laser Scanning Microscope (CLSM). Using antiserum against R200-RapA1, we confirmed by Western blot that this phenotype was due to the secretion of RapA1 since in all cases, RapA1 was either associated to the cell surface or released to the extracellular milieu in a PrsDE-dependent manner. A faint band with a similar molecular weight to RapA1 was also observed in the surface protein fractions of RapA1-defective strains, suggesting that a RapA1 homologue is also located on the cell surface. By indirect immunofluorescence and CLSM of live bacteria, we observed that in the A34 wild type background, RapA1 also localizes at one cell pole, as has been shown in R200. However, overexpression of *rapA1* in A34 resulted in a localization of RapA1 in an extended region around one cell pole. Surprisingly, we observed by CLSM that overexpression of *rapA1* in an EPS-defective mutant caused unusual cell-cell interactions within biofilms, suggesting that even in the absence of EPS, RapA1 is able to interact with the bacterial surface. In line with this observation, a polar localization of RapA1 was observed by immunofluorescence in the *rapA1*-overexpressing cells of an EPS-defective mutant. Taken together, these observations suggest that in A34: i) synthesis of EPS highly influences cell-cell interactions mediated by RapA1, ii) RapA1 localization at one cell pole is EPS-independent and iii) a RapA1 homologue also localizes at one bacterial pole.

EM-P1

DETECTION OF *Bradyrhizobium* sp (*ARACHIS*) CELLS IN SOIL AND NODULES BY A MODIFIED AFLP PROCEDURE

J. Angelini[1], P. Dion[2], F. Belzile[2], A. Fabra[1]
[1]Dpto. Cs. Naturales. Fac. de Ciencias Exactas. UNRC. Ruta Nac. 36 km 601 - 5800 Río Cuarto (Córdoba) [2]Département de phytologie- CHM Pavillon Charles-Eugène-Marchand Université Laval-Québec Canadá (jangelini@exa.unrc.edu.ar)

A modified Amplified Fragment Length Polymorphism (AFLP) procedure was developed for the specific identification of *Bradyrhizobium* bacteria in peanut nodules and soil samples.The technique developed corresponds to a simplified AFLP analysis with one restriction enzyme (*Eco*RI) and one selective primer (*Eco*RI-T). This new AFLP procedure allowed the direct detection and identification of specific *Bradyrhizobium* strains present in peanut nodules and soil samples following greenhouse and field inoculation assays. Hence, a rapid and straight-forward method has been developed for the direct monitoring of specific bacteria in the environment.
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EM-P2

EFFECTS OF AGROCHEMICALS ON PHOSPHATE SOLUBILIZING AND NITROGEN FIXING BACTERIA ASSOCIATED TO PEANUT CROP

María S. Anzuay[1], Silvina Ghio[1], Jorge G. Angelini[1], Tania Taurian[1], Adriana Fabra[1]
[1]Universidad Nacional de Río Cuarto (ttaurian@exa.unrc.edu.ar)

In Argentina, peanut crop production is concentrated in Cordoba province. Intensification of agricultural practices in this area has led to excessive use of agrochemicals. It is well known that plant growth promoting bacteria inhabiting agricultural soils can be affected by the application of these substances. The understanding of soil bacteria diversity and its response to the agrochemical application is necessary in order to implement friendlier practices in agriculture. The aim of this study was to analyze the effect of agrochemical application on nitrogen fixing and phosphate solubilizing bacteria associated to peanut crop. Two soil samples of a peanut cultivated field were used in this study: a soil sample without application of agrochemicals (control) and one where herbicides were introduced (treated soil). Total cultivable bacteria grown in TSA medium, number of phosphate solubilzing bacteria in NBRIP-BPB medium and nitrogen fixation bacteria in NFB and JNF media were determined in both soil samples. Results demonstrated that number of heterotrophic and phosphate solubilizing bacteria was not altered by agrochemicals application while nitrogen fixing bacteria´s number decreased in treated soil sample. Neverheless, even when number of heterotrophic and phosphate solubilizing bacteria was not affected by agrochemicals application, an increase in the proportion of gram positive bacteria in this population was determined. It is possible to conclude that herbicides application in peanut crop fields cause changes in agronomically important microbial population.
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EM-P3

NEW TRIACYLGLYCEROL-ACCUMULATING BACTERIA ISOLATED FROM ALTIPLANO LAKES

Susana Bequer Urbano[1], Roxana A. Silva[1], María E. Farías[2], Héctor M. Alvarez[1,3]
[1]CRIDECIT, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco [2]PROIMI, CONICET [3]CONICET (halvarez@unpata.edu.ar)

Triacylglycerols (TAG) are non-polar, water-insoluble fatty acid triesters of glycerol, which occur in most eukaryotic organisms, including animals, plants, yeast and fungi. For several years it has been considered that the formation of TAG as storage lipids was restricted to eukaryotic cells, and that prokaryotes were unable to synthesize and accumulate them. However, during the last years the occurrence of TAG has been detected in certain groups of prokaryotes as reserve material, such as Gram positive bacteria belonging to the actinomycetes group, such as *Mycobacterium*, *Rhodococcus*, *Nocardia* and *Streptomyces*, and in some Gram negative bacteria belonging to the gamma-Proteobacteria, such as *Acinetobacter*, *Marinobacter* and *Alcanivorax*. TAG accumulation may provide cells of energetic autonomy and a temporal independence from the environment and contribute for cell survival when they do not have access to energy resources in soil, like in deserts. In a previous study, we demonstrated that survival of *Rhodococcus* cells under desiccation conditions depended on endogenous metabolism using intracellular TAG for generating energy and precursors, and also as a reservoir of metabolic water. In this study, we partially analyzed an extremophile culture collection from Andean Altiplano in order to identify new TAG-accumulating bacteria. This place presents arid conditions such low nutrients and other extreme environmental conditions. Lipid accumulation was investigated by thin layer and gas chromatography analyses of cells. Desiccation tolerance was analyzed according to the method of Alvarez et al. (2004. FEMS Microbiol. Ecol. 50:75-86). Interestingly, we were able to identify four new TAG-accumulating

genera among this extremophile culture collection: *Microbacterium arborescens* CH5 and *Agrococcus jenensis* CH9, which belong to actinobacteria group, and *Stenotrophomonas maltophilia* Ver 10 and *Alkalilimnicola* sp. Sv 12.18, which belong to gamma-Proteobacteria. Among these strains, only *M. arborescens* CH5 accumulated significant amounts of storage lipids (app. 20 %, w/w), whereas the other strains produced no more than 2 % (w/w) after cultivation with a carbon source under nitrogen-limiting conditions. In addition, cells of these four strains were resistant to desiccation, and a high fraction of the cells (> 50 %) survived after two weeks under these conditions (20% H. R.).
Resuming, after analyzing partially an extremophile culture collection from Andean Altiplano, we identified four new TAG-accumulating genera, such as *Microbacterium*, *Agrococcus*, *Stenotrophomonas* and *Alkalilimnicola*. As described for other TAG-accumulating bacteria, the studied microorganisms showed high water stress tolerance. Further studies are necessary to determine the relation between the accumulation of TAG and the desiccation tolerance by such bacteria.

EM-P4
LIGNOLITYC AND CELLULOLITYC ACTIVITY OF STRAINS ISOLATED FROM COMPOST OF AGRICULTURAL WASTES

Fabiana M. Bressan[1], *Vanina J. Galzenati*[2,1] , *Cecilia Carmarán*[2], *Diana C. Crespo*[1]
[1]*IMYZA, INTA Castelar* [2] *DBBE, FCEyN, UBA (fbressan@cnia.inta.gov.ar)*

Cellulolityc and lignolityc activity of fungal strains are involved in the degradation of organic matter. The characterizations of enzymatic profiles from isolated organisms from compost allow identify which ones have a potencial use to develop inoculants and new technologies for improvement compost quality and biodegradation efficiency. In the agricultural waste composting, lignocellulose accounts the major part of biomass and its degradation is essential for the process. The aim of this work was to detect and characterize qualitatively cellulolityc and lignolytic activities of strains isolated from agricultural waste composting (poultry manure + rice hulls + wood thin shaving). Four culture media were used as follows: Carboxy-methyl cellulose agar (CMC) for cellulolytic activity and Tannic acid agar (TA), Galic acid agar (GA) and Guaiacol agar (GuA) for lignolytic activity. During the composting 124 isolates were obtained, of which 17% showed positive reaction for at least one of the media used and only one isolate was positive in all tested media. On the whole of positives, a 86% showed exclusively one enzymatic activity, either cellulolityc or lignolytic; so that 23% was reactive in CMC (mainly strains belonging to the genera *Trichoderma* and *Geotrichum*), 38% in GuA and 95% in TA and GA (strains belonging to the genera *Alternaria*, *Epicoccum* and *Cladosporium*). The 14% remaining showed both enzymatic activities and hence have a promissory potential of biodegradation. The most complete profiles were obtained in strains without sporulation (mycelia sterilia). The impact on the technology process is discussed.

EM-P5
STRENGTHS AND LIMITATIONS OF MOLECULAR TECHNIQUES FOR THE ANALYSIS OF BIODIVERSITY OF MARINE PICOPHYTOPLANKTON

Gonzalo Caló[1], *Macarena Perez-Cenci*[1], *Graciela L. Salerno*[1]
[1]*Centro de Estudios de Biodiversidad y Biotecnología de Mar del Plata (CEBB-MDP)-CONICET, CIB, FIBA, (gcalo@fiba.org.ar)*

Very small organisms are fundamental components of marine planktonic systems, often accounting for a significant fraction of the biomass and activity. Marine phytoplanktons are responsible for about half of the global carbon fixation. A significant proportion of this fixed carbon derives from the photosynthetic picoeukaryotes (PPEs), component < 3 µm in size. Compared with the prokaryotic component of the picophytoplankton, the PPEs community is highly diverse, comprising members from virtually every algal class. Despite the evident ecological significance of the PPEs, relatively little is known about diversity in the marine environment. Their identity, has remained elusive mainly due to difficulties in identification by light microscopy and/or in culturing. Only recently, with the advent of molecular techniques picophytoplankton diversity has begun to be revealed. To date little is known about the picophytoplankton diversity in the South Atlantic Ocean. The aim of this work was to identify and molecular characterize the components of the picophytoplankton using molecular approaches. We determined the diversity of marine picoeukaryotes (PEs) by sequence analysis from 18S rDNA from four environmental genetic libraries from samples collected from Argentinean Sea surface waters. Such studies, using universal 18S rDNA gene primers (which target both photo-autotrophic and heterotrophic organisms) have shown the presence of the PPEs affiliated with many different algal classes including the Prasinophyceae, Bacillariophyceae, Dictyochophyceae, Haptophyceae, Cryptophyceae, Dinophyceae, Pelagophyceae y Picobiliphyta. Additionally sequences were associated with heterotrophic forms for Alveolados, Stramenopilos, Cercozoa, Apuzoa and Katablepharidae. In spite of the highly diverse organisms detected the molecular level some groups of picoeukaryotes that were abundant by microscopic observations could not be identify in the genetic libraries with the 18S rDNA primer set. Significant differences in 18S rDNA gene copy number of some components of PPEs

could be responsible of these differences between both analyses. Use of Chloroplastida (green algae) primers instead of universal primers has been a good approach for increase the identification of PPEs species with low 18S rDNA copy number in environmental samples. PCR biased towards the green lineage with specific primers may be significant due to selective amplification of phototrophic over heterotrophic organisms.

EM-P6

CANCHADA YERBA MATE: MICROBIOLOGICAL PROFILE

María L. Castrillo[1], *Ayelen Tayagui*[1], *Marta A. Horianski*[1], *Gladis Jerke*[1], *Carina I. Serpp*[1]
[1]*Laboratorio de Microbiología, Módulo de Bioquímica y Farmacia. FCEyN. Universidad Nac. de Misiones (mlc_827@hotmail.com)*

Argentina produces more than 60% of the worldwide total of canchada yerba mate followed by Brazil and Paraguay. It constitutes the raw material for the mills and it is one of the degrees of transformation in which yerba mate is comercialized. The process of elaboration of yerba mate comprises the harvest, held once a year usually between May and September, the sapecado, where the crop is subjected to direct action of the flame for a short time, causing the stoppage of the enzymatic activity of the biological processes responsible for degradation of plant tissue, the drying through a system of tapes submitted to moderate temperatures, 80 - 100°C to remove residual moisture to ensure the obtaining of a good product: canchado and zaranda, consisting of coarse grinding and sieving of the dried leaves of yerba mate, which are then stationed and afterwards crushed to obtain the traditional yerba mate. The canchada yerba mate is obtained from the stage of canchado and zaranda. The aim of this study was to perform the microbiological profile of canchada yerba mate which came from various establishments in the province of Misiones. We analyzed a total of 20 samples of canchada yerba mate, taken from different shops and markets in the center and the suburbs of the city of Posadas, Misiones. The microbiological profile was performed using the IRAM rule 20517:2004 which includes: counting of total mesophilic aerobic bacteria (BAMT), counting of fungi and yeasts (RHL), counting of total coliform (RCT), counting of fecal coliforms (RCF) and detection of *Escherichia coli*. The results of the countings were subjected to statistical analysis, finding for BAMT an average of 1.19 $x10^2$ CFU/g, a minimum of 7 CFU/g and a maximum of 4.13 $x10^2$ CFU/g; for RHL an average of 1, 25 $x10^2$ CFU/g, a minimum value of 1 CFU/g and a maximum of 4.6 $x10^2$ CFU/g; for RCT an average of 5.5 $x10^1$ CFU/g, a minimum value less than 10 CFU/g and a maximum of 1.1 $x10^2$ CFU/g. Neither the presence of fecal coliform nor *E.coli* was detected in any of the samples. Comparing these results to those obtained in traditional yerba mate in previous studies, a minor microbiological contamination of two orders of magnitude was observed.

EM-P7

REMOVAL OF HEAVY METALS FROM MINERAL WASTE USING RHAMNOLIPIDS AND IRON AND SULFUR OXIDIZING BACTERIA

Mayri A. Diaz De Rienzo[1], *Isora Urdaneta*[1], *Blas Dorta Dorta*[1], *Maria L. Blazquez*[1,2] , *Felisa Gonzalez*[1,2] , *Jesus A. Muñoz*[1,2] , *Antonio Ballester*[1,2]
[1]*Universidad Central de Venezuela* [2] *Universidad Complutense de Madrid (mayriales@gmail.com)*

The most important inorganic chemicals in the study of contaminated soils are are arsenic, cadmium, zinc, copper, chromium, manganese, mercury, lead and cyanide. The soils that remain after the explotation of a mine contain all kinds of residual matter which represents serious problems in the development of the vegetable layer. Given this fact, it is necessary to evaluate decontamination solutions to induce changes in the oxidation state of metals associated with the minerals which are conducive to the process of bioleaching, opening an opportunity for the development of simple, economical and low environmental impact for the restoration of land affected by contamination such metals. The ability to leach and wash iron (Fe) and zinc (Zn) using iron and sulfur oxidizing bacteria as well as rhamnolipids produced by *Pseudomonas aeruginosa* was evaluated from samples of mineral residues originating from the region of Murcia, Eje Cartagena-La Unión axis, Spain. The results show that removal of metals such as iron and zinc can be removed by mixed cultures of iron and sulfur oxidizing bacteria. Similarly in the presence of rhamnolipids the removal of metals is favored, as well as demonstrate that these metals can change phase (residual sulphides in the case of exchangeable iron and zinc in the leachate) in the presence of the surface at pH 8. The mechanism on cycles allows recovery of heavy metals because it involves the effect of bioleaching bacteria mediated by iron and sulfur oxidizers and the effect of washing mediated for rhamnolipids at a concentration of 1mg/mL in the same recovery mechanism. This assay is a novelty because it allows to compare yields in the recovery of metals with existing technologies as well as perform economic balances that allow the establishment of measures that involve metal recovery and the bioremediation of soils affected by mining activities using biological techniques that are more compatible with the environmental like bacterias belonging to the *Acidithiobacillus* genere and the rhamnolipids produced by differents species of the *Pseudomonas* genere.

EM-P8

EVALUATION OF THE CO-INOCULATION OF DSE AND VAM FUNGI ON RYE GRASS (*Lolium perenne*)

Tai En Lo[1], *Carolina Rothen*[1], *Cinthia Alegre*[1], *Mariana Pérgola*[1], *Alicia M. Godeas*[1,2] , *Maria A. Rodriguez*[1,2]
[1] *DBBE, FCEyN, UBA* [2] *CONICET (taienlo@hotmail.com)*

Dark septate endophytes (DSE) is one of the worldwide least studied soil microorganisms. Along with vesicular-arbuscular mycorrhyzas (VAM), they can be hosted in terrestrial plants roots, causing several effects on their host. In order to examine the interaction between these two fungi and to establish possible effects caused by both microorganisms on plants, we co-inoculated a VAM fungus, *Glomus intraradices*, and a strain of DSE fungus on pre-germinated rye grass seeds. Treatments, inoculating either only *Glomus intraradices* or DSE and a control without inoculation, were made for comparison. Plants were kept in a greenhouse and harvested at day 30, 45, 60, 75 and 90 of growth. Parameters like plants total length and weight were measured in each harvest. Since rye grass is commonly used for fodder, we examined its re-sprouting capacity in all treatments, by cutting the plant 1 cm above de soil surface in each harvest. Larger plants scions and higher re-sprouting capacity in control treatments are some of the preliminary results.

EM-P9

INTERFERENCE COMPETITION BETWEEN WILD YEASTS ASOCIATED WITH SOIL AND CANOPIES OF *Larrea divaricata* AND *Bulnesia retama*, IN AN ARID ZONE OF SAN JUAN, ARGENTINA

Natalia P. Oro Castro[1], *Paola Y. Maturano*[2], *María C. Nally*[2], *María E. Toro*[2], *Fabio Vázquez*[2]
[1]*Departamento de geología, FCEFN, UNSJ* [2]*Instituto de Biotecnología, FI, UNSJ (oronatalia@gmail.com)*

Arid ecosystems are dominated by shrubby vegetation, where soil properties are located in "islands of fertility". Among microorganisms associated to these fertility islands are yeasts. The distribution of yeasts in nature is not at random and those communities of species are defined by their habitat and the niche they occupy. Different yeasts belonging to various families and genera show a phenomenon known as *killer*, which implies a potential competition through the production of toxic glycoproteins. Ecological studies on this issue indicate that this phenomenon is a mechanism of interference competition, which involves the production of a toxin from yeasts in order to exclude others from their habitat. The objective of this study was to analyze competitive relationships (considering *killer* phenotype) between wild yeasts isolated from soil and canopies of *Larrea divaricata* and *Bulnesia retama* in a dry area of San Juan, Argentina, in two consecutive years. Samples were taken in sterile conditions from canopies of each shrub and in associated soils. Yeast isolations were from different culture media (Yeast Extract Peptone Dextrose and Yeast Malt), according to the topography of colonies. Taxonomic identification of yeasts was performed. *Killer* character was detected by cross-reactions between isolated yeasts. Data were analyzed by constructing contingency tables. Chi-square method was the most plausible to use (G^2). From 96 yeasts isolated from the first sampling, 232 interactions were determined. The result was lower in the second sample. From 41 yeast isolations were found 98 interactions. From statistical data analysis it was inferred that in both samples the number of interactions depended on microsites from which yeasts were isolated (G^2=56.22, p≤0.0001, G^2=31.22, p≤0.0001). The species that presented the greatest number of interactions K/S (Killer/Sensitive) were *Candida catenulata* (65 K/S interactions) in the first sampling and *Candida sake* and *Sporidiobolus johnsonii* (18 and 19 interactions K/S respectively) in the second sampling. It was found that the number of interactions K/S were dependent of the species considered (G^2=29.04, p≤0.0001; G^2=5.49, p=0.0043). It can be concluded that the number of interactions K/S depends on microsites which yeasts were isolated may be due to competition of microbial communities in habitats with low concentrations of available substrates, such as the study area that tends to produce separations in related ecological populations.

EM-P10

***Aspergillus* SECTION *flavi* IN SOILS WITH RECENT O NO HISTORY OF PEANUT CULTIVATION**

Maria P. Ortiz[1], *Maria L. Ramirez*[1], *Sofia N. Chulze*[1]
[1]*Departamento de Microbiologia e Inmunologia, Fac. Cs. Esxactas Fco-Qcas y Naturales. UNRC (mramirez@exa.unrc.edu.ar)*

The major peanuts-growing region of Argentina is located in the Central-South of Córdoba province. During the last few years the cultivation has been extended to new areas belonging to the Córdoba province and other provinces such as Formosa, and Salta. Many factors, such as phytosanitary problems, climatic conditions and soil degradation have contributed to explain this expansion of peanuts cultivation to new areas. Peanuts is often invaded before harvest by *Aspergillus* section *Flavi*, *A. flavus* and *A. parasiticus*, these species can produce the carcinogenic aflatoxins. Soil is the main reservoir of the inoculum of both species and peanut pods can be contaminated with the toxigenic species. At the present, there is information about soil population of *Aspergillus* section *Flavi* the traditional peanut growing region. The aim of this work was extend the knowledge on these populations from new areas with recent o no history of peanut

cultivation. Soil samples were obtained from fields located in the south of Córdoba province and Formosa. The results showed that soil densities of combined *Aspergillus* species from section *Flavi* were characterized by large variation among fields, with a lowest frequency on the fields without peanuts history. *Aspergillus flavus* was the dominant specie in all the fields. *Aspergillus parasiticus* was found in low frequency, mainly in fields with recent peanuts cultivation history (2-3 years). There were not significant differences in the ratio of toxicogenic and atoxicogenic strains but it was found the lower percentage of toxicogenic strains on the field with no/or recent history of peanuts cultivation. The results are relevant in order to evaluate the risk of peanuts to be contaminated with aflatoxins under conductive conditions.

EM-P11

CHARACTERIZATION OF BIOFILMS PRODUCED BY DISTINCT BACTERIAL GENERA ISOLATED FROM DRINKING WATER

Manuela Pujol[1,2] *, Nancy López*[1]*, Blanca Rosales*[2]

[1]*Lab. Genética y Ecología Bacteriana, Dpto. de Química Biológica, FCEyN, UBA* [2] *Lab. Biocorrosion, CIDEPINT, La Plata (elianamanuela@yahoo.com.ar)*

Anywhere a metallic surface comes into contact with the water in a distribution system, biofilms can be produced. Biofilms consist of microbial cells and their extracellular polymeric matrix (MPE) which facilitates attachment of cells to the surface. MPE consist of a complex mixture of cell derived exopolysaccharides (EPS), proteins, lipids and nucleic acids. MPE or any of its components can affect corrosion rate of the surface. In previous works, we observed that cast iron is a suitable surface for colonization and biofilm development by bacteria from drinking water. To evaluate the relationship between bacterial biofilm and corrosion rate, in this study, we analyzed the biofilm formation capacity (BFC) and MPE of bacterial genera isolated from drinking water of La Plata city on coupons of cast iron for one month. We analyzed three bacterial isolates of drinking water. The isolates were characterized as belonging to the genera: *Pseudomonas* , *Methylobacterium* and *Sphingomonas* according to rRNA 16S gene sequencing. We submerged four coupons of cast iron in non-flow water system for each bacterial isolate. Each week, bacteria attached to the surface were removed by scraping using 3ml steril physiological solution. Aliquots of 1ml were used to determine biomass ($DO_{590\ nm}$), EPS, proteins and corrosion rate. EPS and proteins were analysed by standard colorimetric methods. Corrosion rates were estimated as decrease weight of coupon (RC=ΔWeight /(area*days)). To analyze the BFC we used a microtiter plate assay. We determined the relation between cells growing in the attached state, evaluated by staining with cristal violet, to cells growing in the planktonic state ($BFC=Abs._{attached\ cells\text{-}crystal\ violet}/OD_{planktonic\ cells}$). The highest biofilm performance was observed for *Pseudomonas sp*. (BFC= 7.38), while *Methylobacterium sp*. and *Sphingomonas sp*. showed similar BFC values (3.04 and 3.95 respectively). In the temporal analysis, we observed that the biomass, EPS and protein increased over immersion period whereas corrosion rate decreased. *Pseudomonas* produced the highest amount of EPS; this result is consistent with its high BFC value. The results showed that corrosion was more evident in the early stage of attachment than in the later stage of development of biofilm. These results suggest that EPS could be an important component related with both biofilm formation and corrosion rate on cast iron

EM-P12

PHYLOGENETIC STUDY BASED ON THE 16S RRNA OF HALOPHILIC ACTINOMYCETES ISOLATES FROM HYPERSALINE ENVIRONMENTS OF MEXICO

Ninfa Ramírez Durán[1]*, Araly A. Salgado*[1]*, Hilda V. Silva*[2]*, Hugo C. Ramírez*[3]*, Horacio Sandoval*[3]

[1]*Facultad de Medicina, Universidad Autónoma del Estado de México.* [2] *Departamento de Genética, Colegio de Posgraduados.* [3] *Departamento de Sistemas Biológicos. Universidad Autónoma Metropolitana-Xochimilco. (ninfard@hotmail.com)*

Actinomycetes are a Group of Gram-positive bacteria with branched filaments, mainly found in soil. Currently it is know that some actinomycete species are able to grow under conditions of extreme salinity, however their numbers are low, and in recent years only a few genera of halophilic actinomycetes have been reported. The objective of this work was to determine which genera of halophilic actinomycetes are present in the hypersaline environments of México, by means of sequence analysis of the 16S rRNA and phylogenetic study of isolated strain from salterns, saline soils and salted lagoons of Mexico. To investigate the phylogenetic position of the isolated strains, sequence of 16S rRNA genes was determined and compared with sequences in the GeneBank database using the BLAST program. Phylogenetic trees were built using sequences of 1200 base pairs including representative actinomycetes including halophilic species. The stability of each cluster was determined by bootstrap analysis of 1000 replicates. We report the presence of the genera *Actinopolyspora*, *Sacharomonospora*, *Amycolatopsis*, and *Nocardiopsis*. Genera found include halophilic species that have been found in different geographical locations in the earth.

EM-P13
GROWTH STRATEGIES OF BACTERIA AND YEAST DEGRADERS OF ONION WASTE
María E. Rinland[1], Mónica D. Baldini[2], Marisa A. Gómez[1]
[1]Dpto. de Agronomía, Universidad Nacional del Sur, CERZOS-CONICET. [2] Dpto. de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. (emiliarin@gmail.com)

Over 20,000 tonnes of onion waste (OW) are produced annually in Patagonian protected region in Argentina. Almost all of onion refuses are incinerated with significant amount of carbon dioxide released. In addition, the accumulation of wastes in growing areas and processing centers are a source of odour and pollution. Therefore, new products produced from worthless onions are strongly desired in order to allow both value additions and effective reuses of the onions. One option is to compost it and return it to the land as an organic fertilizer. In the present study we investigated microbial populations during the degradation of OW and examined ways to accelerate this process. We used microcosms (3 kg of waste) to evaluate different OW treatments to identify the optimum waste composition to achieve minimum composting time. We analyzed OW from different sites at different stages of the production process to obtain a wide diversity of degrading microorganisms. Total aerobic heterotrophs were quantified on Nutrient Agar and fungi and yeasts on Yeast Extract Glucose Chloramphenicol (YGC) Agar. Seventy-three colonies were randomly picked. Ability to use onion's nutrients was assessed by inoculating pure cultures (53 bacteria and 20 yeast cultures) into tubes with onion medium (1 g K_2HPO_4, 0,5 g $MgSO_4$, 150 ml onion juice and 1 l distilled water). Each culture was also inoculated in Nutrient broth or YGC broth as control medium. One set of tubes was incubated at 28°C and the other at 4°C for 7 days, as degradation of onion crop residues has been observed in field conditions even at low temperatures. Microbial growth was assessed by absorbance at 620 nm. The OW decomposition may be achieved in two months if the pile is made with chopped bulbs. All samples had a large count of total aerobic heterotrophic bacteria (7,9 to 9,9 $\log_{10}$ CFU g^{-1}), fungi and yeasts (4,6 to 8,6 $\log_{10}$ CFU g^{-1}). Microorganisms showed different growth strategies. All microorganisms were able to develop in onion juice at 28°C and some bacteria grew better in onion juice than in control medium. Some bacteria and yeasts were also able to develop in onion juice at 4°C. In two cases (one bacteria and one yeast) growth pattern changed with temperature. They had greater turbidity in onion medium than in control medium at 28°C but the opposite occurred at 4°C. One gram of waste contains about 10^6 of these isolates. In conclusion, there is a large diversity of bacteria and yeasts which varied with location that are capable of degrading onions between 4 to 28°C.

EM-P14
POPULATION DYNAMICS OF GLYPHOSATE-DEGRADING BACTERIA AGAINST AGRONOMIC PRACTICES
Marcela Rorig[1], Daniel H. Grasso[1]
[1]Instituto de Suelos, CIRN-CNIA INTA. Las Cabañas y De Los Reseros (1712) Castelar (dgrasso@cnia.inta.gov.ar)

The introduction in 1996 of the transgenic soybean tolerant to glyphosate herbicide, was the most important progress in agriculture in recent years Argentina. The increase in area planted to transgenic soybean, also generated an increasing use of glyphosate, which increased from 12 to over 160 million liters in the period 1997 to 2007. From the environmental standpoint the glyphosate-based herbicides are considered of moderate toxicity, mainly due to low mobility and they can be degraded by microorganisms. However, it can interfere with the structure of soil microbial community, either by direct action on a particular group of microorganisms or by the comparative advantages that give others. Glyphosate is an organophosphorus compound characterized by covalent bond C-P which confers great stability. However, many bacteria and even enteric bacteria *Escherichia coli* have the ability to enzymatically break this union and release inorganic phosphate. The aim of this study was to evaluate the effect of the application of the herbicide glyphosate on soil microbial populations under two management systems and compare the bacterial populations capable to use glyphosate as P source. Microbial population of soils samples subject to two management systems: organic (without application of glyphosate at least during 5 years) and conventional (with application of glyphosate) were analyzed using Eco Plates from Biolog system. With this procedure in a greenhouse experiment using both types of soil samples we have detected changes in the metabolic pattern due to application of glyphosate and others variables tested. Cultivable bacterial population capable to use glyphosate as P source was also studied. These bacteria were selected on minimal medium that has glyphosate as the sole source of P, and then genetically analyzed by Rep-PCR. Representatives of each fingerprint pattern were taxonomically classified by sequencing of 16S rDNA. Our results show a pronounced effect of both practices in population size, diversity and identity of the individuals.

EM-P15

BACTERIAL COMMUNITY RESPONSE TO GLYPHOSATE AND ATRAZINE EXPOSURE IN THE WATER OF LOS MOLINOS RESERVOIR (CÓRDOBA, ARGENTINA). A PRELIMINARY STUDY

Ariana A. Rossen[1,2] *, Luis E. Higa*[2]*, Sonia E. Korol*[1]

[1]*Cátedra de Higiene y Sanidad. Facultad de Farmacia y Bioquímica. UBA.* [2] *Instituto Nacional del Agua (INA) (arianarossen@hotmail.com)*

Crops such as soy and corn have grown in order to supply national and international demand. As a result the risk of water pollution in agricultural areas has increased due to the frequent use of crop-associated pesticides like glyphosate (GF) and atrazine (AT). Local and regional information regarding the environmental impact of agricultural activities are still scarce. The main objective of this work was to evaluate the bacterial community response to GF and AT exposure in water samples taken from Los Molinos reservoir, located in an agricultural basin. Water samples were exposed to GF (10 mg/L) and AT (0.050 mg/L) during a five-day assay to evaluate bacterial tolerance. Total and pesticides-tolerant heterotrophic bacterial counts were performed in R2A agar plates. Changes in bacterial community were assessed by a carbon source profile before and after pesticide exposure in microplates. A color changing redox indicator dye was used to quantify the degree of carbon source utilization. The average metabolic response (AMR) and community metabolic diversity (CMD) were determined in order to differentiate among carbon source profiles. Bacterial tolerance to GF (10-400 mg/L) and AT (0.05-10 mg/L) was determined by plate serial dilution technique. Strains able to tolerate maximum concentration were isolated. After GF exposure (10 mg/L) bacterial count increased in two orders of magnitude comparing with the control without pesticides addition. While after AT exposure (0.05 mg/L) bacterial count did not differ from the control. Bacterial community tolerated up to 400 mg/L of GF without differences in bacterial counts comparing with control ($1{,}2x10^6$ bacteria/mL average). The maximum concentration of AT tolerated was 10 mg/L with a decrease in bacterial count (110 bacteria /mL). Six atrazine-tolerant bacteria were isolated. All were Gram negative non-fermentative bacilli. After GF exposure the carbon consumption increased while atrazine caused a decrease in the number of carbon source metabolized. The high number of substrate utilized after glyphosate exposure indicate a higher functional diversity than atrazine-exposed community. This work brings preliminary results regarding bacterial community response to pesticides in the Los Molinos reservoir. Tolerant bacteria play an important role in pesticides polluted aquatic environment since they contribute to restore ecosystems and natural conditions.

EM-P16

BIOFILM FORMATION IN *Mesorhizobium loti*

Alicia B. Supanitsky[1,2] *, Ángeles Zorreguieta*[2,4] *, Viviana C. Lepek*[3]

[1]*INTI (Instituto Nacional de Tecnología Industrial)* [2] *Fundación Instituto Leloir* [3] *IIB-INTECh-UNSAM* [4] *QB, FCEyN, UBA (asupanit@inti.gob.ar)*

Mesorhizobium loti interacts with *Lotus spp*. tissue surfaces during symbiosis relationships. Some previous studies show that biofilm formation plays a role in the interaction between bacteria and its host plant.On the other hand, the biofilm may enhance bacterial survival in soil in the absence of a legume host. Bacterial surface polysaccharides are very important in the establishing of symbiosis and attachment to surfaces. We studied biofilm formation in *M. loti* strain MAFF303099 and *M. loti* strain Ayac 1 BII by microtiter polystyrene-plate assay and CV-stained, under various conditions. We found that biofilm formation was greater when the bacteria were grown in minimal media rather than in rich media (TY) in cultures shaken. Strain MAFF shows maximal biofilm formation when the bacteria were grown in minimal media Y manitol 0.2%, for 4 days to an initial optical density at 595 nm of 0.001 while strain Ayac 1 BII shows the optimal attachment in minimal media AB sacarosa 0.5 %, when were grown for 3 days to an initial optical density at 595 nm of 0.1. Our results show that biofilm formation in *M. loti* differs from one bacterial strain to another under different culture conditions. Previously we had null mutants in *M. loti* Ayac 1 BII affected in the synthesis of lipopolysaccharide (LPS), β(1-2) cyclic glucan and the gene codes for the phosphoglucomutase (pgm). *M. loti lps* β2 mutant strain produces LPS with reduced amount of O-antigen and *M.loti lps* β1 mutant produces LPS totally devoid of O-antigen, *M. loti pgm* mutant contains an altered form of LPS, lacks EPS and cyclic glucan. These null mutants were evaluated in the optimal condition to biofilm formation to *M. loti* Ayac 1 BII. Only *M. loti pgm* mutant was unable to grow in biofilm suggesting that EPS is crucial for biofilm formation in *Mesorhizobium loti* Ayac 1 BII. Based on the genome sequence of *M. loti* strain MAFF303099 we are construing null mutants in EPS to complete this study

EM-P17

CANCHADA YERBA MATÉ: GENERIC IDENTIFICATION OF FILAMENTOUS FUNGI.

Ayelén B. Tayagüi[1], Maria L. Castrillo[1], Gladis Jerke[1], Marta A. Horianski[1], Miriam Marinez[1]
[1] Laboratorio de Microbiología, Módulo de Bioquímica y Farmacia, FCEQyN, UNaM (ayelenbetsabe@yahoo.com)

Canchada yerba maté is one of the ways of marketing of yerba maté that is obtained in the early stages of development of the traditional yerba maté through the coarse grinding of dry leaves after sapecado and drying. Due to the temperatures at which maté is subjected during processing, to direct fire on the stage of sapecado and high temperatures during drying, the bacterioflora is practically eliminated while the mycoflora that, on one hand can withstand these conditions and on the other, can reach the product during its acquisition, is an important microbiological parameter to evaluate. The purpose of this study was the generic identification of isolated fungi in canchada yerba maté that came from various establishments in the province of Misiones. We analyzed a total of 20 samples of canchada yerba maté, taken from different shops and markets in the center and the suburbs of the city of Posadas, Misiones. We performed total fungal counting (molds and yeasts) using the method of dilution in plates by surface spreading in the culture medium of fungi and yeasts after 5 to 7 days of incubation at 25 ± 1 º C. The generic characterization of strains of filamentous fungi was made on the basis of macro-micromorphology of the colonies, taking into account their fruiting bodies according to taxonomic keys. Of all the fungal isolated strains (molds and yeasts), 4% corresponded to fungi yeasts and 96% to filamentous fungi. Among the filamentous fungi the identified genera with their corresponding percentage of occurrence were: *Aspergillus* 75%, *Emericella* 8.4%, *Cladosporium* 5.8%, *Penicillium* 2.1%, *Trichophytom* 2.1%, *Aureobasidium* 1.3%, *Microsporum* 1%, *Alternaria* 1%, *Blastoschizomyces* 1%, *Chrysosporium* 0.7%, *Rhizopus* 0.7%, *Curvularia* 0.5%, *Fusarium* 0.2% and *Phaeoannellomyces* 0.2%. Consistent with findings of previous studies, in canchada yerba maté there was a predominance of filamentous fungi, being *Aspergillus* the genus of more incidence. In substrates such as black tea there was 92% of molds and 66% *Aspergillus* and in traditional yerba maté 90% of filamentous fungi and 82% of *Aspergillus*.

EM-P18

SPATIO-TEMPORAL PATTERNS OF MICROBIAL BIOMASS AND ENZYMATIC ACTIVITIES FROM ARID SOILS OF MONTE DESERT

Angela D. Vega Avila[1], Luciana Fernandez[1], Mario Baigori[2], German Babelis[3], Fabio Vazquez[1]
[1]Instituto de Biotecnología – FI – UNSJ [2] PROIMI. Avenida Belgrano y Pasaje Caseros. Tucumán [3] Instituto Nacional de Tecnología Agropecuaria. EEA Pocito. San Juan (vega_daniela06@yahoo.com.ar)

Soil microbial biomass is the active component of the soil organic matter pool, which is responsible for organic matter dynamics and thus nutrient availability to plants. It is an important indicator of soil fertility and its measurement is often essential in soil ecological studies. Soil microorganisms are the main source of enzymes, and despite their relatively low amounts, play the crucial role of keeping the main nutrient cycles in soil (C, N, P, S) through recycling from organic matter. The objective of this work was to evaluate microbial indicators of soil quality: soil microbial biomass and enzymatic activities associated to soil patches of *Bulnesia retama*, *Larrea divaricata*, and interspaces, in interdune environments. The studied site was located in the Dunes, San Juan, Argentina. This site is a sandy loam soil with 75% sand, 2,88% silt and 5,95% clay. Samples were taken from patches of the two species and interspaces at random in April and August. In 2007, during the first sample period (from February to April) precipitation volume was 70,32 mm and 0,325 mm in the second sample period (from June to August); in 2008, in the first sample period was 53,75 mm and 17,55 mm in the second one. Biomass was determined through the colony count method using differential isolation media. Soil enzymatic activities involved in decomposition of organic matter were determined (xylanases; cellulases; amylases). Results: No significant differences in studied microorganisms biomass were found in patch of *L. divaricata* , and interspace, at both samples of 2007. Bacteria abundance was higher in August than in the period of more precipitation (April) ($p=0,0024$). In samples of August 2008, yeast biomass from patch of *L. divaricata* was significant different from April ($p=0,0001$). In soil samples of 2007 xilanolytic activity was higher in the dry period (August) in relation with April at all microsites studied ($p=0,0009$, $p=0,0002$, $p=0,0001$). On the other hand, cellulolytic activity in patches and uncovered interspaces was significant different in April ($p=0,0001$, $p=0,0002$ y $p=0,0001$). Amylase activities in both sample periods did not show significant differences. In the same way that in 2007, xilanolytic activity evaluated in soil samples of 2008 at *B.retama*, and *L. divaricata* patches, was higher in August (winter, dry period) than in autumn (April) ($p=0,0022$, $p=0,0022$). Nevertheless, cellulolytic activity determined at the three studied microsites was significantly different in August, 2008 respect to the first sampling in the same year ($p=0,0152$, $p=0,0022$y $p=0,0276$). However, amylase activity was higher in patches and interspaces in the first sample period of 2008 ($p=0,02$ y $p=0,0001$). Conclusions: The abundance of bacteria and yeasts and the highest enzymatic activities corresponded to the maximum level of registered precipitations for both sample periods in both years.

EM-P19

EVALUATION OF RHIZOBIAL POPULATIONS ABILITY TO DEGRADE HERBICIDES AND DENITRIFICATE

Magalí Vercellino[1], *Marisa A. Gómez*[1]

[1]*Lab. de Microbiología Agrícola, Dpto. de Agronomía, Universidad Nacional del Sur - CERZOS - CONICET (mvercellino@cerzos-conicet.gob.ar)*

Herbicides are widely used in agriculture worldwide and they can compromise water and soil quality. Besides, excessive use of nitrogen fertilizers can be harmful to ecosystems, resulting in a significant source of nitrate pollution in soil and water. Rhizobia have a wide variety of functions associated with the agricultural environment where they thrive. The objective of the present work was to evaluate the capabilities of rhizobial populations to degrade herbicides and the reduction of nitrates and/or denitrification. We evaluated 81 rhizobial strains belonging to 4 different genera. The ability of rhizobia to use herbicides 2,4-Dichlorophenoxyacetic acid (2,4-D), Glyphosate (GF) and Atrazine (AT) as a source of nutrients was assessed in mineral-based medium (MB). Liquid cultures were inoculated (10 µl strain^{-1}) in the control (MB) and in the following treatments: 1) MB + 2,4-D; 2) MB + GF; 3) MB + AT, and incubated at 28ºC in a moist chamber for 36 days. Nitrate reduction was assessed by inoculating each strain in tubes containing modified Bergersen liquid medium and Durham tubes. Tubes were incubated in anaerobic conditions at 28°C for 14 days. The presence/absence of nitrogen gas bubbles was recorded and nitrate reduction was tested by GRIESS-ILOSVAY's Nitrite Reagent. In this study, herbicide AT was the most degraded by the rhizobial populations assessed. Most strains had normal development in the evaluation of degradation of 2,4-D and GF. Fifty-three strains reduced nitrate (no gas) and seven strains appeared to be potential denitrifiers (gas production). Until now, genes *nor*C and *nos*Z were detected in one of the strains. Three *Bradyrhizobium* strains were both able to degrade AT and denitrificate, and we called them "double capacity strains". Their ability to degrade AT was assessed weekly (during 42 days) determining the absorbance at 620 nm. Turbidity increased with time, demonstrating that strains can use AT as a source of nutrients. It is suggested that "double capacity strains" are important because they might be able to degrade the herbicide under anaerobic conditions. Knowledge of these functions in rhizobia broadens the expectations at the farm and environmental level as well as economic level. The possibility of using these N_2 fixing bacteria as inoculants may be advantageous as it would allow appropriate fertilization management and also help in the biodegradation of pesticides in agricultural soils.

IN-P1
EFFECT OF VOCs PRODUCED BY *Arthrobacter agilis* ON LEGUMINOSE PLANTS
Crisanto V. Becerra[1], *Josue A. Hernandez*[1], *Lourdes M. Rodriguez*[1], *Eduardo V. Cantero*[1]
[1]*IIQB, Universidad Michoacana de San Nicolas de Hidalgo, Morelia Michoacán, México (crixvb@hotmail.com)*

The plant growth-promoting rhizobacteria (PGPR) are bacteria that can modulate plant growth by various mechanisms such as biocontrol, biofertilization and phytostimulation. During this decade, the emission of volatile organic compounds (VOCs) provided by the bacterial strains PGPRs results play a role in the development and plant architecture, some studies have demonstrated that bacterial strains use the volatile compounds during the interaction with other organism in order to influence a symbiosis, in the case of plant interaction has been capable of module root architecture. In our laboratory we have showed the plant *Medicago sativa*, modifies its root architecture when growing in the presence of the rhizobacterium *Arthrobacter agilis* UMCV2. When we inoculate the bacterial strain at a distance of 3.5 cm form the roots tip, the primary root growth is reduced in comparison with the uninoculated control, and the lateral roots increase in number and length. The plant shoot is also increased. When using a system of separate compartments in petri dishes for grow the bacteria and plant abolishing any physical contact between them, we observed that the VOCs produced by the strain UMCV2 alter the root and shoot length of plants treated in postembryonic stage. In this system, the plants growth in presence of the bacteria produced primary roots with length of 1 cm or less, while plants growth without the bacteria produced primary roots with length of 2 cm or more. In deep, by exposing the plant to VOCs from strain UMCV2 after 96 hours of germinated seeds, the changes of the root architecture are more dramatic, increased the number and length of lateral roots and shoot length. Also, we analyze the VOCs effects from *Arthrobacter agilis* UMCV2 in the plant *Medicago truncatula*. This plant showed an statistically significant increase a in parameters such as length shoot, fresh weight and chlorophyll concentration when was growth in presence of the strain UMCV2 compared with the controls. It was very clear in the chlorophyll parameter where the plants grown in presence of the strain UMCV2 showed chlorophyll concentrations of almost twice the concentration present in control plants. With these data is clear that VOCs from strain *Arthrobacter agilis* UMCV2 have high biological activity, as was having the ability to change the overall architecture of our plants, with a dose-dependent effect on the amount of VOCs emitted by the strain. We were unable to detect 2,3- butanediol and acetoin in the mixture of VOCs produced by the strain UMCV2 but the effect of other VOCs produced by the bacteria are been for their biological effect.

IN-P2
INOCULATION METHOD INFLUENCES COMPETITIVENESS AND EFFECTIVENESS OF BIOLOGICAL NITROGEN FIXATION IN THE INTERACTION PEANUT-RHIZOBIA
Pablo Bogino[1], *Fiorela Nievas*[1], *Erika Banchio*[1], *Walter Giordano*[1]
[1]*Dpto. de Biología Molecular. FCEFQyN. Universidad Nacional de Río Cuarto. (wgiordano@exa.unrc.edu.ar)*

Inoculation of legume plants with effective rhizobial strains constitutes a beneficial alternative in order to increase the productivity in these crops. Introduction of viable rhizobia into the agricultural ecosystems can be achieved by means of seed inoculation or by direct application into the soil, technology termed in furrow inoculation. The inoculation of the seedbed shows several advantages as compared to seed inoculation because it favors the survival and competitiveness of introduced strain. *Arachis hypogaea* L. (peanut) is one of the most important legume crops cultivated in the central area of Argentina. Inoculation of peanut is a controversial practice because nodulation by native bacteria is usually assumed to be sufficient. By using a laboratory model which makes possible to reproduce the application of seed inoculation or in furrow inoculation conditions, we evaluated the effect of these technologies on the efficiency of nitrogen fixation and competitiveness between the recommended as inoculant C-145 strain and indigenous strains. In furrow inoculation was found more effective than seed inoculation for the majority of the parameters of efficiency studied. It significantly increased weight and number of nodules, nitrogenase activity and shoot dry weight respect to seed inoculation. Competitiveness assays for nodule occupancy clearly showed the interference caused by native strains on the ability of a recommended strain to nodulate the legume, independently of the inoculation method applied. The effect was more marked for seed inoculation, C-145 strain inoculated on the seed was incapable of competing with native established strains which occupied the majority of nodules (~ 90 %) independently of their localization on principal or lateral roots. For in furrow inoculation nodule occupancy by C-145 strain was slightly augmented when that strain increased its proportion in the inoculum. Results obtained for efficiency and competitiveness according the technique of inoculation applied, support the superior benefits that in furrow inoculation technology provides to an introduced recommended strain and finally to the legume crop.

IN-P3

THE PGPR *Arthrobacter* sp. MRP$_1$-24 PROMOTES WHEAT GROWTH UNDER SALINE STRESS AND INDUCES THE EXPRESSION OF *TNHX1*

Marina G. Castro[1], Edgardo Jofré[1], Analía Príncipe[1], Gladys B. Mori[1]
[1]Dpto. Cs. Nat., FCEFQyN. Universidad Nacional de Río Cuarto (mcastro@exa.unrc.edu.ar)

Salt stress is one of the most significant abiotic stresses affecting plant physiology and metabolism. Different plant strategies have evolved to minimize adverse effects associated to high salinity. Among them, selective ion uptake and differential ion compartmentalization into vacuoles are efficient mechanisms to avert the deleterious effects of Na+ in the cytosol. Under stressing conditions the plant hormone ethylene regulates plant homeostasis resulting in a reduced root and shoot growth. The inoculation of PGPRs bacteria containing the enzyme 1-aminocyclopropane-1-carboxilate (ACC) deaminase can reduce deleterious effect of stress-induced ethylene. The aim of the present study was to analyze if the inoculation of plant-growth-promoting bacterium *Arthrobacter* sp. MRP$_1$-24, containing the enzyme ACC deaminase, can promote wheat growth under saline stress conditions and/or modifies *TNHX1* (Na+/H+ vacuolar antiporter) expression in wheat roots. In order to select microorganisms containing ACC deaminase we performed a first screening, within a PGPR collection, and selected those strains capable to grow in minimal medium with ACC as the only nitrogen source. The presence of the *acdS* gene was confirmed in the strain MRP$_1$-24 by PCR assays. Moreover, ACC deaminase activity was determined in this strain. The strain MRP$_1$-24 was identified as belonging to the *Arthrobacter* genus by sequencing the *16S rRNA* gene. Plants assays were carried out in hydroponics cultures supplemented with 150mM of NaCl and inoculated with *Arthrobacter* sp. MRP$_1$-24 (107 UFC/ml). After 15 days post-inoculation the dry weight of shoots and roots was determined. The results showed that the inoculation of *Arthrobacter* sp. MRP$_1$-24 to salt stressed plants increased shoot dry weight compared to non inoculated plants under saline stress conditions. The analysis of gene expression by RT-qPCR was performed using total RNA from wheat roots subjected to the conditions described before. *Actin* was used as a normalizer gene. Expression of *TNHX1* gene was found up-regulated in wheat roots inoculated PGPR *Arthrobacter* sp. MRP$_1$-24 under saline stress. In contrast the expression of *TNHX1* gene was found down-regulated in roots from plants grown under saline stress conditions as well as in roots from plants inoculated grown under non saline conditions. Our data are in agreement with previous reports in which ACC deaminase-containing PGPR can reduce plant stress and induce changes in the expression of genes associated to stress tolerance.

IN-P4

GENETIC DIVERSITY OF NATIVE *Pseudomonas* spp. ISOLATED FROM MAIZE PLANTS.

Paula V. Cordero[1], Sonia E. Fischer[1], Edgardo C. Jofré[1], Gladys B. Mori[1]
[1]FCEFQyN - Universidad Nacional de Rio Cuarto (pcordero@exa.unrc.edu.ar)

The genus *Pseudomonas* is one of the best-studied bacterial groups in soil, and includes numerous species of environmental interest. Some species exhibit plant growth-promoting and pathogen-suppressing functions and may be exploited for use in biological control. The PGPR traits and persistence of bacteria in the roots may be affected by biotic and abiotic factors. Therefore the assessment of the genetic structures of microbial populations is important for understanding their ecological role in natural environments. In our lab, we isolated bacterial strains on King B and Gould's mS1 media from maize plants (at two stages of growth: young and old plants). Several isolates identified as belonging to *Pseudomonas* genus showed PGPR traits, such as production of indole acetic acid, siderophores and extracellular enzymes or solubilization of phosphates. In this work ERIC and BOX-PCR (rep-PCR) were performed to estimate the genetic differences among 53 strains of *Pseudomonas* spp. In addition, the bacterial strains isolated were evaluated by an amplified ribosomal DNA restriction analysis (ARDRA) with the enzymes: *Hae*III, *Taq*I, *Hinf*I, *Rsa*I. Gel images were digitized, converted, normalized with DNA size markers, and analyzed with GelComparII software (version 5.1; Applied Maths, Kortrijk, Belgium). For rep-PCR fingerprint and ARDRA analysis, a band-matching algorithm (tolerance of 1% and 3% respectively) was used to calculate pairwise similarity matrices with the Dice coefficient. Cluster analyses of similarity matrices were performed by the unweighted pair group method using arithmetic averages (UPGMA). Cluster analysis from rep-PCR showed 29 unique *Pseudomonas* genotypes out of 53 isolates. These strains were clustered into five major groups: A, B, C, D and E. Group A included only strains isolated from young plants while group E only strains of the old plant. Most isolates from both growth stages fell within the B and C groups. D group have only one strain (DGE5). The restriction analyses (ARDRA) revealed two main groups A and B. The strains DKR6 and DKR11 shared a high similarity (100%), and DKS4 and *Pseudomonas putida* KT2440 were closely related with an 80% of similarity.

IN-P5

DIFFERENT CAPACITIES OF *Staphyloccocus aureus* AND *Staphyloccocus epidermidis* ISOLATED FROM PATIENTS, TO ACTIVATE INNATE IMMUNE CELLS

Silvia Dávila[1], Sara Satorres[1], Julio Arce Miranda[2], María G. Paraje[2], Claudia M. Mattana[1], Virginia Rivero[3,4]
[1] Área Microbiología, FQByF, UNSL. [2] Dpto Farmacia, FCQ, UNC. [3] CIBICI, CONICET. [4] Dpto Bioq. Clínica, FCQ, UNC. (davilasdv@yahoo.com.ar)

Most of species of the genus *Staphyloccocus* are widely distributed in the nature, and are essential commensal of the normal bacterial flora in human skin and mucosa. Nevertheless, *Staphyloccocus epidermidis* is important as the main pathogen in foreign body infections, where it does not induce a significant systemic or local inflammatory response. On the other hand, *Staphyloccocus aureus* is a well characterized specie, known to have a diverse arsenal of virulence factors and to cause skin superficial or invasive serious infections accompanied with a prominent inflammatory response. In the present work, we studied the capacity of different clinical strains of *S. epidermidis* and *S. aureus* to stimulate macrophages or epithelial cells. We analyzed the production nitric oxide (NO) or urea, two intermediaries from the arginine metabolism that involve nitric oxide synthase enzyme (which generates NO, an inflammatory mediator) and arginase enzyme (which generates urea, depleting the milieu of arginine with an anti-inflammatory outcome). Sixteen clinical strains of *S. aureus* and 11 clinical strains of *S. epidermidis* isolated from blood cultures and catheters of hospitalized patients, were included in our study. Bacteria were incubated with RAW 264.7 or A549 cells and 48 hours later NO production and arginase activity were evaluated by the Griess's technique or measuring urea levels respectively. Our results showed significant differences in the capacities of *S. epidermidis* or *S. aureus* to stimulate macrophages and epithelial cells. Strains of *S. aureus* included in our study showed higher capacity to induce NO in RAW 264.7 ($p \leq 0.05$) and A549 cells. When we analyzed the abilities of both species to induce arginase activity, we observed that *S. aureus* and *S. epidermidis* were poor inductors of arginase activity exhibiting no differences among species. Our results demonstrate that *S. aureus* was good inductor of NO while *S. epidermidis* exhibited scarce ability to induce this inflammatory mediator in macrophage and epithelial cells. Our results also demonstrated that the low capacity of *S. epidermidis* to induce NO, was not related to a deviation bias the arginase metabolic way. In conclusion, our results show that *S. aureus* and *S. epidermidis* present different capacity to stimulate cells of innate immunity, and suggest that this characteristic might explain the different clinical presentation observed in patients affected by these microorganisms.

IN-P6

NITRIC OXIDE AND TNF-A INDUCTION BY INNATE IMMUNE CELLS STIMULATED BY *Staphyloccocus aureus* SLIME-POSITIVE AND MULTI-RESISTANT STRAINS

Silvia Dávila[1], Virginia Rivero[3,4] , Julio Arce Miranda[2], María G. Paraje[2], Lucía E. Alcaráz[1], Sara Satorres[1]
[1] Área Microbiología, FQByF, UNSL. [2] Dpto Farmacia, FCQ, UNC. [3] CIBICI, CONICET. [4] Dpto Bioq. Clínica, FCQ, UNC. (davilasdv@yahoo.com.ar)

Staphylococcus aureus is a well defined and more common coagulase-positive specie of the genus *Staphyloccocus* being one of most significant pathogen for the human. Epithelial colonization by *S. aureus* is an important risk factor for infection by this organism in both community and hospital settings. The gradual increase of multi-resistant and methicillin-resistant *S. aureus* strains has complicated the therapeutic antibiotic for these infections. In addition, biofilm formation is considered to be a virulence factor because bacteria in biofilms are less accessible to antibiotics and immune defenses. In the present work, we analyzed the capacity of slime-positive or slime-negative *S. aureus* strains to stimulate the induction of nitric oxide (NO) by macrophages. Moreover, we analyzed the ability of multi-resistant and methicillin-resistant *S. aureus* strains to stimulate the induction NO and inflammatory cytokines in macrophages and epithelial cells. Sixteen clinical strains of *S. aureus* isolated from blood cultures and catheters of hospitalized patients were included in our study. Slime formation was detected by three methods that include tissue culture plate, tube method and Congo red agar. Antibiotic susceptibility was tested by the disc diffusion method (CLSI). Bacteria were incubated with RAW 264.7 or A549 cells and 48 hours later the production of NO and TNF-α was detected by the Griess's technique and capture ELISA respectively. NO levels produced by macrophages incubated with slime-positive or slime-negative *S. aureus* strains did not demonstrate differences, indicating that discrimination of *S. aureus* based on the expression of this virulence factor did not correlate with a higher capability to activate immune cells. When we analyzed NO and TNF-α levels produced by macrophages or epithelial cells incubated with multi-resistant or methicillin-resistant *S. aureus* strains, we observed that these strains were poor inductors of NO and TNF-α ($p \leq 0.05$). Our results show that multi-resistant and methicillin-resistant *S. aureus* strains have reduced ability to activate innate immune cells, fact that may be related to their high virulence.

IN-P7
ASSAYS OF SURVIVAL AND INOCULATION OF *Burkholderia tropica* ON TOMATO
Jesica Frik[1], Roberto D. Moyano[1], Gimena Garcia Ferreyra[1], Manuel Couyoupetrou[1], María F. Luna[1,2]
[1]CINDEFI (UNLP; CCT-La Plata, CONICET), Facultad de Cs. Exactas [2]CIC-PBA (mafla@quimica.unlp.edu.ar)

The use of Plant Growth Promoting Bacteria (PGPB) as biofertilizer and biocontrol organisms is being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture. The growth stimulation by the microorganisms can be a consequence of nitrogen fixation, the production of phytohormones, biocontrol of phytopathogens in the root zone or by enhancing availability of minerals. Bacteria on roots and in the rhizosphere benefits from root exudates, but some bacteria are capable of entering the plant as endophytes that do not cause harm and could establish a mutualistic association. In both cases a key feature of plant-beneficial bacteria associations is the efficient colonization of root surfaces. To ensure an effective colonization it is necessary to use a successful inoculant and an adequate inoculation process. In this way and with the knowledge of *Burkholderia* strains are promising candidates for biotechnological applications, in the present study has been performed experiment of seed and seedlings inoculation (*Lycopersicon esculentum, cv. platense italiano*) with *Burkholderia tropica* to investigate the colonization behavior on gnotobiotically grown. Survival microorganism experiments on tomato seeds were carried out to complete this approach. Microscopy results indicated that *B. tropica* was able to colonize tomato plants independently of the inoculation form but surfaced sterilized root bacterial enumerations was lower in the case of seed inoculation. The evaluation of viability in tomato seeds showed that *B. tropica* survived during germination period in filter paper or soft agar.

IN-P8
MACROPHAGE INFLAMMATORY RESPONSE TO *Clostridum chauvoei* INFECTION .
Giovanna L. Gallo[1], Alba E. Vega[1], Maria C. Villa[1], Claudia S. Cáceres[1], Teresa I. Cortiñas[1], Ana M. Stefanini[1]
[1]Área Microbiología. Fac. Química, Bioquímica y Farmacia. UNSL (ticor@unsl.edu.ar)

Clostridium chauvoei is an anaerobic bacillus, which is the causative agent of blackleg, a clostridial myonecrosis that has an important impact on the sheep and cattle industry worldwide. Blackleg is considered a gangrene gaseous, which is an acute disease with a poor prognosis and often fatal outcome. Initial trauma to host tissue damages muscle and impairs blood supply. Initial symptoms are generalized fever and pain in the infected tissue. As the clostridia multiply, various exotoxins (including hemolysins, collagenases, proteases, and lipases) are liberated into the surrounding tissue, causing more local tissue necrosis and systemic toxemia. Infected muscle is discolored (purple mottling) and edematous and produces a foul-smelling exudates with gas bubbles formed from the products of anaerobic fermentation. In many inflammatory diseases many immuno-mediators are involved in this process, amongst them, we can remark cytokines TNF-α, IL-6 and IL-1β. IL-6 is a pleiotropic citokine of complex biological activity and TNF-α is a pro-inflammatory cytokine which main function is the recruitment and stimulation of neutrophils and monocytes and the induction of apoptosis, giving rise to inflammation and connective tissue destruction. We hypothesize that *C. chauvoei* may induce an inflammatory cascade which stimulates tissue destruction mediated by the host. Until now it is unknown which are the immune inflammatory response involved in *C. chauvoei* infection. The aim of this work was evaluate the capability of *C. chauvoei* to induce the expression of IL-1β, IL-6 y TNF-α pro-inflammatory citokynes in peritoneal mouse macrophage. β- actin was used as housekeeping gene. Macrophage (1 x 106 cell/well) were co-cultures with *C. chauvoei* cells obtained in log phase at different multiplicity of infections (MOI): 3:1, 10:1 y 20:1 and treated with culture supernatants at 5% and 10%, for 5, 12 and 21 h of incubation. Total RNA was obtained using TRIZOL reagent. For RT-PCR, RNA was reverse transcribed into cDNA and used for RNA amplification with specific primers. The bands were semi-quantitative analyzed by Scion image software. The results showed that *C. chauvoei* induces an increased expression of TNF-α between 3 to 10 times higher than the control at 21 h of post- infection. Meanwhile for IL-1β expression the increase observed was between 3 times at 12 h to 10 times at 21h. The IL-6 showed a remarkable increased of 8 times higher than the control at 12 h. The expression was dose dependent in all cases and the lost of viability was observed in macrophages infected at higher MOI and treated with culture supernatants. Our results showed that the pro-inflammatory cytokines must play an important role in blackleg outcome.

IN-P9

***Lactobacillus delbrueckii* susp. *Lactis* (STRAIN CIDCA 133) ENHANCES UPTAKE OF PATHOGENS BY MACROPHAGES.**

Ayelen A. Hugo[1], Pablo F. Pérez[1,2]
[1]CIDCA-UNLP-CONICET [2] Cátedra de Microbiología, FCE. UNLP. (ahugo@cidca.org.ar)

Phaghocytic activity not only contributes to the elimination of potential dangerous microorganisms but also constitute a key step in antigen presentation. Lactobacilli are widely used as probiotic because of its health-promoting effects. Beneficial effects of probiotics could be ascribed, at least partially, to the activation of professional immune cells such as macrophages. In the present study, we evaluated the phagocytic activity of human macrophages, against two attaching and effacing pathogens: Enterohaemorrhagic *E. coli* (EHEC) and *Citrobacter rodentium*. We performed flow citometry studies with the human cell line U-937 in the presence of viable *Lactobacillus delbrueckii* subsp. *lactis* (strain CIDCA 133) and its extracellular products. Cells were cultured in RPMI medium supplemented with 10% (v/v) fetal bovine serum, at 37ºC in a 5% CO_2atmosphere. Differentiated cells were obtained by adding 1 % (v/v) DMSO. Lactobacilli were grown in MRS broth at 37 ^{0}C. Supernatants were obtained by centrifugation. They were filter sterilized, neutralized and diluted. EHEC strain 69160 and *C. rodentium* ICC 180 were grown in LB medium at 37^{0}C. They were labelled with 0.3 mg/ml of flourescein isothiocyanate. Opsonization was conducted by incubation in RPMI containing 10 % (v/v) human serum. Lactobacilli were labelled with carboxifluorescein-succinimydylester. Microorganisms and eukaryotic cells were suspended in RPMI medium with a multiplicity of infection of 20:1 and incubated at 37 ^{0}C for 30 min. To determine internalized bacteria, fluorescence of externally attached microorganisms was quenched by adding 2 % (w/v) trypan blue before acquisition by flow cytometry. Uptake index was defined as the percentage of cells positive in FL1 (green) x mean fluorescence intensity. In the presence of lactobacilli, uptake of EHEC and *C. rodentium* by U-937 cells increased in 40 %. These results were obtained in both differentiated and undifferentiated cells. Uptake index of *C. rodentium* was 2 – 3 times higher than that for EHEC. When experiments were performed in the presence of culture supernatants of lactobacilli instead of whole bacteria, uptake was not modified. These findings suggest that the enhancement of the phagocytic activity is not due to extracellular factors secreted by lactobacilli. Interestingly, low uptake indexes were obtained for strain CIDCA 133 and uptake was not modified in the presence of the gramnegative bacteria assayed. Results demonstrate that *Lactobacillus delbrueckii* susp. *lactis* (strain CIDCA 133) are capable to increase the uptake of the enteropathogens under study. This ability could be very relevant not only for the elimination of the pathogens but also to prime immune system to generate a protective response.

IN-P10

IMMUNOMODULATORY EFFECT OF *Lactobacillus johnsonii* LA1 IN A MURINE MODEL OF INFECTION WITH G*iardia intestinalis*.

Martín A. Humen[1], Pablo P. Pérez[1,2]
[1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos - CIDCA, CCT La Plata -CONICET [2]Facultad de Ciencias Exactas. Cátedra de Microbiología . UNLP (mhumen@cidca.org.ar)

The ability of probiotic microorganisms to antagonize intestinal bacterial pathogens has been widely documented. However, little is known on the effect of probiotics on intestinal protozoa such as *Giardia intestinalis*. We have demonstrated, in vivo, the ability of *Lactobacillus johnsonii* La1 to antagonize *Giardia* infection. In the present work we sought to gain insight on the effect of the administration of lactobacilli on the balance of relevant cellular populations in a murine model of giardiasis. *Lactobacillus johnsonii* La1 was administered (5.10^8 CFU/day) to C57 BL/6 mice for 7 days prior to the infection with 10^7 trophozoites/mice with *Giardia intestinalis* (strain GS, clone H7). After 7 and 14 days, samples of mesenteric lymph nodes (MLN), Peyer´s patches (PP), and spleen were collected. Ratio of CD4+/CD8+, mast cells, B cells and the expression of MHC-II were evaluated by flow cytometry. As compared with untreated controls, administration of *Lactobacillus johnsonii* La1 for 7 days prior the infection with *Giardia*, leads to a decrease in CD4+/CD8+ ratio in PP (P=0.02). This results seem to be related to a diminution of the ratio of CD4+ cells. In PP, an increase in the expression of MHC-II was observed in both B220+ (B cells) and B220- cells. In contrast, in MLN, there was a decrease in the expression of MHC-II in B220+ cells due to the probiotic treatment. Seven days after infection with the parasite, ratios of CD4+ and CD8+ cells showed no changes as compared with the infected group without probiotic treatment. In the same time-point, a decrease in the ratio of CD4+ cells in MLN was observed. Concerning the expression of MHC-II and ratio of mast cells, no differences were detected between the probiotic and placebo groups. Our results show that probiotic administration modifies the ratio of relevant immune cells in a murine model of giardiasis. Higher differences were found at day 7 of probiotic treatment and prior to the infection with the parasite. Probiotic treatment increases expression of MHC-II in PP thus indicating a modification of antigen presentation that could improve immune response against the parasite. Since no changes were found in MLN, it can be hypothesize that there is no antigen translocation from the intestinal mucosa. Presented results could explain, at least partially, the decrease in the infection rates observed in a murine model of giardiasis treated with *Lactobacillus*

johnsonii La1. Thus, we showed the capacity of *Lactobacillus johnsonii* La1 to modify the distribution of different cellular populations preparing the host in a better manner to deal with this parasitic infection.

IN-P11

EFFECTS OF INOCULATION WITH *Bacillus simplex, Pseudomonas fluorescens* AND *Azospirillum brasilense* ON BIOMASS PRODUCTION, GRAIN YIELD AND NATIVE MICROBIAL COMMUNITY OF WHEAT PLANTS

María de los Ángeles H. Ortega[1], *Mariana Kade*[1], *Luciana P. Di Salvo*[1], *Jhovana S. Escobar Ortega*[1], *Inés E. García de Salamone*[1], *Atilio J. Barneix*[1]

[1]*Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (INBA-CONICET-FAUBA) (ortega@agro.uba.ar)*

The prospect of manipulating crop rhizosphere microbial populations by inoculation with beneficial bacteria in order to increase plant growth has shown to be promising. The potential environmental benefits of this approach, leading to a reduction in the use of agricultural chemicals and the fit with sustainable management practices, are driving this technology. Plant growth promoting rhizobacteria (PGPR) enhance plant growth by direct and indirect means. Direct mechanisms include: fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones. Indirect mechanisms involve the ability of PGPR to reduce the deleterious effects of plant pathogens on crop yield. The purpose of this work was to study the effect of inoculation with different PGPR strains on biomass, yield and native microbial community of *Triticum aestivum* L. cv. ProINTA Isla Verde. The experiment was performed in a greenhouse in a completely randomized block design (four plants per pot). Inoculation treatments consisted of no inoculation, *Bacillus simplex* 10-1B, *Pseudomonas fluorescens* Sp233 and *Azospirillum brasilense* Sp245. Plants were sampled at: 100% emergence of the principal ears, 10 days after anthesis and maturity. Plant material was dried at 60ºC for 48 h up to constant weight. The physiology of rhizosferie microbial community was studied through profiles of use of carbon substrates (CLPPs). *A. brasilense* increased root dry weight in the first sampling date. In the second one, *A. brasilense* and *P. fluorescens* increased aerial, ear, and root biomass; grain yield, however, showed no differences. On the other hand, the average of inoculation treatments detected differences in the physiological profiles of carbon substrate utilization only at the first sampling date. Our results show that the PGPR included here had a temporary impact, because these rhizobacteria were probably not competent and could be quickly displaced by the native microbial community.

IN-P12

EFFECT OF INOCULATION WITH THREE DOSES OF *Azospirillum brasilense* IN *Lolium multiflorum* LAM. (ITALIAN RYEGRASS)

Estela B. Postulka[1], *Maria E. Olivera*[1], *Liliana Ferrari*[1], *Liliana R. Galián*[2], *Mariana A. Marrero*[2], *Cyntia Szemruch*[1], *Cristian E. Esposito*[2]

[1]*Cátedra de Forrajicultura.* [2]*Cátedra de Microbiología.Facultad de Ciencias Agrarias. Universidad Nacional de Lomas de Zamora (epostulka@yahoo.com)*

The use of biofertilizers with *Azospirillum brasilense* (Az) as seed treatment is a practice increasingly studied since it helps to enhance crop growth. But technology needs to be adjusted to achieve maximum efficiency. For this purpose the effect of three doses of biofertilizer in interaction with nitrogen fertilization was evaluated. The trial was sown on 28/5/08 using 4 viable seeds / pot of *Lolium multiflorum* Lam. Doses were derived from the recommended dose of biofertilizer for wheat. Three doses of Az (1 / 2, 1 and 2 wheat dose) were combined with and without nitrogen fertilization (200 kg N / ha as urea). The treatments were: fertilized with N (CF), control without fertilization (CsF), ½ wheat dose fertilized with N (1/2F), ½ dose unfertilized (1/2sF), 1 wheat dose fertilized with N (1F), 1 dose unfertilized (1SF), 2 wheat doses fertilized with N (2F), 2 doses unfertilized (2SF). DCA was applied with five replications. Measurements were made of four plants per pot at 180 days after sown. The biomass data was expressed in grams of dry matter (gDM). Thus were obtained ADW values (aerial dry weight), RDW (root dry weight), SDW (seedling dry weight) and number of tillers. Data was analyzed by ANOVA and Tukey test ($p > 0.05$). While the main effect on the variables studied corresponded to nitrogen fertilizer application, significant interactions were found between both types of fertilization. The higher values for ADW were achieved with treatments fertilized with N, standing out 1F (10. 30 (±) 0.81 gDM) followed by CF with 8.98 (±)1.47 gDM. For RDW the highest values with no significant differences between them were obtained for CF (8.73 (±) 0.43 gDM) and 2SF (7.26 (±) 2.03 gDM). On SDW the highest values were obtained for CF and 1F (16.40(±) 2.07 gDM). Furthermore there was no difference between 2SF and 2F (13.74(±)1.74 gDM). The higher number of tillers were always obtained with treatments fertilized with N, regardless of the dose of Az. Preliminary results indicate that the combination of both fertilizers would be effective, however further research is needed in order to find the most appropriate dose of Az for this forage species.

BF-P1

PRODUCTION OF EXTRACELLULAR (α)-L-RHAMNOSIDASE BY *Neurospora sp.* IN SOLID-STATE FERMENTATION.

Silvina E. Adárvez[1], *María L. Martín*[1], *María L. Correa-Daneri*[1], *Silvia C. Gouiric*[1], *Martha D. Vallejo*[1]
[1] *Instituto de Biotecnología- Facultad de Ingeniería-Universidad Nacional de San Juan. (mvallejo@unsj.edu.ar)*

(α)-L-rhamnosidase (EC 3.2.1.40) is an enzyme potentially useful in food, pharmaceutical, and flavoring applications. Solid state fermentation (SSF) is a technology that allows the use of agroindustrial waste as feedstock to obtain many high valuable bio-products like enzymes, pesticides, fertilizers, colourings, organic acids, alcohols, etc. At the same time, SSF allow to minimize the final waste mass and a safe final disposition. Sugar beet pomace (SBP) is the solid residue left from sugar beet processing (80% on weight); it contains abundant cellulosic fibers and carbohydrates. The present work describes the SSF production of (α)-L-rhamnosidase ((α)-L-rh) activity by the fungus *Neurospora sp*, using SBP as substrate without nutrient supplementation. SBP was prepared as follows: fresh sugar beet was milled (particle size 2-5 mm), soluble sugars extracted by maceration in cold (70 ºC) distilled water, washed, and dried at 50 ºC until constant weight. Then, SBP was stored. For SSF, SBP was humidified to water content 50 %, 60 % , 70 % and 80 %, initial pH adjusted to 4.5, sterilized at 121 ºC for 20 minutes; then, substrate was inoculated with 10^6 spore/g, placed in Petri dishes, and incubated at 28 ºC for 10 days. Each day, three dishes were taken for analysis. In solid samples, water content was determined. Aqueous extracts from SSF were done, and tested for pH, sugar concentration and (α)-L-rh activity. Enzyme activity was assayed in sodium-acetate buffer pH 5, using p-nitrophenyl-(α)-L-rhamnoside as substrate, incubating the reaction at 45 ºC, for 1 h; then, reaction was stopped by addition of 0.5 M (HO)Na and absorbance at 400 nm was measured. Results: profiles for water and sugar content, wet and dried weight loss, pH, and enzymatic activity are presented. Best microorganism growth was attained in cultures with 70 % initial water content. In this case, maximum (α)-L-rh production was found at 7 day of culture, reaching 60 U/g. *Neurospora sp.* depleted, at 7 days of culture, about 50 % of the initial dried mass, which is also interesting from the waste management point of view. Optimizations on the culture and extraction conditions are our next aim.

BF-P2

SCREENING OF FILAMENTOUS FUNGI FOR PRODUCTION OF EXTRACELLULAR (α)-L-RHAMNOSIDASE ACTIVITY, BY SOLID-STATE FERMENTATION ON SUGAR BEET POMACE.

Silvina E. Adárvez[1], *María L. Martín*[1], *María L. Correa-Daneri*[1], *Silvia C. G*[1], *Martha D. Vallejo*[1]
[1] *Instituto de Biotecnología- Facultad de Ingeniería-Universidad Nacional de San Juan. (mvallejo@unsj.edu.ar)*

Microorganisms are the major source of rhamnosidases; many fungal and bacterial species synthesize these enzymes. One of the main differences between fungal and bacterial (α)-L-rhamnosidase (EC 3.2.1.40) is their optimum pH: acidic for fungal and alkaline for bacterial. This suggests different uses for each one, for example, the fungal (α)-L-rhamnosidase ((α)-L-rh) could be applied to wine and citric juice production; while the bacterial (α)-L-rh, to the production of L-rhamnose from hesperidin. Compared to studies on submerged culture, solid-state fermentation (SSF) has been less investigated for production of (α)-L-rh. Sugar beet pomace (SBP), an agroindustrial waste rich in organic compounds, can serve as substrate for microorganism growth and biosynthesis of high-value products in SSF. The aim of the present work was to select filamentous fungi capable of produce (α)-L-rh activity on SBP, by SSF. Twenty-nine fungal isolates collected from agricultural habitats were screened. These fungi were from the genus *Aspergillus*, *Penicillium*, *Mucor*, *Neurospora*, *Trichoderma*, *Cunninghamella*, and *Byssochlamys*. *A. kawachii* and *P. decumbens* were used as positive controls for growth and (α)-L-rh production on SBP. Fungi were assayed for growing in medium containing salts and L-rhamnose as the sole carbon source. SSF was prepared as follows: SBP (particle size 2-5 mm) humidified to water content 70 %, initial pH adjusted to 4.5, sterilized at 121 ºC for 20 minutes; then, substrate was inoculated with 10^5 spore/g, placed in Petri dishes, and incubated at 28 ºC for 2, 4, and 7 days. Aqueous extracts from SSF were done, and tested for the presence of (α)-L-rh activity. Enzyme activity was assayed in sodium-acetate buffer pH 5, using p-nitrophenyl-(α)-L-rhamnoside as substrate, incubating the reaction at 45 ºC, for 1 h; then, reaction was stopped by addition of 0.5 M (HO)Na and absorbance at 400 nm was measured. Results: fourteen strains, including controls, were positive for growth and (α)-L-rhamnosidase production. High enzyme activity was found in five strains. The best producer was *Neurospora sp.*, attaining 40.6 U/g (enzyme units per gram of dried mass) at 7 days cultures. This is an encouraging result, if compared with the (α)-L-rh activity found for *A. kawachii* (23.8 U/g) and for *P. decumbens* (23.4 U/g).

BF-P3

SELECTION AND CHARACTERIZATION OF COLD-ACTIVE A-L-RHAMNOSIDASE PRODUCER STRAINS WITH APPLICATION IN WINE INDUSTRY

Adriana E. Alvarenga[1], *María I. Arévalo*[1], *Carlos M. Abate*[1,2]
[1] *PROIMI-CONICET* [2] *Fac. de Bioqca., Qca. y Fcia. UNT (cabate@proimi.org.ar)*

α-L-rhamnosidases catalyze hydrolysis of rhamnoside substrates, releasing L-rhamnose. Several technological applications of these enzymes in industrial rhamnoside hydrolysis have been reported, including removal of bitterness from citrus juices. In addition, α-L-rhamnosidases are used in the release of flavour compounds from terpenyl glycosides for the enhancement of aroma in grape juices and derived beverages.The aims of this work were selection and taxonomical characterization of marine microorganisms isolated from Beagle Channel. Enzymatic activity was determined at 25 ºC and 5 and 7 pH values.58 isolates were cultivated in Brunner Minimal Medium (MMB) with rhamnose or naringine. 21 strains were selected at 8 and 20 ºC, and 5 and 7 pH values. The selected isolates were separated into groups according to ARDRA profiles. 16S rDNA amplifications were sequenced and the phylogenetics trees were constructed. The gene sequences allowed us to determine their association with the genera *Serratia*, *Shewanella*, *Pseudomonas*, *Pseudoalteromonas*, *Halomonas* and *Aeromonas*, members of the *Proteobacteria* class. Enzymatic activity was determined using 4-Nitrophenyl α-L-rhamnopyranoside (U and A.E.). Selected strains present promissory qualities related to α-L-rhamnosidase expression at low pH values and temperatures.

BF-P4

OREGANO (*Origanum vulgare*) INHIBITS BIOFILM FORMATION AND DECREASES VIABLE *Helicobacter pylori* OF ESTABLISHED BIOFILM.

María C. Dalfovo[1], *Alba E. Vega*[1], *Teresa I. Cortiñas*[1], *Humberto J. Silva*[1]
[1] *Área Microbiología. Fac. de Química, Bioquímica y Farmacia. UNSL. (aevega@unsl.edu.ar)*

Helicobacter pylori is a strict human pathogen that colonize half the world population causing chronic infections such as chronic gastritis, peptic ulcers and gastric carcinoma. Bacterial attachment to stomach epithelium is considered the initial step for *H. pylori* pathogenesis. A triple-therapy regimen employing a proton-pump inhibitor and two antimicrobials, clarithromycin (Cla) and metronidazole (Mtz), is often adopted for eradication of this microorganism. Pathogen microorganisms in a biofilm have a remarkable protection against antimicrobial agents and the reduced susceptibility is recognized as an important factor in the persistence of chronic infections. Antibiotic therapy together with activated host defenses can kill planktonic cells of *H. pylori*, however they are not effective in cells within a biofilm, determining the basis for chronic infections and thus contributing to the development of resistance. The antibiotic resistance problem spurs the search for alternative methods to treat *H. pylori* infection. In this sense, herbs and spices are used to increase the shelf lives of foods with a very well known antimicrobial effect. In fact many natural plant extracts have anti-*H. pylori* activity, including garlic, broccoli and cranberries. The aim of this study was to analyze the effect of sub inhibitory concentrations of an oregano extract on both *H. pylori* biofilm formation and established *H. pylori* biofilms. Previously the bactericidal activity of oregano was determined at 10 mg/ml by standard death-curve. The reference strain NCTC11638 and clinical strain HP796 (resistant to Cla and Mtx) were grown in Mueller-Hinton Broth supplemented with a) 5% fetal calf serum (reference) and b) 2,5mg/ml oregano extract. The cultures were incubated under microaerophylic conditions for 48 h at 37ºC. The effect of oregano extracts on *H. pylori* adhered to an abiotic surface was determined using plate counting. The morphologic changes were observed by optical microscopy in cells stained with crystal violet. The results showed that sub inhibitory concentrations of oregano extract significantly inhibited the biofilm formation of reference and clinical strains with a decreased of 0.6 and 2 log units respectively. In established *H. pylori* biofilms the oregano extract decreased biofilm biomass ($p \leq 0.05$) of both reference and HP796 strain in more than 1 log units, additionally the extract was able to induce 100% *H. pylori* cocoid forms after 26 h of treatment. The results indicate that oregano (*Origanum vulgare*) may be envisaged as a food additive that could reinforce present therapies. On the other hand people with asymptomatic gastritis would certainly benefit from a nutritional approach aimed at maintaining a low level of infection, since an increased density of *H. pylori* in the gastric mucosa is associated with more severe gastritis and high incidence of peptic ulcer.

BF-P5

ENHANCED ENZYMATIC HYDROLYSIS OF XYLAN BY *Paenibacillaceae* ISOLATED FROM SUGARCANE BAGASSE.

María J. Dávila Costa[1], Daniel H. Valdeón[1], Alicia L. Ragout[1,2] , Nora I. Perotti[1], Carlos M. Abate[1,3] , María A. Martínez[1]

[1] PROIMI- CONICET [2] Fac.de Cs. Exactas y Tecnología [3] Fac. de Bqca, Qca. y Fcia. [4] Fac. de Cs. Nat e IML UNT (jdavila@proimi.org.ar)

The recycling of photosynthetically fixed carbon through the action of microbial plant cell wall hydrolases is a key biological process. Xylan, one of the most abundant plant structural polysaccharides, is a heterogeneous polymer that requires multiple xylanolytic enzymes to be efficiently hydrolyzed. Moreover, a tight interaction between the enzymes and their substrates and the cooperation of multiple enzymes are also required to enhance hydrolysis due to the complex structures of their substrates, such as endo-1,4-β-xylanase (1,4-β-D-xylanylanohydrolase [EC 3.2.1.8]) and β-xylosidase (EC 3.2.1.37) that cleave the backbone chain and α-L-arabinofuranosidase (EC 3.2.1.55), acetyl esterase (EC 3.1.1.6), and α-D-glucuronidase (EC 3.2.1.1). Several bacteria were isolated from both, sugar cane bagasse and from the liquor that flows through the sugar cane bagasse pile during its pre-treatment for paper production. In this work we report the improvement of xylanase production during aerobic growth of selected isolates preliminary characterized as novel members of *Paenibacillaceae* according to their 16S rDNA sequence as well as morphologycally and biochemically by using standard procedures. Our results showed that the use of hemicelluloses extracted from sugar cane bagasse as substrate, xylanolytic activity is higher than that obtained with birchwood xylan as a carbon source, which could indicate the adaptation to their natural environment. Enzymatic activities were better when minimal media was supplemented with calcium and magnesium. Furthermore, addition of tween 80 at concentrations ranging from 0.02 to 0.4% to the media led to increments of xylanolytic activities of 50% to 90% for members to *Paenibacillaceae*, showing different magnitud of effect, no effect or growth inhibition for other bacilli isolated in this work.

BF-P6

USE OF POROUS BEDS BASED ON SILOXANE AS CARRIERS FOR THE DEVELOPMENT OF BACTERIAL BIOFILMS FOR A BIOREMEDIATION PROCESS.

Marcelo R. Fernandez[1,2] , Maria G. Casabona[1,2] , Delia L. Bernik[1,2]

[1] Instituto de Química Física de los Materiales, Ambiente y Energía (INQUIMAE), CONICET [2] Departamento de Quimica Inorganica, Analítica y Quimica Fisica. FCEyN. UBA (mfernandez@qi.fcen.uba.ar)

It is well known that the use of bioreactors is considered as an advantageous method to degrade organic pollutant compounds. Bacteria immobilization on inert carriers by biofilm production has demonstrated to improve bioremediation process. The biofilm is produced when microorganisms adhere and start duplicating on a material surface. Materials used for this purpose should satisfy some conditions, porous materials have been confirmed to be the most efficient. The use of these materials in bioreactors requires that these carriers are able to move through the growth medium to obtain a well mixed solution. Having this in mind, it is desirable to obtain particles with the following characteristics:

1- High porosity to maximize contact surface.
2- Low density to improve mixing.
3- Resistance and stability but at the same time biodegradability in middle – long time.

This work describes the use of porous particles for biofilm formation in favour of a bioremediation process (Casabona et al, poster presented in the Physicochemistry Congress in Salta, May 2009). The capability of *A. ferroxidans* to form biofilm on three different silicon surfaces was studied. Three diverse carriers based on polydimethylsiloxane (PDMS) were synthesized for this purpose, varying in their chemical composition. Scanning Electron Microscopy (SEM) showed that chemical composition has an influence on surface roughness. Biofilm formation on these beds was analyzed by Confocal Laser Scanning Microscopy (CLSM) using as staining method a viability kit LIVE/DEAD BacLight (Invitrogen). Finally, preservation techniques, such as lyophilisation, were studied after biofilm development.

BF-P7

INDIGENOUS DOG LACTIC ACID BACTERIA AND THEIR POTENTIAL PROBIOTIC PROPERTIES

María G. Fernández Juri[1], *Julián A. Muzzolón*[1], *Carla L. Barberis*[1], *Ana M. Dalcero*[1], *Carina E. Magnoli*[1]

[1] *Dpto. de Microbiología e Inmunología, Fac. Cs Exactas, Fco.- Qcas y Naturales, UNRC (gfernandezj@exa.unrc.edu.ar)*

Probiotic utilization is becoming increasingly popular in veterinary medicine. However, only a few probiotic products are commercially available for use in dogs in our market. Potential probiotic bacteria are classified, and generally regarded as safe as opposed to antibiotics, which have a number of recognized adverse effects. Lactic acid bacteria (LAB) are a broad group of Gram-positive, non-sporing rods and cocci, usually non-motile, catalase and oxidase negative that ferment carbohydrates forming lactic acid as the major end-product. A variety of microorganisms, typically lactic acid bacteria such as lactobacilli, bifidobacteria, and enterococci, have been evaluated as potential probiotics. According to this, the aims of this work were: 1) LAB isolation and identification from the intestine of healthy dogs, b) to analyze the capability of the strains of auto- aggregate and co- aggregate, c) tolerance of gastrointestinal enzyme and pH. The strains were isolated from feces of health dog in MRS agar (Mann Rogosa, Britania) and incubated in a micro-aerophilic chamber at 37°C for 72 h. Colonies were identified as LAB based on colonial morphology, Gram stain appearance, catalase, oxidase, indol and gelatinase reaction. Auto-aggregation and co-aggregation was performed on 6 selected strains using *Enterobacter* spp., *S. aureus*, *E. coli* and *Klebsiella* spp. isolated from ill dogs as indicators strains. The resistence of the strains to pH, different digestives enzymes and bile salts were evaluated. Twenty-three strains of possible LAB were isolated. The 78% of the studied strains were Gram (+) cocci in pairs. The rest were long and thin Gram (+) non- sporing rods. Catalase, oxidase, indol, gelatinase and motility test were negative. The 60% of the strains maintained in glycerol were recovered. The total of the strains assayed showed auto-aggregation capacity. In the evaluation of tolerance at low pH levels the strains survived at pH 4 during 4 h at 37ºC. These results suggest that some of the isolated strains from feces of healthy dogs can be selected for forward studies that may, in the future, corroborate their efficiency as benefic microorganisms in order to supplement the dog diets to improve their quality of life.

BF-P8

EFFECT OF NITROGEN SOURCE CONCENTRATION ON THE HYALURONIC ACID (HA) PRODUCTION BY *Streptococcus equi subsp. equi*

Dario G. Gomez[1,2] , *Faustino Siñeriz*[2,3] , *Joaquin A. Orejas*[1]

[1] *UNRC (Universidad Nacional de Rio Cuarto)* [2] *CONICET* [3] *Proimi (Planta Piloto de Procesos Industriales Microbiológicos) (dgomez@ing.unrc.edu.ar)*

The HA is an increased-in-value natural biopolymer (U$S 55.000/kg) and because of it´s exclusives rheological characteristics is extensively used in the industry (cosmetic and pharmaceutical). Microbiological HA production is nowadays more important than animal sources. *Streptococcus equi* (*subsp. zooepidemicus* and *subsp. equi*) is one of the most utilized microorganism for HA production but the disadvantage is the complex nutritional requirements of the strain (peptides, vitamins and nucleotides). HA is a primary metabolite and requires the same nutrients as biomass synthesis. The aim of this work was to study the effect of the nitrogen source on the biomass growth and HA production by a strain *S. equi subsp. equi* previously isolated in our laboratory. Experimental procedures were run in a 3L (Applikon) fermentor under controlled ambient conditions in order to improve HA productivity. In a culture medium with 5 gL^{-1} yeast extract and 20 gL^{-1} glucose there were obtained 0.8 g (p.s.)L^{-1} biomass and 0.43 gL^{-1} AH. Glucose was not completely exhausted during growth so nitrogen source concentration was increased. With 10 gL^{-1} yeast extract and 20 gL^{-1} glucose, 92.5 % and 62.8 % increase for biomass and HA concentrations respectively were achieved. Maximum HA productivity was 0.06 a 0.08 [$gL^{-1}h^{-1}$] and the glucose was exhausted between 8-10 hs. It is planned to use techniques of experience designs in order to find the optimal production conditions.

BF-P9

OPTIMIZATION OF INDIGO FORMATION BY A MARINE *Pseudomonas* STRAIN

Paula Isaac[1], Juan P. Riva Mercadal[1], Marcela A. Ferrero[1]
[1] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET) (ferreromar@hotmail.com)

Pseudomonas sp. J26 was isolated from intertidal sediment of Patagonia, Argentina, during a selective enrichment with naphthalene as a sole carbon and energy sources. It was selected as a dark blue colony by indole test, and was identified as *Pseudomonas* sp. closely related to *P. plecoglossicida* using biochemical tests and sequencing of 1400 pb of 16S rRNA gene (99.7 % similarity). Attempts at microbial bioindigo formation for industrial purposes started in 1983, when naphthalene dioxygenase genes from *P. putida* G7 were expressed in a recombinant *E. coli* strain that efficiently converted glucose to indole. After that, several oxidoreductase enzymes like monooxygenases and dioxygenases have been reported to yield indigo from many substrates.A technique developed to determine naphthalene dioxygenase (NDO) activity was optimized and used to study the biotransformation of indole to indigo by *Pseudomonas* sp. J26 whole cells. To provide an accurate indigo determination, a polypropylene vial scarification method was developed. Indigo formation reaction was started simultaneously in 30 polypropylene vials containing 0.5 ml of washed cells (OD=1.0) previously grown in JPP medium, by adding indole from a 100 mM indole in dimethylformamide (DMF) stock solution. Assays were performed by adding indole 0.25; 0.5; 1; 2.5; 4 and 5 mM, and samples were incubated at several temperatures within the mesophilic range. Tubes were collected over a 10 hours period. After that, reaction mixtures were centrifuged and supernatant were carefully pipetted off. The cell pellets were resuspended in 1 ml of DMF to ensure removal and dissolution of indigo associated to cells. The $A_{600}$of the supernatants were determined and the concentration of indigo extracted from J26 whole cell was calculated. Indigo formation was not detectable when indole concentrations lower than 1 mM were used, reaching a maximum at 2.5 mM. Activity dropped rapidly at higher concentrations, and was not detectable at 5 mM. The highest NDO activity was achieved at 25 °C according with previous data obtained for indigo production by different *Pseudomonas* strains. Finally, a maximum rate of indigo production (0.56 nmol min^{-1} dry mg $biomass^{-1}$) was achieved at 60 minutes from the beginning of the reaction and 75.5 µM indigo was produced in the next 8 hours. However, the maximal indole concentration (138.1 µM) was reached after 20 hours of incubation. In this work, we used a high-throughput and easy technique to determine NDO activity. In addition, indigo production was performed in a micro-batch culture from a whole cell system. To our knowledge, this is the first report of NDO activity measures using indole biotransformation and indigo production by a *P. plecoglossicida* related strain. *Pseudomonas* sp. J26 shows promise for bioindigo production with industrial purposes, avoiding the use of recombinant strains

BF-P10

ITACONIC ACID PRODUCTION BY *Aspergillus terreus* MJL05: CULTURE MEDIUM COMPOSITION EFFECT USING EXPERIMENTAL DESIGN TECHNIQUES

Mariana I. Juy[1,3] , María E. Lucca[2,3] , Joaquín A. Orejas[1]
[1] Grupo Ingeniería de Reacciones, Facultad de Ingeniería, Universidad Nacional de Río Cuarto [2] Microbiología Superior, Facultad de Bioquímica, Qca. y Farmacia, Universidad Nacional de Tucuman [3] PROIMI (Planta Piloto de Procesos Industriales Microbiológicos) CONICET (mjuy@ing.unrc.edu.ar)

Itaconic acid (IA) is an unsaturated dicarbonic organic acid. It can easily be incorporated into polymers and may serve as a substitute for petrochemical-based acrylic or methacrylic acid. It is used as a comonomer in resins and also in the manufacture of synthetic fibres, in coatings, adhesives and thickeners. IA is obtained by carbohydrates fermentation with a filamentous fungus, *A. terreus*. Glucose is usually used as the only carbon source. In our previous work, *A. terreus* MJL05 strain was tested in IA production with glycerol as carbon substrate in immobilized systems and stirred liquid cultures and the results obtained showed no significant differences in IA yield. In this work, with the purpose improve the productivity of the process a theoretic-experimental study is described using experimental design and response surface techniques. The factors to study were initial concentrations of three nutrients (potassium dehydrogenate phosphate, ammonium nitrate and glycerol). The responses were IA production, biomass production, conversion of glycerol and yield. The selected procedure was a 2^3 two- level complete compuest central factorial design that was implemented at two blocks or stages. The statistical analysis of the experimental results allowed define two additional zones which were examined also with a compuest central factorial design but only including two factors (ammonium nitrate and glycerol). Each experiment was carried out in 250 ml Erlenmeyer flasks with 100 ml of medium inoculated with a spore suspension of 10^8 spores/ml and incubated at 30ºC for 8 days on a rotary shaker at 200 rpm. Influence of each factor and possible interactions between them were analyzed. Depending on initial nutrients concentrations tested, IA final concentration obtained was as low as 2.0 $gr.l^{-1}$ or increased up to 25.0 $gr.l^{-1}$. Glycerol transformed in IA was varied between 15 to 55 %. The nitrogen source had a relevant impact on IA production and IA concentration was increased in 10 $gr.l^{-1}$ by adding a plus of 2 $gr.l^{-1}$ in the nitrogen concentration source. It might be concluded that IA concentration showed a linear relation respect of biomass and that considering a specific glycerol conversion factor, IA yield referred to carbon source is higher than the corresponding one for biomass.

BF-P11

***Yersinia enterocolitica* RECOVERY BY IMMUNOMAGNETIC SEPARATION**

Valeria S. Lazarte Otero[1], Cecilia S. Lucero Estrada[1], Gabriela I. Favier[1], Giovanna Gallo[1], María E. Escudero[1], Ana M. Stefanini de Guzmán[1]

[1] *Microbiología General, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis (vslazarte@unsl.edu.ar)*

Yersinia enterocolitica, a human enteropathogen, is transmitted through contaminated water and food. Its recovery by culture is difficult when a low number of this bacterium is found. The immunomagnetic separation (IMS) is a separation and concentration method which uses paramagnetic polystyrene particles (PMP) covered with specific antibodies against surface antigens of microorganisms under study. In the present work, IMS was performed for evaluating the *Y. enterocolitica* recovery from an enrichment broth. The local virulence plasmid bearing strains, *Y. enterocolitica* 2/O:9 and *Y. enterocolitica* 3/O:3 were grown in trypticase soy broth (TSB) at 22° C overnight. Their initial concentrations were standardized at OD_{600} 0.2. Serial dilutions of each strain were performed and spread on trypticase soy agar (TSA), and countings of 2 x 10^8 CFU/ml for 2/O:9 and 1x 10^9 CFU/ml for 3/O:3 were obtained. One milliliter of each dilution was transferred to 9 ml of TSB and these bacterial suspensions were utilized on the same day (day 0) and 24 h after being incubated at 22° C (day 1). Dilutiones 10^{-2} to 10^{-5} were assayed on day 0 and dilutions 10^{-6} to 10^{-9} were assayed on day 1. *Y. enterocolitica* counting obtained without IMS treatment (wIMS) were compared to those obtained after IMS treatment (aIMS). The 2,8 µm diameter PMP covered with sheep anti rabbit-IgG (Dynalbeads) reacted with rabbit anti *Y.enterocolitica* 2/O:9 IgG and rabbit anti *Y.enterocolitica* 3/O:3 IgG and were ready for the challenge against suspensions of *Y.enterocolitica* O:9 and *Y. enterocolitica* O:3. The antibody-antigen reaction was performed at 35° C for 30 min with gentle agitation. The procedure was concluded by performing three washes with PBS-0.02% Tween 20 and final suspensions in 100 µl PBS. A measured volume of each Eppendorf tube was spread on Mac Conkey agar for estimating the *Y. enterocolitica* recovery after IMS. This counting was compared to that obtained before performing IMS. The best performance of IMS in the *Y. enterocolitica* recovery was observed for the highest bacterial dilutions (dilutions 10^{-6} on day 0 and 10^{-9} on day 1). Thus, counts of 80 CFU/ml (wIMS) and 1322 CFU/ml (aIMS) for the strain 2/O:9 and 70 CFU/ml (wIMS) and 1535 CFU/ml (aIMS) for the strain 3/O:3 were obtained on day 0. Also, counts of 4 x 10^4 CFU/ml (wIMS) and 6,3 x 10^4 CFU/ml (aIMS) for the strain 2/O:9 and < 200 CFU/ml (wIMS) and 6090 CFU/ml (aIMS) for the strain 3/O:3 were obtained on day 1. The *Y. enterocolitica* recovery was up to 30 times more effective by culture after IMS than by culture without IMS.

BF-P12

PECTINOLYTIC ACTIVITY EXPRESSED BY YEASTS ISOLATED FROM OENOLOGICAL ENVIRONMENTS.

Paola Y. Maturano[1], Leticia A. Rodríguez Assaf[1,2] , María E. Toro[1], Lucía I. Castellanos de Figueroa[3,4] , Fabio Vázquez[1]

[1] *IBT- FI- UNSJ- San Juan* [2] *FCEFyN- UNSJ- San Juan* [3] *PROIMI- CONICET- Tucumán* [4] *FBQyF- UNT- Tucumán (paolamaturano@yahoo.com.ar)*

Introduction: Pectolytic enzymes play an important role in the winemaking process due to the fact that they improve the extraction of colour and aroma compounds. They also improve clarification and filtration processes of musts and wines. These enzymes break up pectin and weaken the cell wall, reducing the viscosity of musts and improving the extraction of the different compounds. Pectinases used in the food industry are commercially produced by *Aspergillus niger*. *Saccharomyces* and non- *Saccharomyces* yeasts present an alternative source for the large-scale production of commercial enzymes. Yeasts have advantages compared to filamentous fungi with regard to the production of pectinases, because they are unicellular, their growth is relatively simple, and in some species the growth medium does not require an inducer. The aim of this work was to study the pectinase activity of 162 isolated yeasts (47 non-*Saccharomyces* and 115 *Saccharomyces* sp.). Qualitative assay was carried out on plates with pectin as substrate, at 25ºC, pH 4.0 and 6.5 during 72h, in order to select yeasts having pectinolytic activity. Results were considered positive when colonies were surrounded by a degradation halo. After that, those isolates were grown anaerobically, for 72 h at 30ºC using two media: an inducing medium (0,67% YNB; 0,5% pectin and 10% glucose), and a non-inducing medium (0,67% YNB; and 10% glucose). For determination of pectinolytic activity, a reaction mixture of 0.1 ml of supernatant, 0.9 ml of 0.5% (w/v) pectin in 0.05 M sodium acetate buffer (pH 5) was used. It was prepared and incubated in a water bath at 37°C for 1 h (yeasts inoculated in inducing medium) and 24 h (yeasts inoculated in non-inducing medium). Pectinolytic activity was determined by estimation of reducing sugars by DNS technique. Results: of the 162 yeasts, 24 isolations were able to hydrolyze pectin under both pH conditions (14 *Saccharomyces* sp. and 10 non- *Saccharomyces*). Highest amounts of yeast isolations were detected at pH 6.5. All yeasts, developed in inducing and non- inducing media, expressed pectinolytic activity. Fifty percent of species from *Saccharomyces* genus registered the greatest values of pectinolytic activity growing in inducing medium. All species belonging to the non-*Saccharomyces* genera which were cultured in non-inducing medium expressed highest activity. This suggests that pectinase synthesis may be

partially constitutive for these non- *Saccharomyces* yeasts. Conclusion: this study clearly revealed the potential of indigenous yeasts to produce useful enzymes to catalyze desired biotransformations during wine fermentation and they offer an alternative source of these enzymes as well.

BF-P13
SELECTION OF INDIGENOUS YEASTS ACCORDING TO THEIR OENOLOGICAL AND VINIFICATION CHARACTERISTICS

Paola Y. Maturano[1], Leticia A. Rodríguez Assaf[1,2] , María E. Toro[1], María F. Sardella[3], María L. Martín[1], Lucía I. Castellanos de Figueroa[4,5] , Fabio Vázquez[1]
[1] IBT- FI- UNSJ- San Juan [2] FCEFyN- UNSJ- San Juan [3] IIQ-FI-UNSJ- San Juan [4] PROIMI- CONICET- Tucumán [5] FBQyF- UNT- Tucumán (paolamaturano@yahoo.com.ar)

In Oenology exists a controversy about differences between pure culture wine fermentations in comparison to those performed with indigenous yeasts. Despite the advantages of using pure cultures of *Saccharomyces cerevisiae* with regard to the easy control and homogeneity of fermentations, wine produced with pure yeasts monocultures lacks the complexity of flavour, stylistic distinction and vintage variability caused by indigenous yeasts. Mixed cultures could assure the maintenance of the typical sensory properties and characteristic profile of the wine of each area. The aims of this work were a) to evaluate oenological characteristics, b) to detect enzymatic activities and c) to compare vinification results of pure and mixed cultures of *Saccharomyces* / non-*Saccharomyces* indigenous yeasts. One hundred and sixty two yeast isolations were used. Qualitative enzymatic assays were carried out on plates with specific substrate, at 25ºC, pH 4.0 and 6.5 during 72h, in order to select yeasts expressing enzymatic activities. Based on these results, 15 strains were selected and oenologically characterized according to protocols of Vazquez et al. (2001). To be tested as starters in wine fermentations, three of these strains were selected by their valuable oenological characteristics, BSc562-04 (*S.cerevisiae*), BHv438-92 (*Hanseniaspora vinae*) and BTd259-04 (*Torulaspora delbrueckii*) in pure and mixed cultures of *Saccharomyces* / non-*Saccharomyces* (1-99% and 10-90%) were seeded in 3 l Pedro Ximenez grape must, without press. Final values (g/l): residual reducing sugars, residual total sugars, volatile acidity, total acidity, glycerol, density, and ethanol (%v 20ºC), viscosity (Pa s), conductivity (mS) were determined in all fermentations. Yeasts employed in all fermentations were selected by their relevant enzymatic activities (xylanase, celulase, amylase, β-glucosidase, protease, pectinase, esterase and lipase) and desirable oenological characters (tolerance and high ethanol production, efficient sugar consumption, growth at high sugar concentration, resistance to killer toxins). Significant differences were found in analyzed parameters of pure and mixed conditions. Pure *T.delbrueckii* fermentations showed the greatest volatile acidity, viscosity and density. It also presented the highest concentration of residual reducing sugar and residual total sugars. The highest total acidity and glycerol production was produced by *H.vinae*. However, mixed assays of all strains do not negatively influence the evolution of fermentation and analyzed parameters. In all mixed cultures, dry wines were obtained with similar or better analytical profiles to those produced by pure *S.cerevisiae*. To enhance the complexity of wine flavours in winemaking, multistarter fermentations could be an interesting alternative to a guided fermentation using a starter culture of *S.cerevisiae*.

BF-P14
EFFECTS OF THE GLOBAL REGULATOR ROB IN THE PRODUCTION OF PHB IN RECOMBINANT *E.coli* STRAINS.

Mariela P. Mezzina[1], Julia Pettinari[1]
[1] Depto de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA (marielamezzina@hotmail.com)

Polyhydroxyalkanoates (PHAs) are thermoplastic biopolyesters accumulated by numerous microorganisms under unfavorable growth conditions. These polymers, that act as energy reserve and electron sink in the cells, have drawn much attention as environmentally-friendly plastics because they are completely biodegradable. Poly(3-hydroxybutyrate) (PHB) is the best characterized PHA. Accumulation of PHB in recombinant *E. coli* has many advantages, including the capability to synthesize it from several carbon sources. In the last years, there has been an important increase in the production of glycerol, a by-product in the synthesis of biodiesel. For this reason, glycerol has become an attractive substrate for bacterial fermentations. The manipulation of metabolic fluxes is a strategy used to redirect the flow of carbon and reducing power to increase the use of substrates for the synthesis of desired products. Traditionally this is done through genetic manipulations that inactivate competing pathways, or enhance the efficiency of reactions involved in the synthesis of intermediaries for the biosynthesis of the products. In recent years an alternative that involves introducing modifications in global metabolic control has been established. This affects several pathways at the same time, altering carbon and reducing power availability. There are several regulators that affect carbon and energy flow in *E.coli*, among them are redox regulators, such as *arcA*, and regulators that affect catabolism, such as *cre*. Mutations in both of these regulators, which are in the same chromosomal region, have been seen to affect PHB production in recombinant *E.coli*. The poorly known *rob* regulator is located in the same region, close to *arcA* and

cre. It was first described as a regulator of antibiotic, heavy metals and organic solvent resistance, and some results suggest that it could affect the redox state of the cells and carbon flow. Because of this, it is a good target for genetic manipulations to improve the synthesis of PHB. The growth of wild-type *E. coli* and a mutant lacking the *rob* regulator in aerobic and microaerobic conditions in cultures containing glucose or glycerol as a carbon source were characterized. Deletion of *rob* did not have a significant effect on growth or production of PHB in the conditions tested. On the other hand, overexpression of *rob* has been shown to confer multidrug, organic solvent and heavy metal resistance. To assess the effect of an overexpression of *rob*, we cloned the *rob* gene under an inducible promoter in a plasmid compatible with the one that carries the *pha* genes, in order to be able to maintain both plasmids in the recombinant. This plasmid permits the analysis of the effect of the overexpression of *rob* on growth and PHB synthesis in recombinant *E.coli*.

BF-P15

BIOETHANOL PRODUCTION FROM SUGAR BEET PRODUCED IN SAN JUAN: SUBMERGED AND SOLID - STATE FERMENTATION.

Laura A. Rodríguez[1], *María L. Correa-Daneri*[1], *María L. Martín*[1], *Silvia C. Gouric*[1], *Martha D. Vallejo*[1]
[1] *Instituto de Biotecnología - Facultad de Ingeniería - Universidad Nacional de San Juan (mvallejo@unsj.edu.ar)*

Sugar beet (SB) *(Beta vulgaris)* is an appropriate crop to perform bioethanol production by direct fermentation (without previous hydrolysis) of its sugars (sucrose). In San Juan, SB has been proposed as raw material to producing bioethanol, but there is no information about performance of SB locally produced. In this work, we report laboratory scale bioethanol production by *Saccharomyces cerevisiae* PM16, in submerged fermentations (SmF) of sugar beet juice (SBJ), and solid-state fermentations (SSF) of milled sugar beet (MSB). SmF were carried out in 250 ml Erlenmeyer flasks containing 100 ml of sterile SBJ; SSF were done placing in Petri dishes about 50 g sterile MSB (particle size 1 x 10 mm, water content 0.59 g/g). Both of the fermentation media, containing 255 mg/g fermentable sugars, were inoculated with 5 x 10^7 cells/g, and incubated in anaerobic environment, at 28 ^oC, for 72 h. Results: time-courses of viable cells, carbon dioxide evolution, fermentable sugar content, and ethanol concentration are presented. Also, enzyme activities xylanase and polygalacturonase were measured. Maximum ethanol concentration was attained at 48 h of cultivation: 58 mg/g for SmF and 72 mg/g for SSF. Ethanol yield based on substrate consumed was about 0.4 g/g (78 % of the theoretic al yield) for SmF and 0.7 g/g for SSF. Polygalacturonase activity reached 6.0 U/ml for SmF, and 24,64 U/ml in the liquid contained in the SSF; while for xylanase activity, 0.38 U/ml in SmF and 6.19 U/ml in SSF were found. As a conclusion, high concentrations and yields were attained in SSF. Also, an important decrease of waste mass is achieved in SSF. Technological and economical advantages of SSF added to the results obtained in this work, are encouraging. Studies in order to optimize variables and to scale up the process are our next purpose.

BF-P16

SELECTION AND IDENTIFICATION OF ETHANOL HIPERPRODUCER YEASTS FROM SUGAR CANE MOLASSES

Macarena Rodriguez Prados[1], *Maria L. Muruaga*[1], *Nora I. Perotti*[2], *Carlos M. Abate*[1,3]
[1] *PROIMI- CONICET* [2] *Fac. de Cs. Exactas y Tecnología* [3] *Fac. de Bqca, Qca. y Fcia. (cabate@proimi.org.ar)*

The decrease of the petroliferous stock and gas in the world, coupled with environmental pollution and global warming, caused mainly by the excessive use of such fuels has generated the need for further studies to become feasible to use new energy sources. The bioethanol consumption produce lower evaporative emissions due to its higher octane concentration and vapor pressure in consequence the pollution levels are much lower than the observed with fossil fuels. The entry into force of Law 26,093 of biofuels in Argentina from 2010 will mean an opportunity for the sugar sector to expand ethanol production to supply 5% of it to all the naphthas. In that regard, Tucumán has a privileged position since its large capacity of ethanol production associated to sugar mills. Our work proposes a microbiological approach to use fermentative microorganisms with high tolerance to alcohol in order to increase ethanol concentration of 11% that is currently obtained in the factories by the alcoholic fermentation of molasses. To take up this, isolation and identification of ethanol hyper-producer strains of yeasts from sugar cane molasses was addressed. Molasses samples were taken from different sugar factories of Tucuman and used to inoculate YPS and YPD media with antibiotics. The microorganisms were inoculated in YPS medium with 50g/L sucrose, incubated at 30ºC with agitation. The fermentations assays were carried out in Erlenmeyers flasks with 200 ml of YPS with 250 g/L of sucrose and incubated at 30 ºC without aeration. Total and direct reducing sugars, biomass and ethanol concentration were determined. Three isolates were selected by your high ethanol production and named as A2, A10 and A11, which produced 11.74, 12.81 and 13.20% of ethanol, respectively. The isolates were identified by sequence analysis of their ARNr 28S intergenic spacer sequences, which allowed assigning identities of 99 and 100% to *Saccharomyces cerevisiae*. Promising results obtained with isolates A10 and A11 justify further studies leading to an optimization of bioethanol production.

BF-P17

THE USE OF *Shewanella oneidensis* MR-1 IN THIRD GENERATION MICROBIAL FUEL CELLS (MFCS). POSSIBILITIES OF DEVELOPING A BIOCHEMICAL OXYGEN DEMAND (BOD) BIOSENSOR.

Natalia J. Sacco[1,2] , *Maria C. Bonetto*[1,2] , *Eduardo Cortón*[1]
[1] *CONICET* [2] *QB.FCEyN.UBA (nsacco@qb.fcen.uba.ar)*

Microbial fuel cells (MFCs) have been designed for energy production purposes from chemical reactions as the more known hydrogen oxidation fuel cells. Even though an interesting application for MFCs is as electrochemical transducers of microbial reducing power (ability, capacity), related with metabolism and designed as an alternative for conventional energy generation methods when needed in small scale applications. MFCs operational principle is based in the electrons capture and transference from microbial metabolism (electron transport chain) to an anode chamber. The anode, negative, is coupled through a load resistance (RL) to a cathode, positive, by an external electrical circuit through where electrons travel from the anode to the cathode because of an existing potential difference between both electrode chamber solutions, producing currents (I). It has been demonstrated that direct electron transference from microbial cells to electrodes happens with low efficiency unless some special bacteria groups are used as *Geobacter* or a few more. MFCs has been classified in those named first generation, where redox mediators, as neutral red or metilene blue, are capable to pass through plasmatic membrane and reduce themselves to reduce an anode afterwards. In those of second generation, electrons are transported within the reduction and oxidation of sulfur compounds. Third generation MFCs employ metals reducing bacteria, *Geobacteraceae* y *Shewanellaceae* family members with membrane cytochromes capable to transfer electrons to electrodes surface. MFCs has been used as electrical energy production methods for very low power applications where can have advantages in front of conventional power fonts. In this work we propose its use as microbial metabolism transducers; several bio-analytical parameters as BOD and toxicity are closely related to microbial metabolic activity or changes in culture media or solutions are related to microbial activity. We are characterizing the *Shewanella oneidensis* MR-1 strain in a third generation MFC prototype, developed in our laboratory. When a two-chamber MFC with an anode, cathode a Nafion proton exchange membrane was inoculated with *S.oneidensis* MR-1 over a period of four days, electricity generation gradually increased to a maximum power density of 14.3 mW/m ^{2} (47000Ω resistance). The potential values obtained with this system are approximately 600 mV, this result is higher than those reported for these types of MFCs, which are in the order of 400-500 mV. We have also developed a stack system consisting in four complete individual fuel cells, as the one described above. It will also be used to the influence of the electrical circuit (series or parallel) on the power, voltage and current output of the overall stack and the MFC units in the stack and to monitor the evolution between the microbial community and electrochemical characteristic of the individual MFCs

BF-P18

***Pichia membranifaciens* AS SPOILAGE YEAST IN PATAGONIAN WINES: ISOLATION, ORIGIN AND VOLATILE PHENOLS PRODUCTION**

Julieta S. Saéz[1], *Christian A. Lopes*[1], *Marcela P. Sangorrín*[1]
[1] *Laboratorio de Microbiología y Biotecnología, IDEPA (CONICET-U.N.Comahue)*
(sangorrinmarcela@conicet.gov.ar)

Yeasts belonging to the species *Dekkera bruxellensis* have long been associated with serious economic damages in winemaking due to its ability to grow and to produce phenolic compounds. In order to evaluate the potential contamination of two wines, different yeast isolation protocols were evaluated. Wine samples were filtered using 0.2 µm pore size membranes and subsequently seeded in two selective agar media: A (GPY+cicloheximide+ampicillin) and B (GPY+ CaCO3 +ampicillin). Alicuots of the same wines were inoculated into two selective liquid media: C (yeast extract+glucose+NO3K+ampicillin) and D (YNB+glucose+cicloheximida+ampicillin). After incubation at 26ºC, yeast cultures were isolated in the same (A and B) solid media. Yeast colonies from each wine were selected according with their macroscopic features and frequencies and identified by ITS1-5.8S rDNA-ITS2 PCR-RFLP analysis. Only two yeast species (*Saccharomyces cerevisiae* and *Pichia membranifaciens*) were detected in both wines using three out of four different media. In medium A only *S. cerevisiae* colonies were observed. Because *P. membranifaciens* has been related to food spoilage, we evaluated the spoilage potential of our isolates (production of volatile phenols in wine). We also evaluated the possible source of contamination (vineyard or cellar surfaces). Healthy and damaged grapes located in both shadowed and sunny vineyard areas were sampled before harvest in the same familiar cellar. Samples from 13 fermentation vats and filters were obtained by streaking with sterile cotton plugs and incubation using selective liquid medium. After growth, samples were seeded in the same solid media and identified. *Hanseniaspora uvarum* and *Zygoascus hellenicus* were detected in both healthy and damaged grapes from sunny areas; the same two species as well as *Pichia guilliermondii* were isolated from damaged grapes in the shadow and only *H. uvarum* in healthy grapes in the shadow. Thirty-eight percent of the samples from vat surfaces did not showed yeast isolates; however, 62% evidenced the presence of *Candida boidinii* and the species *P. guilliermondii*,*Rhodotorula mucilaginosa* and *H. uvarum* were only detected one vat each. The only species detected in filters was *P. membranifaciens*. The intraspecific characterization of the *P. membranifaciens* isolates by mtDNA-RFLP demonstrated that a same strain was detected in both vines and filter.

Finally, the capacity of producing volatile phenols by different *P. membranifaciens* isolates was evaluated by HPLC in laboratory scale fermentations using sterile wine added with the precursor p-coumaric acid (100 mg/L). Average values of 0,6 mg/L of 4-etilphenol and 2,7 mg/L of 4-vinilphenol were detected, evidencing for the first time the capacity of this yeast species to produce these compounds in wine conditions.

BF-P19

Larrea cuneifolia (JARILLA MACHO) ANTIFUNGAL PROPERTIES AGAINST PLANT PATHOGENS

María V. Vogt[1], Carolina Cravero[1], Carlos E. Tonn[2], Liliana I. Sabini[3], Susana B. Rosas[1]
[1] Departamento de Biología Molecular. Universidad Nacional de Río Cuarto. Río Cuarto. [2] INTEQUI – CONICET. Universidad Nacional de San Luis. San Luis. San Luis. [3] Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto. Río Cuarto. (vvogt@exa.unrc.edu.ar)

At the present time plant pathogenic fungi cause serious economic damage of crop plants, been necessary to use large quantities of fungicides. This use has eventually led to an increase in the level of toxic residues in foodstuffs, as well as environmental pollution. Therefore, alternatives are needed for crop protection with less environmental impact. Medicinal plants are known to produce bioactive molecules which react with others organisms inhibiting bacterial or fungal growth and can be used as natural fungicides. The aim of this work was to determine the potential activity of *Larrea cuneifolia* popularly known as "Jarilla macho" on phytopathogenic fungi growth. From the aerial part of *L. cuneifolia* were obtained 5 extracts (hexane, clorophorm, methanol, cold aqueous and warm aqueous extract) and evaluated against *Fusarium graminearum*, *Fusarium solani*, *Fusarium verticillioides*, *Macrophomina phaseolina* and *Sclerotium rolfsii*. Activities were assayed by disc diffusion method, agar dilution technique and broth dilution method. The Inhibitory Concentration 50% (CI_{50}) for *M. phaseolina* and *S. rolfsii* in broth dilution method were also determined. A positive control of captan was included. The results obtained with disc diffusion detect inhibition on the clorophorm, methanol and warm aqueous extract against *F. solani*, *F. verticillioides* and *M. phaseolina*. The agar dilution technique detected inhibitory activities in all the extract of *L. cuneifolia* (concentration 1000 µg/ml). The clorophorm and methanol extract performed best, being more active against *M. phaseolina* (inhibition of 91 and 76% respectively) and *S. rolfsii* (inhibition of 98 and 76% respectively). Captan inhibition at 6,43 mg/Kg for *M. phaseolina* and 0,643 mg/Kg for *S. rolfsii* were lower. *Fusarium graminearum*, *F. solani* and *F. verticillioides* inhibition with clorophorm extract were of 64, 58 and 81% respectively. Captan inhibition at 64,3 mg/Kg for those species was lower. The CI_{50} of clorophorm extract for *M. phaseolina* in broth dilution method was of 34 µg/ml. It is conclude that *L. cuneifolia* extracts showed to possess an important capacity of inhibiting the growth of phytopatogenic fungi and may have potential applications as novel agrochemical agents in agriculture.

BF-P20

CONSTRUCTION OF STABLE _Escherichia coli_ STRAINS FOR POLYHYDROXYALKANOATES SYNTHESIS

Manuel S. Godoy[1], María J. Pettinari[1]
[1] Dpto de Qca. Biológica, Facultad de Ciencias Exactas y Naturales, UBA. (msgodoy@qb.fcen.uba.ar)

This study is centered on the construction of *E. coli* strains for the stable and efficient production of PHB from glycerol by inserting *phaBAC* genes in the bacterial chromosome and manipulating cellular metabolism to optimize carbon and energy usage in polymer synthesis. We have previously constructed a recombinant *E. coli* strain (K24) carrying *phaBAC* genes from *Azotobacter* sp. FA8, on plasmid pJP24. Strategies to redirect carbon flow include mutations in structural genes and regulators such as *arc* and *cre*. In previous work we have observed that double mutants increased PHB production when grown in microaerobic conditions. Studying the effect of mutations in *cre* within an *arc+* background will help elucidate the role of this particular regulator on growth and PHB accumulation under several conditions. On the other hand, the current recombinant strains carry the *phaBAC* genes in a plasmid, so a selective pressure needs to be maintained with antibiotics to prevent plasmid loss. The construction of a recombinant strain containing the genes in a chromosomal location will avoid these problems. Our studies with *ldhA* mutants indicated that this gene is a potential target for chromosomal location as well as a useful key for carbon and electron flow manipulation. In order to create an *E. coli* strain carrying *pha* genes within its chromosome, we developed a strategy involving four steps. In the first one, we constructed plasmid pJP24KF containing the *pha* genes and a kanamycin cassette flanked by FRT sequences. For the second step, three DNA fragments containing a conditional origin of replication next to a FRT (ORI-FRT), plus two different regions of *ldhA* gene (ldhA1 and ldhA2), were amplified by PCR using primers with restriction sites. Fragments ldhA1 and ldhA2 were cut with BamHI to make a head-tail ligation which was re-amplified in a new round of PCR, cut with EcoRI and ligated to ORI-FRT. In the third step, a new plasmid (pMSG) was created by ligating this three-in-one PCR amplicon with the *phaBAC*-kanamycin cassette fragment released from restriction of pJP24KF. Cutting pMSG by its single EcoRV site, generated a DNA fragment capable of recombining in *E. coli* chromosome by means of the *red* genes from phage lambda. In the last stage, the

resistance cassette and the conditional ORI are eliminated using another yeast recombinase. The final product is a recombinant *E. coli* strain containing the *pha* genes inserted in the chromosomal *ldh*, and no antibiotic resistance. This strain will be adequate for the efficient and stable synthesis of PHB (a bioplastic) from glycerol, the main byproduct of biodiesel synthesis. On the other hand, the use of this strain minimizes the potential environmental and sanitary risks implied by the industrial use of bacterial strains containing transferrable antibiotic resistance gen

BF-P21

DIACETYL PRODUCTION BY *Oenococcus oeni* STRAINS FROM WINES DURING GROWTH UNDER DIFFERENT CULTURE CONDITIONS.

Carmen Maturano[1]; Fabiana M. Saguir[1]
[1]Facultad de Bioquímica, Química y Farmacia. UNT. Tucumán, Argentina.(fabianasaguir@fbqf.unt.edu.ar)

Lactic acid bacteria (LAB) are associated with the wine making process by inducing malolactic fermentation (MLF), that consists in the conversion of L- malate to L-lactate and carbon dioxide. As a consequence of this reaction, the total acidity decreases and the organoleptic properties and biological stability generally improve. Diacetyl is considered one of the most important flavor produced during MLF. This compound at low concentrations (and in combinations with other wine aroma compounds) will impart yeasty, nutty, toasty aroma. Diacetyl can be reduced further by wines LAB to acetoin and 2,3- butanediol. In a previous work we selected the MS9 strain of *Oenococcus oeni* from Argentina wine by its ability to produce diacetyl, acetoin and 2,3 butanediol in concentration lower than 5 mg/l at the end of exponential growth in MRS medium. The aim of this study is to analyze the influence of some components normally found in wine on the diacetyl, acetoin and 2,3 production during growth of the MS9 strain in basal medium. At the same time the behavior of the commercial PSU strain is investigated. Cells are grown in MRS broth with tomato juice, 15%, pH 4.8, (BM) and added with (g/l): L -malate 2; citrate 0.7; SO_2 0.08 and/or grape juice 10% in place of tomato juice. Growth is measured by determining the colony forming units (cfu), diacetyl, acetoin and 2,3-butanediol and L-malic acid spectrophotometrically. In BM, the MS9 strain develops with a maximum growth rate and final cell concentration of 0.29 h^{-1} and $5.01x10^8$ cfu/ml, respectively. The PSU strain shows similar growth rate but a final cell population 9.6% higher than MS9 strain. Organic acids and/or grape juice addition to BM stimulate the final biomass, especially when all substrates are included. Thus, in this last condition, the MS9 and PSU strains grow at $6.03x10^8$ and $1.39x10^9$ cfu/ml, respectively. In BM, both strain produce 4.6 and 4.0 mg/l of diacetyl, acetoin and 2,3 butanediol, respectively. Only citric acid stimulates the aroma compounds formation as compared to BM and this effect is maximum in stationary growth phase (4.98 and 4.68 mg/l for the MS9 and PSU strains, respectively). In both strains, L-malic acid is always completely consumed.
In conclusion, the MS9 strain that displays in general, a similar behavior than the commercial strain as regards to its ability to produce the aroma compounds and malolactic activity under the tested conditions could have adequate characteristics for its potential use in winemaking. Finally, the assessment of the biochemical property in study such as diacetyl production should be considered as a quality criterion for selecting starter cultures for the wines aroma improvement.

BF-P22

***Lactobacillus plantarum* ISOLATED FROM KEFIR GRAINS AND ITS ANTAGONIC EFFECTS AGAINST *Shigella* sp. INVASION**

Kakisu, E.J[1,2], Bolla P.[1,2], Fernandez M.[1,2], De Urraza P[2], Abraham A[1], De Antoni G.L.[1,2]
(1) CIDCA-CONICET, 47 y 116 (1900) La Plata, (2) Cátedra de Microbiología General, Facultad de Ciencias Exactas, UNLP, 47 y 115 (1900) La Plata.(emilianok@cidca.org.ar)

Shigella sp. is one of the most important causes of acute diarrhoea in developing countries. In our country the most frequent species identified are *S. flexneri* and *S. sonnei*. *Shigella* sp. is the etiological agent of a disease known as shigellosis or human dysentery. The early essential step to cause shigellosis is the invasion of colonic epithelial cells, followed by intracellular bacterial multiplication and spread of invading bacteria into adjacent epithelial cells. *In vitro* models have been used to study cell invasion and subsequent events that allow efficient bacterial colonization of the epithelial layer
It is known the use of probiotics to prevent gastrointestinal disorders, including infectious diseases caused by enteric pathogens. In our laboratory has been reported the ability of some bacteria isolated from kefir grains to antagonized different enteropathogenic bacteria such as *Salmonella* and enterohemorragic *Escherichia coli*.
For this study we selected two enteroinvasive *Shigella* strains, obtained from clinical cases: *Shigella flexneri* 72 and *Shigella sonnei* 45. Three potentially probiotic microorganisms were used to study their inhibitory activity against *Shigella*: *Lactobacillus plantarum* (CIDCA 83114), *Kluyveromices marxianus* (CIDCA 8154) and *Streptococcus thermophilus* (CP2 strain). Invasion of Hep-2 cells was studied with and without previous incubation of *Shigella* with *Lb. plantarum* and the mixture of lactic acid bacteria and yeast. Both, *Shigella* and inhibitory microorganisms at

concentration of 10^8 CFU/well were added to Hep-2 monolayer and incubated during one hour. Similar experiments were performed by pre-incubating bacterial walls obtained from *Lb. plantarum.* Enumeration of *Shigella* was performed by plate-count on nutrient agar. Unlike the control in which the pathogen had invaded the Hep-2 cells, the results showed a significant reduction of *Shigella* invasion when cells were treated with lactobacilli and the mixture of potentially probiotic strains. In order to explore the protection mechanisms, co-aggregative properties of strains and the action of proteolytic enzymes were tested. Co-aggregation was determined by measuring optical density. The results obtained indicate that protection against *Shigella* could not be attributed to co-aggregation with lactic acid bacteria or yeast since any OD reduction or aggregation of microorganisms has been detected. An anti-invasive effect has been observed with *Lb. plantarum* bacterial walls. Treatment of *Lb. plantarum* with pepsin decreased the antagonic effect against *Shigella*. The results obtained demonstrate that certain protection effects against invasion may be ascribed to bacterial surfaces.
In conclusion, we have found that *Lb. plantarum* isolated from kefir lactobacilli has the ability to protect Hep-2 cells against invasion by *Shigella flexneri* and *Shigella sonnei*. This property could be associated to the presence of lactobacilli surface proteins.

BF-P23

EFFECT OF BUTYLATEDHYDROXYANISOLE ON OCHRATOXIN A PRODUCTION BY *Aspergillus* SECTION *Nigri* ON PEANUT GRAINS.

Carla Barberis, Guillermina Fernandez-Juri, Ana María Dalcero, Carina Magnoli.
Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico- Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional Nº: 36 Km 601 (5800) Río Cuarto, Córdoba, Argentina.

Peanut (*Arachis hypogaea L.*) is one of the most important agricultural products in the Argentinean economy. About 94 % of the national yield is produced in the centre-south region Córdoba province. An important percentage of total production is left outside the external market every year, due to post-harvest fungal contamination and mycotoxin contamination. Ochratoxin A (OTA) is one of the most important fungal toxic metabolites of worldwide concern for human and animal health, due to its implication in a diverse range of toxicological effects, including renal toxicity, mutagenicity, teratogenicity and immunotoxicity.The control of environmental factors - such as water activity (a_W) and temperature - during storage could prevent *Aspergillus* section *Nigri* growth and OTA production in several agricultural products. Previous studies demonstrated that this antioxidant could control fungal growth, fumonisins (FBs) and aflatoxins (AFs) production on natural substrates.The aim of the present study was to evaluate the effect of one of the best food grade antioxidant, butylatedhydroxyanisole on i) the lag phase to growth, ii) growth rates and iii) OTA production by *Aspergillus niger* aggregate strains under different environmental conditions on peanut grains. *A. niger* aggregate strain (RCP191) and *A. carbonarius* (RCPG) were evaluated. Irradiated peanut grains (7 kGy) were dehydrated to 0.980, 0.950 and 0.930 a_W. The antioxidant 2,3-ter-butil-4-hidroxianisol (BHA) was added to the irradiated grains to obtain the required concentrations (1, 5, 10 and 20 mmol g^{-1}). Peanut grains were inoculated centrally with 5 µl of spore suspensions (1 x 10^6 spores ml^{-1}) and incubated at 18 and 25°C for 30 days. Growth parameters and OTA concentration were determined. Ochratoxin A extraction was carried out using a clean up column (MycoSep ®229 Ochra column, MFC, Romer Labs®, Inc., MO., USA) and detected by HPLC. The antioxidant BHA at 5, 10 and 20 mmol g^{-1} inhibited completely OTA production by *A. carbonarius* strain at 0.93 and 0.95 a_W at 18ºC, whereas for *A. niger* aggregate strain this fact was observed only at 0.95 a_W and at the same temperature. However, a significant reduction in OTA production was observed at this temperature with 5 and 10 mmol g^{-1} and 0.93 a_W. In general at 25ºC BHA was ineffective to reduce OTA production in all conditions assayed. The use of antioxidants could be an alternative to control ochratoxigenic fungi in peanut grains during storage before they are destined to internal and external market and industrialized.

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Ortiz Marquez JC	BF-O3
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Rodríguez MA	EM-P8
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Tirante L	MM-P19
Tolmasky ME	MM-P4 MM-P18
Tonelli ML	BB-P24
Tonn CE	BF-P19
Toro ME	BB-P15, BB-P16 BF-P12 BF-P13 EM-P9
Torres AM	BB-P18 BD-P4
Torres Sanchez RM	BB-P17
Tranchida MC	BB-P25
Trejo FM	BB-O5
Tribelli P	MM-P17
Tymczyszyn EE	BB-P5 PM-P3
Urdaneta I	EM-P7
Valdeón DH	BF-P5 PM-P8
Valdez JC	BB-O6 PM-P13
Vallejo MD	BF-P1 BF-P2 BF-P15
Valverde C	BD-P1BD-P12 IN-O4
Vargas WA	PM-O4
Vay C	MM-O4 MM-O5 PM-P6
Vázquez F	BB-P15, BB-P16 BF-P12 BF-P13 EM-P9 EM-P18